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Sherma Zibadi *Editors*

Bioactive Dietary Factors and Plant Extracts in Dermatology

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Preface

Historically, as well as recently, research is showing that foods, dietary supplements, and some nutrients are important in skin cancer prevention and skin health. Within the 48 chapters of this book two major needs are fulfilled by defining the role of dietary supplements, foods, and nutrients in treatment and prevention of skin cancer and dermal damage. A major focus is on the primary causes of dermal damage: aging and solar exposure in seven focused areas of skin health promotion.

Initially broad overviews of diet and food in skin health are reviewed. Thus the role of foods found in the Mediterranean diet and probiotics are documented to affect the skin and prevent damage. In addition overview chapters are included on the effects of ultraviolet irradiation (UV) which cause significant damage and cancer in were included the skin.

The book's second section describes the role of selected nutrients in promoting skin health and preventing dermal diseases. Diet and nutrition are vital keys to controlling morbidity and mortality from chronic diseases. Thus taurine and omega 3 fatty acids can aid in ameliorating psoriasis and this is documented in reviews. In addition, antioxidant actions of vitamin C and nitric oxide produced from supplemental arginine are keys to dermal health.

Then key researchers in the third section describe the role of herbs and plant spices in skin health. Turmeric and ginger are documented to function in skin care. In addition an Indian indigenous berry and Aloe vera's roles in dermatology are described.

The fourth section has detailed reviews of selected dietary components in dermal health. Polyphenols as a group with components from grapes, chocolate, and other nutrient rich botanicals are explored as examples. Antioxidants also play roles in skin functions. Specifically, resveratrol, rice bran, and coenzyme Q10 are discussed. Antioxidants in dietary supplements and nutriceutical foods counteract some of the damaging effects of UV (ultraviolet) radiation (light) in skin and other tissues. They play key roles in preventing the development of skin cancer. UV light is clearly the major cause of skin cancers as well as aging, damaged skin. Therefore experts reviewed the roles of bioactive foods and their constituents to reduce UV-induced skin cancer and dermal damage.

The fourth section focuses on historic vitamins with well-defined effects on skin and skin cancer where new research is providing new insights. Thus folate, vitamin D, and vitamin E on skin cancer are reviewed. These are readily available agents that have multiple effects on health and frequently used as supplements.

The fifth and major section investigates research and focuses on the two major types of skin cancer: melanoma and basal cell carcinoma. Skin cancer is the most common form of cancer and dietary materials can play a key role. The U.S. National Institutes of Health report that only 18 % of adults meet the recommended intake of vegetables. Increasingly, Americans, Japanese, and Europeans are turning to the use of dietary vegetables, medicinal herbs, and their extracts or components to prevent or treat cancer. It has been known for decades that those populations with high plant consumption

have reduced risks of cancers. Therefore important foods in skin health and cancer prevention are reviewed. These include Indian foods, chocolate, green tea and its components, licorice, fruit antioxidants, mangosteen, soybeans, and polyacetylenes in carrots. In addition the multitude of complex biomolecules as dietary extracts in dietary fruits and vegetables play a crucial role in skin health maintenance. Experts review dietary supplements in general as well as specific ones including *N*-acetylcysteine, turmeric, and polyphenols in general.

The final section is extensive in its review of plants and their components in preventing and treating skin diseases. There is a huge cosmetic and skin care industry for damage that does not result in cancer. Here antioxidant dietary materials may be particularly useful in prevention or as ingredients in medications to combat solar and aging effects. Specific issues confronting older Americans include challenges of how to deal with changes in skin texture, health, and, especially, cancer. The U.S. Bureau of Census predicts that seniors are increasing dramatically and will more than double to 80 million by 2050, at which time there will be nearly 2 billion seniors worldwide. It is critical that these additional years are productive, enjoyable, and disease free. Antioxidants and their food and herbal sources should play critical roles in this process. Antioxidants in dietary vegetables and their products often have limited harmful side effects. This stands in stark contrast to many drugs that are promoted and studied for possible disease-preventive activity. A wide variety of herbs including ginger, vitamins, Indian native plant remedies, foods, including chocolate, and well-recognized herbs, including aloe vera, are reviewed by experts. Mechanisms of actions including molecular sensors and mediators in skin cancer, and insulinotropic signaling in psoriasis and atopic dermatitis are defined.

Plant extracts as dietary supplements are now a multibillion-dollar business, built upon limited research data. Common dietary vegetables and herbs and their over-the-counter extracts are readily available. Therefore this book is useful to the growing nutrition, food science, and natural product research and development community. This book focuses on the growing body of knowledge on the role of various dietary plant constituents that reduce oxidative damage as part of chronic disease. Expert reviews define and support the actions of bioflavonoids, antioxidant vitamins, and similar materials that are part of dietary vegetables, dietary supplements, herbs, and nutriceuticals.

Finally, the volume editors would like to extend their appreciation to Springer and their staff for providing the professional platform of communication for new, challenging ideas and hypotheses in nutritional sciences. Similarly appreciation is extended to the series editor Adrianne Bendich for her personal input in positioning the book toward the right audience and also her incisive and pertinent comments, recommendations, and suggestions for improving the presentation, content, and cohesion.

Tucson, AZ, USA

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Series Editor Page

The great success of the Nutrition and Health Series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes: (1) a synthesis of the state of the science, (2) timely, in-depth reviews by the leading researchers in their respective fields, (3) extensive, up-to-date fully annotated reference lists, (4) a detailed index, (5) relevant tables and figures, (6) identification of paradigm shifts and the consequences, (7) virtually no overlap of information between chapters, but targeted, inter-chapter referrals, (8) suggestions of areas for future research, and (9) balanced, data-driven answers to patients' as well as health professionals' questions, which are based upon the totality of evidence rather than the findings of any single study.

The Series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The editor(s), whose training(s) is (are) both research and practice oriented, has the opportunity to develop a primary objective for their book, define the scope and focus, and then invite the leading authorities to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

"Bioactive Dietary Factors and Plant Extracts in Dermatology," edited by Dr. Ronald Ross Watson and Dr. Sherma Zibadi, is a very welcome addition to the Nutrition and Health Series. The 48 chapters in this comprehensive volume include chapters that examine the role of essential and nonessential dietary components on skin health, skin care, skin cancers, and other dermatological diseases. The book is logically organized into seven sections and begins with an overview section that includes five informative chapters. The first chapter, on the Mediterranean diet and skin health, informs us that people from Mediterranean regions have one of the lowest melanoma rates in Europe. Several of the bioactive plants and plant components of the Mediterranean diet, including herbs, vitamin C, vitamin E, *n*-3 polyunsaturated fats from extra virgin oil and blue fish, and anthocyanins and flavonoids from red oranges, are reviewed in depth in subsequent chapters. The second chapter provides important safety data concerning the potential for certain dietary factors and plant extracts to trigger some immune-mediated skin disorders including autoimmune and hypersensitivity reactions. Sun exposure provides UV radiation that can adversely affect the skin if exposure is prolonged and skin is not protected. Several dietary components have been shown to protect skin from UV damage and these are reviewed in the next chapter. Skin changes have been documented as consequences of metabolic diseases including diabetes and obesity, as reviewed in the fourth chapter. Diabetes-associated skin changes include inflammation, immune dysfunction, imbalanced epidermal homeostasis, and other skin disorders. Obesity is associated with changes in skin barrier function, sebaceous gland and sebum production, wound healing as well as acanthosis nigricans, and skin tags that may be associated with

insulin resistance. The final chapter in the overview section is unique in its examination of the role of probiotics in skin health. Several clinical trials have shown an association between probiotic supplementation and the management of atopic dermatitis. Thus, the chapter reviews the immunological mechanisms affected by probiotics, beyond the gut, that may benefit the skin.

The second section contains four chapters that describe the roles of certain vitamins, fatty acids, and amino acids in maintaining the health of the skin as well as their effects in certain skin diseases. The chapter on the importance of vitamin C to skin health reminds us that vitamin C is essential to the formation of collagen and that its antioxidant properties enhance the potential for reduction in UV skin damage. Omega-3 fatty acids, given as a supplement or as an intravenous infusion, have been shown to reduce psoriatic lesions of the skin. This new area of clinical research is summarized in the next chapter. Arginine, a nonessential amino acid (and sometimes considered as a conditionally essential amino acid), is metabolized to nitric oxide. This nitric oxide metabolic pathway has been found in several cell types that reside in the skin including keratinocytes, melanocytes, Langerhan's cells, fibroblasts, and endothelial cells. Taurine is also considered as a conditionally essential amino acid, and recent clinical studies with taurine-containing compounds have shown some benefits when given orally or used topically in several skin conditions.

The third section on dietary components and skin care contains four chapters that examine the potential for certain foods and food components to affect skin functions. The plant components are used internally, externally, or in some cases, can be used both ways. The substances reviewed in individual chapters include turmeric, ginger, amla, and aloe vera. These plants and the plant parts have been used to treat various skin ailments for centuries in Chinese, Ayurvedic, Tibetan, Unani, Srilankan, Arabic, and African traditional medicines. The authors of these chapters review the mechanisms of action of the plants and plant extracts, as well as the major bioactive components and examine preclinical studies and, where available, clinical data related to the assessment of skin quality as well as wound healing, UV blocking, treatment and/or prevention of psoriasis, skin aging and skin cancer. Although all of these plants have been used to treat conditions affecting many aspects of health, with respect to the skin, observational as well as preclinical and some clinical studies have shown these four plants to have skin care properties such as preventing signs of skin ageing, acne, psoriasis, enhancing wound healing, reducing UV-induced skin damage, and reducing the effects of chemically induced carcinogenesis in animal models.

There are many dietary components that affect skin health. The next section highlights eight dietary components and their effects on the functioning of the skin. This Series has a whole volume devoted to "*Chocolate in Health and Nutrition*," edited by Ronald Ross Watson, Victor R. Preedy, and Sherma Zibadi. The first chapter in the section examines the role of chocolate in skin health. Other substances reviewed include oats and its constituents (*Avena sativa*) in a chapter that includes excellent tables, figures and 86 up-to-date references, resveratrol, grape seed extracts, coenzyme Q10, and rice bran. Skin health is discussed with respect to increased elasticity, tonicity and/or firmness, reduced wrinkle width and/or volume, reduced fine lines, increased hydration, decreased skin roughness, decreased scaling, improved skin structure and barrier function, and depigmentation of age-related skin spots. Conditions discussed include extra dry, itchy skin (xerosis), and dry skin associated with diabetes, as well as associated pruritus (itching), eczema, UV-induced skin damage and acne. Data included in these chapters are derived from preclinical as well as clinical studies where available. Administration of the substances can be via oral intake and/or topical applications. Several chapters examine novel oral and topical delivery systems to enhance the bioavailability of the substances.

Skin cancer is generally divided into two categories: melanoma and non-melanoma skin cancers. Non-melanoma skin cancers include basal cell carcinoma and squamous cell carcinoma. Basal cell carcinoma is the most common form of skin cancer and rarely spreads to other tissues. Squamous cell carcinoma cells have the ability to invade other tissues of the body. Melanoma is not as common as the two types of carcinomas but it is the most lethal form of skin cancer. This volume contains 14

chapters that review the role of dietary factors in skin cancer development and treatment. Several chapters specifically examine melanoma as this is considered the most serious type of skin cancer.

The fifth section, containing three chapters, reviews the data on essential nutrients (folate, vitamin D, and vitamin E) and their potential to affect skin cancer as preventive as well as therapeutic agents. The chapter on folate, with nine excellent figures and 135 relevant references, indicates that folate nutrient levels support many biochemical processes important for the maintenance and function of healthy skin. When dietary folate intakes are low and/or skin folate levels are depleted, there are associated findings of increased risk of psoriasis, vitiligo, dermatitis, and skin cancers. The precursor of the active form of vitamin D is synthesized in the sun-exposed skin. Although low vitamin D status is associated with increased risk of many cancers, increased sun exposure is associated with increased risk of skin cancers. This well-referenced and illustrated chapter carefully balances the recommendations for safe levels of UV exposure with the dietary recommendations for assuring optimal vitamin D status.

Eleven chapters are devoted to examining the role of dietary factors and their components and dietary supplements in skin cancer development and/or treatment. The first three chapters in this section provide broad overviews of the potential mechanisms of action of plant molecules and describe the models used to test the activities of these bioactive components. Specific chapters review findings using components of grapes, milk thistle, citrus peel, tomatoes, bitter melon, marigold, peach, soy, carrots, chocolate, turmeric, and green tea. Certain components that are found in a number of these foods include flavonoids that have been shown to have chemopreventive properties. Specifically discussed are apigenin, genistein, silymarin, quercetin, and *N*-acetylcysteine. Certain non-flavonoid polyphenols also have been shown to have chemopreventive activities such as resveratrol, curcumin, and epigallocatechin-3-gallate (EGCG). These chapters examine the current body of research and include detailed information about cellular targets, research from in vitro and laboratory animal studies and clinical data when available. Safety data from clinical studies are included in addition to potential efficacy findings.

The final section of the volume mainly examines the importance of plants and plant extracts in the care of non-cancerous skin diseases. The majority of the unique chapters describe plants that have been used in India as part of the Ayurvedic medicine tradition. Each plant is described using its botanical name and the uses of each plant part are described. This section contains 13 chapters that review skin diseases including psoriasis, atopic dermatitis, acne, parasitic skin diseases, fungal, viral, and bacterial skin diseases as well as systemic diseases that result in skin changes. Substances not examined in earlier chapters include licorice, Indian gooseberry, East Indian globe thistle, mangosteen, neem, karanja oil, heartleaf moonseed and Indian ginseng. A number of the chapters review findings from non-Western cultures where tropical fruits, herbs and spices have been used as components of salves and lotions as well as oral solutions for thousands of years but without the benefit of well-controlled trials that would provide valuable information with regard to dosing, duration of use as well as safety data.

In addition to the chapters on plants, the in-depth chapter by Melnik, which includes over 200 references, examines the overall effect of Western diets and obesity on the development of the major classes of skin diseases. This chapter, as well as the chapter by Sabetisoofyani that links leptin levels with increased risk of melanoma, remind us that total dietary caloric intake above recommended intake levels increases the risk of many serious diseases including some that affect the skin. The final chapter in this comprehensive volume examines the potential for orally administered probiotics to be of benefit in the treatment of atopic dermatitis. The chapter includes an extensive review of the immunology of the skin and the complexity of treating atopic dermatitis as this disease may involve fungal as well as bacterial infections and is thought to have a genetic component as well as neuropathology. The chapter includes over 130 relevant references and detailed tables that summarize both the laboratory animal models as well as clinical studies using specific probiotics in the treatment of chronic dermatological diseases.

The logical sequence of the sections as well as the chapters within each section enhance the understanding of the latest information on the role of dietary components and therapeutically used plants in the care of the skin under healthy as well as skin disease condition. The volume contains unique chapters that are helpful for clinicians, related health professionals including the dietician, nurse, pharmacist, physical therapist, behaviorist, psychologist, and others involved in the treatment of many different racial and ethnic population groups with skin conditions as well as serious skin diseases. This comprehensive volume has great value for academicians involved in the education of graduate students and postdoctoral fellows, medical students, and allied health professionals who plan to interact with patients and/or clients with relevant dermatological disorders.

The volume contains over 100 detailed tables and figures that assist the reader in comprehending the complexities of skin physiology as well as the details of many of the plants used to treat skin conditions. The overriding goal of this volume is to provide the health professional with balanced documentation and awareness of the newest research and therapeutic approaches including an appreciation of the complexity of the skin's responses to UV-induced trauma, infections, and diseases. Hallmarks of the 48 chapters include key words and bulleted key points at the beginning of each chapter, complete definitions of terms with the abbreviations fully defined for the reader and consistent use of terms between chapters. There are over 2,000 up-to-date references; all chapters include a conclusion to highlight major findings. The volume also contains a highly annotated index.

This unique text provides practical, data-driven resources based upon the totality of the evidence to help the reader understand the basics, treatments and preventive strategies that are involved in the understanding of how skin conditions may affect healthy individuals as well as those with chronic diseases. Of equal importance, critical issues that involve patient concerns, such as UV exposure, potential effects of immunological, psychological, and neurological functions, are included in well-referenced, informative chapters. The overarching goal of the editors is to provide fully referenced information to health professionals so they may have a balanced perspective on the value of various preventive and treatment options that are available today as well as in the foreseeable future.

In conclusion, "*Bioactive Dietary Factors and Plant Extracts in Dermatology*," edited by Ronald Ross Watson, Ph.D. and Dr. Sherma Zibadi, M.D., provides health professionals in many areas of research and practice with the most up-to-date, well-referenced, and comprehensive volume on the current state of the science and clinical practice involving plants used as foods as well as therapeutics in the maintenance of healthy skin and the treatment of skin diseases. This volume will serve the reader as the most authoritative resource in the field to date and is a very welcome addition to the Nutrition and Health Series.

Adrianne Bendich, Ph.D., F.A.C.N., F.A.S.N.

Series Editor Bios

Dr. Adrianne Bendich has recently retired as Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare where she was responsible for leading the innovation and medical programs in support of many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has coauthored over 100 major clinical research studies in the area of preventive nutrition. Dr. Bendich is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich, who is now President of Consultants in Consumer Healthcare LLC, is the editor of ten books including "*Preventive Nutrition: The Comprehensive Guide For Health Professionals*," fourth edition, coedited with Dr. Richard Deckelbaum, and is the Series Editor of *Nutrition and Health* for Springer/Humana Press (www.springer.com/series/7659). The Series contains 40 published volumes—major new editions in 2010–2011 include *Vitamin D,* second edition edited by Dr. Michael Holick; *Dietary Components and Immune Function* edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi, and Dr. Victor R. Preedy; *Bioactive Compounds and Cancer* edited by Dr. John A. Milner and

Dr. Donato F. Romagnolo; *Modern Dietary Fat Intakes in Disease Promotion* edited by Dr. Fabien DeMeester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; *Iron Deficiency and Overload* edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; *Nutrition Guide for Physicians* edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; *Nutrition and Metabolism* edited by Dr. Christos Mantzoros, and *Fluid and Electrolytes in Pediatrics* edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include *Handbook of Drug-Nutrient Interactions* edited by Dr. Joseph Boullata and Dr. Vincent Armenti; *Probiotics in Pediatric Medicine* edited by Dr. Sonia Michail and Dr. Philip Sherman; *Handbook of Nutrition and Pregnancy* edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch, and Dr. Elliot Philipson; *Nutrition and Rheumatic Disease* edited by Dr. Laura Coleman; *Nutrition and Kidney Disease* edited by Dr. Laura Byham-Grey, Dr. Jerrilynn Burrowes, and Dr. Glenn Chertow; *Nutrition and Health in Developing Countries* edited by Dr. Richard Semba and Dr. Martin Bloem; *Calcium in Human Health* edited by Dr. Robert Heaney and Dr. Connie Weaver, and *Nutrition and Bone Health* edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich served as Associate Editor for *Nutrition,* the International Journal; served on the Editorial Board of the *Journal of Women's Health and Gender-Based Medicine*, and was a member of the Board of Directors of the American College of Nutrition.

Dr. Bendich was the recipient of the Roche Research Award, is a *Tribute to Women and Industry* Awardee, and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences, 2000–2001. In 2008, Dr. Bendich was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. Dr. Bendich holds academic appointments as Adjunct Professor in the Department of Preventive Medicine and Community Health at UMDNJ and has an adjunct appointment at the Institute of Nutrition, Columbia University P&S, and is an Adjunct Research Professor, Rutgers University, Newark Campus. She is listed in Who's Who in American Women.

Volume Editors Bios

Ronald Ross Watson, Ph.D., attended the University of Idaho but graduated from Brigham Young University in Provo, Utah, with a degree in chemistry in 1966. He earned his Ph.D. in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained 2 years of postdoctoral research experience in immunology and nutrition.

From 1973 to 1974, Dr. Watson was assistant professor of immunology and performed research at the University of Mississippi Medical Center in Jackson. He was assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982 Dr. Watson joined the faculty at the University of Arizona Health Sciences Center in the Department of Family and Community Medicine of the School of Medicine. He is currently professor of health promotion sciences in the Mel and Enid Zuckerman Arizona College of Public Health.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and alcoholism research societies. Among his patents he has one on a dietary supplement; passion fruit peel extract with more pending. He had done DHEA research on its effects on mouse AIDS and immune function for 20 years. He edited a previous book on melatonin (Watson RR. *Health Promotion*

and Aging: The Role of Dehydroepiandrosterone (DHEA). Harwood Academic Publishers, 1999, 164 pages). For 30 years he was funded by Wallace Research Foundation to study dietary supplements in health promotion. Dr. Watson has edited more than 100 books on nutrition, dietary supplements and over-the-counter agents, and drugs of abuse as scientific reference books. He has published more than 500 research and review articles.

Dr. Sherma Zibadi received her Ph.D. in nutrition from the University of Arizona and is a graduate of the Mashhad University of Medical Sciences, where she earned her M.D. She has recently completed her post-doctoral research fellowship awarded by the American Heart Association. Dr. Zibadi engages in the research field of cardiology and complementary medicine. Her main research interests include maladaptive cardiac remodeling and heart failure, studying the underlying mechanisms and potential mediators of remodeling process, which helps to identify new targets for treatment of heart failure. Dr. Zibadi's research interest also extends into alternative medicine, exploring the preventive and therapeutic effects of natural dietary supplements on heart failure and its major risk factors in both basic animal and clinical studies, translating lab research finding into clinical practice. Dr. Zibadi is an author of multiple research papers published in peer-reviewed journals and books, as well as coeditor of several books.

Acknowledgments

The work of Dr. Watson's editorial assistant, Bethany L. Stevens, and Humana's project manager, Maureen Alexander, in communicating with authors, working with the manuscripts and the publisher was critical to the successful completion of the book and is much appreciated. Their regular responses to queries and collection of manuscripts and documents were extremely helpful. Support for Ms. Stevens' work was graciously provided by the National Health Research Institute (nonprofit). It is part of its mission to communicate to scientists about bioactive foods and dietary supplements was vital (http://www.naturalhealthresearch.org). Such support and was part of the Institute's efforts to educate scientists and the lay public on the health and economic benefits of nutrients in the diet as well as supplements. Finally Mari Stoddard of the Arizona Health Sciences library was instrumental in helping find the authors and their addresses in the early stages of the book's preparation. The support of Humana Press staff as well as the input by the series editor, Adrianne Bendich, is greatly appreciated for the improved organization of this book.

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Part I
Overview

Chapter 1
Mediterranean Diet and Skin Health

Laura Primavesi, Marta Piantanida, and Valerio Pravettoni

Key Points

- Mediterranean diet includes similar cooking traditions from regions bordering the Mediterranean sea. This diet encourages high intake of fruits, vegetables, and legumes and low consumption of red meat and saturated fats.
- As people from Mediterranean regions present one of the lowest melanoma rates, Mediterranean diet can be extremely helpful to providing skin protection.
- This dietary approach may be recommended particularly during early childhood, for populations with high risk to develop skin diseases, and for individuals with high UVR exposure and/or compromised immunity.
- The most interesting bioactive components of the Mediterranean diet include carnosol from herbs like sage and rosemary, vitamin C together with vitamin E, *n*-3 polyunsaturated fats from extra virgin oil and blue fish, and anthocyanins and flavonoids from red oranges.

Keywords Mediterranean diet • Dietary antioxidant supplementation • Carnosol • Red orange complex • Vitamin C • Vitamin E • Carotenoids • Hydroxytyrosol • *n*-3 Polyunsaturated fats • UV radiation

Mediterranean Diet: Definition

The Mediterranean diet (MD) represents a complex series of centuries-old nutritional habits typical of the regions bordering the Mediterranean Sea. One of the first scientists to study the MD was Ancel Keys (1904–2004), who hypothesized that different kinds of dietary fats could have different effects on health. He observed a lower mortality rate due to cardiovascular diseases in Southern Europe and Northern Africa than in Northern Europe and the United States [1].

The most commonly known version of the MD is graphically represented by a food pyramid, first presented in the mid-1990s and based on food patterns typical of Crete and Southern Italy. Briefly, this diet emphasizes high consumption of vegetables, fruit, legumes, and unrefined cereals, moderate

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consumption of dairy products (mostly as cheese and yogurt), moderate to high consumption of fish, low consumption of meat and meat products (in particular reducing consumption of red meat), and moderate wine consumption. Fats represent approximately 25–35% of the MD total calories, almost exclusively constituted by olive oil instead of butter, margarine, and other saturated fats comprising only 8% or less of the daily caloric intake [2].

We aim to highlight that the MD constitutes not only a mere food chart but represents a set of skills, knowledge, practices, and traditions promoting social interaction and a healthy lifestyle. Latest versions of the MD include at the base of the food pyramid the following concepts: physical activity, conviviality, and seasonal and local consuming. On account of this fact in 2010, the United Nations Educational, Scientific and Cultural Organization (UNESCO) included the MD in the representative list of the intangible cultural heritage of humanity.

In the following paragraphs, we focus our attention on peculiar MD food components considered to have a photoprotective effect. We prefer to start with major natural sources because nutraceutical properties of bioactive molecules are often enhanced by a synergic effect that occurs in the food matrix.

Mediterranean Herbs

In the MD, an important role is played by Mediterranean herbs, including rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.), and oregano (*Origanum vulgare* L.). Their flavorful leaves are commonly used to season meals and reduce salt content. These herbs are very rich in diterpenes, recently discovered to be phytochemicals because of their functional properties, such as their antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. One of the most investigated and promising molecules is carnosol, an ortho-diphenolic diterpene with an abietane carbon skeleton with hydroxyl groups in the C11 and C12 position and a lactone moiety across the B ring. This compound is derived from the oxidative degradation of carnosic acid. Carnosol and carnosic acid together represent approximately up to 5% of the dry weight of rosemary leaves and account for more than 90% of their antioxidant properties [3].

Huang and colleagues [4] evaluated the effect of a methanol extract from dried rosemary leaves on tumor onset and promotion in mice skin. To induce tumor onset, the mice were topically treated on their backs with benzo(a)pyrene (BP) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) once or twice a week. In a group of animals topically treated with 1.2 mg or 3.6 mg of rosemary extract 5 min prior to each application, the number of tumors per mouse decreased by 54–64%. In contrast, topical application of BP + TPA in mice not treated with rosemary extract resulted in 7.1 tumors per mouse. The application of rosemary extract on mouse skin also inhibited TPA-induced ornithine decarboxylase activity, TPA-induced inflammation, arachidonic acid-induced inflammation, TPA-induced hyperplasia, and TPA-induced tumor promotion. Other mice were treated with 7,12-dimethylbenz(a)anthracene (DMBA) and TPA. In addition, in mice pretreated with rosemary extract (0.4, 1.2, and 3.6 mg), the number of TPA-induced skin tumors was reduced per mouse by 40%, 68%, and 99%, respectively. In contrast, mice without rosemary extract application developed an average of 17.2 skin tumors per mouse. The authors also investigated the effect of a topical application of carnosol or ursolic acid isolated from rosemary. Carnosol (1, 3, and 10 μmol) and ursolic acid (0.3, 1, or 2 μmol) inhibited the number of skin tumors per mouse in DMBA-pretreated mice by 38%, 63%, and 78%, and by 45–61%, respectively. Thus, rosemary extract seems to have a stronger inhibitory effect on tumor promotion than carnosol or ursolic acid alone, suggesting that combinations of these compounds together with other rosemary constituents are responsible for inhibitory effects against tumors. In conclusion, topical application of rosemary extract inhibited the covalent binding of BP to skin and the tumor onset by BP and DMBA.

Offord and colleagues [5] investigated the protective effect of carnosic acid against UVA-induced photodamage in a cell culture system. Human dermal fibroblasts, derived from a young male with skin type III (brown hair, brown eyes), were treated with several nanoparticle formulations containing dietary antioxidant alone or in mixture to preserve their properties. This treatment was performed 24 h before UVA irradiation of a typically minimal erythemal dose in human skin to allow penetration into cells. UVA irradiation led to a 10- to 15-fold increase in metalloproteinase 1 expression, considered a marker of potential collagen degradation and photoaging. This increase was suppressed in the presence of low micromolar concentrations of vitamin E, vitamin C, or carnosic acid. Heme-oxygenase 1 expression, a general marker of cellular oxidative stress, was also strongly induced by UVA irradiation but none of the antioxidants inhibited this effect at the concentrations used in this study.

Recently, Russo and coinvestigators [6] demonstrated the ability of a rosemary extract, containing 31.7% of carnosic acid, 0.4% rosmarinic acid, and 5.9% of carnosol, to counteract the adverse effects of UV radiation. Consistent with prior studies, at concentrations of 10–80 μg/mL, this extract was able to significantly reduce the growth of two melanoma cell lines in a dose-dependent manner. In addition, this study provided the first evidence that rosemary extract reduces the growth of human cancer cells by triggering an apoptotic process. In fact, melanoma cells exposed to methanol rosemary extract demonstrated high DNA fragmentation but no necrosis, which was indicated by no statistically significant increase in cytoplasmic lactate dehydrogenase (LDH) release.

Citrus Fruit

In the MD, citrus fruit is much more important than any other fruits and vegetables. Seven countries in the Mediterranean basin, including Spain, Italy, and Egypt, are in the top 20 producers of the sweet orange (*Citrus sinensis* L.), the most commonly grown fruit tree in the world. A total of 68.5 million tons of this orange was produced worldwide in 2008 (http://faostat.fao.org). Red or blood oranges are named for their juice, which is reminiscent of blood, and cultivated since the fifteenth century in Sicily (Italy); they represent a natural variety of sweet oranges with an abnormal pigmentation that gives the pulp a streaked red color. Important varieties of blood oranges are *Tarocco*, *Sanguinello*, and *Moro*, which grow almost exclusively in Sicily's Etna area. From these fruits, a "red orange complex" (ROC) has been chromatographically purified and investigated [7] because of its peculiar composition, which is characterized by high levels of anthocyanins, flavanones, ascorbic acid, and hydroxycinnamic acids. A possible explanation of the peculiar photoprotective role of these compounds is that they naturally play an important resistance to the UVA and UVB sun irradiation by the plant photosynthetic apparatus.

Cardile and colleagues [7] analyzed the *in vitro* anti-inflammatory activity of ROC at concentrations of 10 and 100 μg/mL on normal human keratinocytes exposed to interferon-gamma (IFN-γ) and histamine, used to enhance the effects of IFN-γ. Keratinocytes initiate and regulate inflammatory and immune skin responses, releasing different cytokines and expressing membrane molecules that are able to modulate permanence and activation of T lymphocytes into epidermis. Normal keratinocytes stimulated by IFN-γ and histamine expressed membrane molecules, such as intercellular adhesion molecule-1 (ICAM-1), and released inflammatory soluble factors, such as monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8). Unstimulated cell lines did not produce these inflammatory molecules.

Moreover, the authors compared ROC activity to hydrocortisone. Incubation of cell lines with IFN-γ and histamine for 48 h induced strong expression of ICAM-1, and the addition of ROC at different concentrations together with IFN-γ and histamine induced dose-dependent inhibition of ICAM-1 expression. At the highest concentration (100 μg/mL), ROC blocked 40 ± 4% of ICAM-1 expression. Hydrocortisone led to a reduction of 28 ± 6% of ICAM-1 expression, not significantly

different from the decrease induced by 10 μg/mL ROC. ROC also markedly inhibited IFN-γ- and histamine-induced release of MCP-1, ranging from −75 ± 3% to −80 ± 5% inhibition for the lower and the higher concentrations of ROC, respectively. Hydrocortisone provoked a more modest inhibition (−36 ± 2%). Induced IL-8 was markedly decreased by ROC in a dose-dependent manner. With respect to ROC, hydrocortisone decreased IL-8 release at lower concentrations. In conclusion, the authors demonstrated that in human keratinocytes stimulated with both IFN-γ and histamine, low and high concentrations of ROC can inhibit the synthesis of ICAM-1 and the release of MCP-1 and IL-8 more efficiently than hydrocortisone.

Another ROC extract, obtained by a patented process from the three pigmented orange varieties mentioned above, was evaluated by Cimino and colleagues [8]. They studied the *in vitro* photoprotective effect of ROC in human keratinocytes after UVB exposure. The authors investigated the modulation of redox-regulated transcription of nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) activation, which play an important role in cell differentiation and carcinogenesis. In addition, they also studied the expression of pro-inflammatory cytokine IL-8, and the effect on caspase-3 activation and DNA fragmentation. The authors demonstrated that ROC counteracted NF-κB and AP-1 activation and that pretreatment with ROC (15 and 30 μg/mL) was associated with a significant decrease of precaspase-3 cleavage after UVB irradiation.

In a previous study [9], the same research team evaluated the *in vitro* protective efficacy of cyanidin-3-*O*-glucoside (C3G), an important citrus anthocyanin, on cellular responses after UVB exposure in human keratinocytes. Effects elicited by UVB exposure were clearly inhibited by pretreating skin cells with 40–80 μM C3G in a dose-dependent manner. The authors concluded that C3G can protect skin cells against UVB-adverse effects and suggested that this molecule could be employed as a skin photoprotective agent.

Another component of citrus fruit, i.e., the peel, was recently studied for its mutagenicity-reducing activity. In comparison to juice, citrus peel contains a higher concentration of ascorbic acid and active components, including D-limonene, which comprises more than 90% of the citrus peel oil, and hesperidin [10]. Hakim and colleagues [11] correlated the history of squamous cell carcinoma (SCC) of the skin and the citrus consumption patterns in an Arizona population with a higher non-melanoma cancer risk than the US population. A total of 470 individuals reported weekly citrus consumption, in particular orange juice (78.5%), orange (74.3%), and grapefruit (65.3%). Peel consumption was common, as 34.7% of all subjects reported citrus peel intake. Although no association was found between the overall consumption of citrus fruits (odds ratio (OR) = 0.99, 95% confidence interval (CI) = 0.73–1.32) or citrus juices (OR = 0.97, 95% CI = 0.71–1.31) and skin SCC, photoprotection induced by citrus peel consumption emerged (OR = 0.66, 95% CI = 0.45–0.95). Moreover, a dose–response relationship between increased citrus peel consumption and decreased SCC risk was detected.

Citrus fruits are also a good source of a well-known natural antioxidant, vitamin C, or L-ascorbic acid or L-ascorbate. Vitamin C is a strong reducing agent, which rapidly scavenges a number of reactive oxygen species (ROS) to perform its reducing function and is subsequently converted into the oxidized form, L-dehydroascorbate. L-dehydroascorbate can be newly reduced to the active L-ascorbate form in the body by enzymes and glutathione. L-ascorbic acid is an essential nutrient for humans, and its deficiency causes scurvy. It acts as an antioxidant by protecting the body against oxidative stress and a cofactor in several enzymatic reactions, such as collagen synthesis.

Oral supplementation of vitamin C does little to increase its skin concentration and appears to have no effect on the UVR-induced erythemal response [12]. Instead, topical application of vitamin C is the preferred method to increase its presence in the skin.

Although there is controversy surrounding the use of oral vitamin C for cancer chemoprevention, there is substantial evidence that topical L-ascorbic acid provides at least some photoprotection for the skin. Studies were previously performed to compare the photoprotective effects of L-ascorbic acid and α-tocopherol (vitamin E) in combination on porcine skin. Antioxidants were applied for 4 days together and alone, and the porcine skin was irradiated with a solar simulator (295 nm). On day 5, the

antioxidant protection factor, in terms of erythema, sunburn cells, and thymine dimers, was measured. The combination of 15% L-ascorbic acid and 1% α-tocopherol provided superior photoprotective effects that were progressive over the 4-day period [13]. Both antioxidants conferred photoprotection when applied alone but to a lesser degree than in combination. It is important to note that topically applied antioxidants must be used prior to ultraviolet exposure to photoprotect [14].

Vitamin C is also known to have potent anti-inflammatory properties. Vitamin C suppresses activation of transcription factor NF-κB by inhibiting tumor necrosis factor α (TNF-α). NF-κB is the transcription factor responsible for the production of a number of pro-inflammatory cytokines, such as TNF-α, interleukin-1 (IL-1), IL-6, and IL-8 [15]. Thus, cutaneous benefits of topical L-ascorbic include promoting collagen synthesis, photoprotection from UV A and UV B, lightening hyperpigmentation, and improvement of a variety of inflammatory dermatoses [16].

Lin et al. [17] reported that the topical association between vitamins C and E is recommended, and that the addition of ferulic acid significantly improves their stability. In fact, its incorporation (0.5%) into a topical solution of 15% L-ascorbic acid and 1% α-tocopherol improved the chemical stability of the vitamins and doubled their photoprotection effects to solar-simulated irradiated skin from fourfold to approximately eightfold, as measured by both erythema and sunburn cell formation. Inhibition of apoptosis was associated with reduced induction of caspase-3 and caspase-7. This antioxidant formulation also reduced thymine dimer formation.

Tomato

Tomato (*Lycopersicon esculentum* L.) is one of the most cultivated and consumed vegetables all over the world. In the MD, it represents a key component and is consumed fresh or after being household cooked or industrially processed. Antioxidant properties of raw tomatoes and processed tomato products could be mainly ascribed to lycopene [18], a peculiar acyclic carotenoid with several conjugated double bonds, representing approximately 80–90% of the total tomato content in carotenoids. This peculiar tomato component is the single most potent oxygen scavenger among all carotenoids and receives much attention for its potential health properties in preventing cancer and cardiovascular diseases [19].

A recent randomized controlled trial [20] evaluated the dietary photoprotective effect of a lycopene-rich tomato paste (approximately 293 ppm) against UV-induced skin damage. A group of healthy nonsmoking white women were randomly assigned to either an active or a control meal. Supplemental foods were administered daily for 3 months. An active meal consisted of 55 g of tomato paste with 10 g of olive oil on white bread. In contrast, the control group was provided 10 g of olive oil on white bread alone. Olive oil was used as a carrier, as bioavailability of lycopene is greater when derived from processed tomato products rather than fresh fruits and its absorption is better in an oily medium [21, 22].

Before and after UV radiation, the authors [20] evaluated short- and long-term photodamage indicators, such as different erythema measurements and the expression of molecules implicated in photoaging, including procollagen I (pCI), fibrillin-1, matrix metalloproteinase-1 (MMP-1), and mitochondrial DNA (mDNA) damage.

Erythema evaluation was visually performed to determine a minimal erythema dose (MED), i.e., the lowest UV dose producing a perceptible erythema, and by means of a reflectance instrument to give a UVR erythema dose–response D_{30}, i.e., the UVR dose resulting in a ΔE of 30 arbitrary units. Although MED did not present a statistically significant difference between the two groups, D_{30} presented a significant difference between pre- and post-supplementation for the active supplement group. This event accounts for substantial protective effects against UVR damage demonstrated by a significant shift in the erythema slope in active supplemented group.

After 3 months of lycopene supplementation, a small but significant increase in pCI was observed in the actively supplemented group compared with the unirradiated baseline site. Fibrillin UV-induced reduction was abolished after supplementation in both groups without significant differences. Before supplementation, UV radiation induced a significant increase in skin MMP-1 expression. In contrast, after 3 months of supplementation, UVR-induced MMP-1 expression was significantly reduced in the active group compared to the control group. Similarly, mDNA damage was reduced after supplementation in the active group. In conclusion, lycopene-rich tomato paste, as well as probably other processed tomato products with similar lycopene content, provides protection against acute and potentially longer term aspects of photodamage.

Another study [5] also considered the photoprotective effect of lycopene in human dermal fibroblasts exposed to UVA radiation. Lycopene was prepared in special nanoparticle formulations, which allowed a better stabilization in cell culture medium and an efficient cellular uptake compared to dimethylsulfoxide. The authors demonstrated that vitamin C, vitamin E, and carnosic acid showed photoprotective potential, while lycopene (0.5–1.0 μM) and β-carotene led to a further 1.5- to 2-fold rise in the UVA-induced MMP-1 mRNA and heme-oxygenase I (HO-1). Because these molecules are not protective on their own but in the presence of vitamin E, their stability in culture is improved by supplementing them with vitamins. Vitamin supplementation suppresses MMP-1 mRNA expression, suggesting a requirement for antioxidant protection of these carotenoids against formation of oxidative derivatives, which influence cellular and molecular responses. This apparent contradiction between the two studies may be explained by the varied food matrices, which often influence the potential properties of such molecules. It is likely that some synergistic effects due to different antioxidant compounds naturally present in tomato and tomato products could allow lycopene to be more protected and "active" in a natural product rather than in a stabilized solution or formulation.

Other carotenoids are well represented in tomato fruit, such as β-carotene (bC). This molecule reduces the deleterious effects of UVA via multiple mechanisms. In fact, in unirradiated keratinocytes, bC reduces expression of the stress signals, extracellular matrix (ECM) degradation, and promotes keratinocyte differentiation. In irradiated cells, bC inhibits gene regulations by UVA, which promote ECM degradation, and enhances UVA-induced protease-activated receptor-2, suggesting that bC enhances tanning. The combination of bC-promoted keratinocyte differentiation with the cellular "UV response" results in a synergistic induction of cell cycle arrest and apoptosis. In conclusion, at physiologic concentrations, bC interacted with UVA in keratinocytes and was able to quench singlet O_2 [23]. These findings have important implications not only on skin photoaging but also on skin diseases in which cellular differentiation is crucial, such as skin cancer and psoriasis.

Henrich et al. [24] compared the photoprotective effect of oral bC supplementation (24 mg/day) to that of a carotenoid mix consisting of the three main dietary carotenoids, bC, lutein, and lycopene (8 mg/day each). Three groups of 12 volunteers with skin type II received bC, the carotenoid mix, or placebo for 12 weeks. Although no changes occurred in the control group, carotenoid levels in serum and skin (palm of the hand) significantly increased for both types of supplementation. In particular, serum bC concentration significantly increased three- to fourfold in the bC group, whereas in the mixed carotenoid group, the serum concentration of each of the three carotenoids significantly increased one- to threefold. The erythema intensity 24 h after irradiation diminished in both groups receiving carotenoids and was significantly lower than the baseline intensity after 12 weeks of supplementation. Long-term supplementation of a carotenoid mix (bC, lutein, and lycopene) ameliorates UV-induced erythema in humans, and its effect is comparable to daily treatment with 24 mg of bC alone. This finding is very interesting because it questions the safety of high-dose supplementation of bC. In fact, at higher levels, pro-oxidant reactions have been observed when carotenoids are applied *in vitro*. UV irradiation of human fibroblasts in the presence of high amounts of bC increases lipid peroxidation and stimulates the expression of HO-1 and IL-6. In particular, for people with a high risk for lung cancer, high-dose bC supplementation has been questioned, and safety issues have been

addressed with regard to long-term intake of bC. Thus, the use of a carotenoid mixture with low doses of individual carotenoids instead of a high dose of a single carotenoid (bC) may be a possible alternative against UV-mediated skin damage.

Olive Oil

Olive oil, representing the major fat source in the MD and generally extracted by cold grinding olive-fruits (*Olea europaea* L.), is a traditional food product of the Mediterranean basin. The highest worldwide production is in Spain, Italy, and Greece, which together account for more than 75% of the world production (http://faostat.fao.org). The antioxidant effects of olive oil are probably due to a combination of a high content of oleic acid (low oxidation potential compared with linoleic acid) and a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, and tyrosol [25].

Budiyanto et al. [26] examined photoprotective effects of extra-virgin oil (EVO) on UVB-induced skin carcinogenesis in hairless mice. EVO was topically applied before or after repeated UVB exposures. The mice were divided in three groups: the first receiving only UVB, the second pretreated with EVO before UVB exposure (pre-UVB mice), and the last treated with EVO after UVB exposure (post-UVB mice). The onset of UVB-induced skin tumors was delayed in EVO-treated mice. However, with increasing number of UVB exposures, differences in the mean number of tumors between control mice and pre-UVB mice were lost. In contrast, post-UVB mice showed significantly lower number of tumors per mouse than the controls and pre-UVB mice throughout the experiment. The authors concluded that topically applied EVO after UVB exposure effectively reduced UVB-induced murine skin tumors.

Dietary vitamin E has been found to protect against DNA miscoding and lipid peroxidation-induced DNA lesions [27]. Furthermore, as already mentioned, vitamin E has also been found to protect and enhance bC, lycopene, and vitamin C activity [5, 13, 14, 17].

N-3 polyunsaturated fats (PUFAs) substantially inhibit UVR-induced immunosuppression and photocarcinogenesis in mice, especially when compared to an equivalent level of *n*-6 PUFAs, which are highly oxidizable and associated with DNA damage and the expression of UVR-induced tumors [27]. In addition, lowering the *n*-6/*n*-3 PUFA ratio in tissue through dietary modifications has been recommended as an effective approach towards attenuating melanoma growth by reducing inflammatory, cancer-promoting, *n*-6-derived eicosanoids and increasing *n*-3-derived tumor suppressors [28].

This finding corroborates case–control and population-based observations that show a consistent trend towards lower risk of melanoma and SCC with higher intake of *n*-3 PUFAs and elevated *n*-3-to-*n*-6 PUFA ratios. In fact, Hakim and coauthors [29] investigated the association between dietary *n*-3 and *n*-6 PUFA intake and SCC risk in the Southeastern Arizona population. They reported an association between higher intakes of *n*-3 PUFAs and a lower risk for SCC. Regarding the ratio of *n*-3 to *n*-6 PUFA, the authors suggested a tendency towards decreased SCC risk and increased intake of foods with high ratio of *n*-3 to *n*-6 PUFAs.

Olive products (table olives and olive oil) are also rich sources of polyphenols, whose major representatives are hydroxytyrosol (3,4-dihydroxyphenilethanol) and derivatives thereof accounting for approximately 50% of the total EVO phenolic compounds [30]. D'Angelo et al. [31] found a protective effect of hydroxytyrosol in preventing the onset of typical oxidative stress markers, such as lipid peroxidation products in UVA-irradiated melanoma cells. These protective effects were dose dependent, reaching a maximal effect at 400 μM hydroxytyrosol. At higher concentrations, hydroxytyrosol acted as a proapoptotic stimulus by activating caspase-3. This study suggests that hydroxytyrosol may exert differential effects on melanoma cells based on the applied dose.

Regarding refined virgin oil and seed oils, EVO contains significantly higher amounts of other powerful antioxidant components, such as squalene and lignans ((+)-1-acetoxypinoresinol and (+)-1-pinoresinol). Lignans may be present in up to concentrations of 100 ppm [32]. Squalene is to large extent transferred to the skin (sebum is reported to contain 12%), and its major protective effect is thought to be against skin cancer. This effect is supported by studies showing neoplasm inhibition in rodents via topical application and a low incidence within Mediterranean population. The mechanism is probably by scavenging singlet oxygen generated by UV light [32].

Seafood

In the MD, seafood represents a good source of proteins and an alternative to meat. Traditionally "blue fish," i.e., small pelagic species colored blue or green on the back and silver-bellied, is highly consumed. Their tissues and the belly cavity could contain up to 30% oil, contrasting with white fish, such as cod (*Gadus* spp.), which contain oil only in the liver. Examples include small forage fish, such as sardine (*Sardina pilchardus* W.), anchovy (*Engraulis encrasicholus* L.), mackerel (*Scomber scombrus* L.), garfish (*Belone belone* L.), and Mediterranean horse mackerel (*Trachurus mediterraneus* S.).

These fishes are excellent sources of *n*-3 PUFAs, especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). Two different studies evaluated the successful prevention of systemic immunosuppression in mice after UVB irradiation by topical [33] and dietary EPA [34]. In particular, the second study evaluated the influence of dietary supplementation with different *n*-3 PUFA on systemic immunosuppression induced by UVB radiation in mice using the contact hypersensitivity response to trinitrochlorobenzene. UVB-induced changes in the skin were also studied. Mice received high-fat (25% w/w) diets enriched with (1) oleic acid (control diet), (2) EPA, (3) DHA, or (4) EPA + DHA. Immunosuppression induced by UVB radiation was 53% in mice fed the oleic acid diet alone, and 69% in mice fed the DHA diet. In contrast, immunosuppression was only 4 and 24% in mice fed the EPA and EPA + DHA diets, respectively. Thus, dietary EPA, but not DHA, seems to protect mice against UVB-radiation-induced immunosuppression. Another study [35] examined the effect of PUFA supplementation on a range of indicators of UVR-induced DNA damage in humans and assessed the effect on basal and post-UVR oxidative statuses. In a double-blinded randomized study, 42 healthy subjects took 4 g daily of purified EPA or monounsaturated oleic acid (OA). EPA skin content after 3 months showed a significant eightfold increase from baseline. No consistent alteration (glutathione, vitamins E and C, or lipid peroxidation) in basal and UVR-exposed skin content was observed on EPA supplementation, which also reduced sunburn sensitivity and raised UVR-induced erythemal threshold. Moreover, UVR-induced skin p53 expression, assessed at 24 h post UVR exposure by immunohistochemistry, fell significantly after EPA supplementation. Peripheral blood lymphocytes (PBL) sampled on three successive days pre- and post-dietary supplementation showed no change with respect to basal DNA single-strand breaks or oxidative base modification (8-oxo-dG). However, when susceptibility of PBL to UVR was examined using the comet assay, a significant reduction in tail moment emerged after EPA supplementation. No significant changes were seen in any of the above parameters following OA supplementation. Reduction in this range of early markers, i.e., sunburn, UVR-induced p53 in skin, and strand breaks in PBL, indicates protection by dietary EPA against acute UVR-induced genotoxicity. Longer term supplementation might reduce skin cancer in humans.

Seafood is also a good source of selenium, a cofactor for glutathione peroxidase and a dietary trace element essential for effective immunity and protection from UVB-induced oxidative damage. Selenium also exhibits antiphotocarcinogenic activities. Rafferty et al. [36] determined the effects of dietary selenium levels on Langerhans (LC) cell number in two mice groups characterized by a Se-lacking or

a Se-adequate diet. After 5 weeks, the skin LC count was 49% lower ($p<0.05$) in Se-deficient mice than in Se-adequate mice. Moreover, skin glutathione peroxidase activity in Se-deficient mice was only 39% ($p<0.01$) of that of Se-adequate mice.

Because low plasma selenium levels have been linked to an increased risk of non-melanoma skin cancer in humans, Pence et al. [37] evaluated the relationship between selenium levels in the diet (0, 0.1, or 0.5 ppm) and development of UVR-induced skin tumors in hairless mice. UVR exposure resulted in skin tumors in all mice groups, and after cessation of UVR exposure, the tumors continued to increase in Se-deficient mice and in those fed only 0.1 ppm selenium but they stabilized for mice fed 0.5 ppm selenium. Skin antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase were monitored. Selenium deficiency decreased glutathione peroxidase and resulted in an early increase in superoxide dismutase and catalase in response to UV treatment. These results indicate that dietary selenium may be an important chemopreventive agent for skin cancer.

Wine

In the MD, one or two small glasses of wine a day, preferably red wine, are often consumed during the main meals. According to Food and Agriculture Organization (FAO), approximately 71% of world grape production is used for wine (*Vitis vinifera* L.), 27% as fresh fruit, and 2% as dried fruit (i.e., raisins and sultanas). Among the top ten wine producers, four countries border the Mediterranean basin: Italy, France, Spain, and Turkey.

Principal antioxidant grape components are resveratrol (*trans*-3,5,4′-trihydroxystilbene), a polyphenolic phytoalexin amply present in grape peels and seeds, proanthocyanidins from seeds, and polyphenols from both grape peels and seeds. These compounds have been shown to inhibit UVR-induced skin cancer and to have a synergistic photoprotective effect when taken in combination. They also inhibit the adverse effects of both acute and chronic UV exposure and skin tumors in murine models via several mechanisms including (1) protection against depletion of endogenous antioxidant defense enzymes, such as glutathione peroxidase and catalase; (2) suppression of oxidative stress, such as hydrogen peroxide and nitric oxide production and lipid and protein oxidation; and (3) inhibition of apoptosis mediated by p53 activity and NF-κB-responsive genes [28]. Topical application of resveratrol to hairless mice resulted in significant inhibition of UVB-induced skin edema. Resveratrol pretreatment caused a decrease in UVB-induced generation of hydrogen peroxide and leukocyte infiltration. Moreover, topical application of resveratrol substantially reduced UVB-induced lipid peroxidation, cyclooxygenase and ornithine decarboxylase activities, and protein expression of the latter enzyme. In normal human epidermal keratinocytes, resveratrol blocked UVB-mediated activation of NF-κB in a dose- and time-dependent manner [38].

Conclusions

The traditional MD contains many bioactive nutrients found to be effective for providing internal protection against UVR when taken as supplements. These compounds have been found to offer synergistic benefits when taken together, particularly as whole foods and in food combinations [28].

MD includes high levels of antioxidants, i.e., vitamins C and E, bC, selenium, flavonoids, and phenolic compounds. Other important MD features include the following: high levels of folate; a greater anti-inflammatory/photo-oxidative FA profile, with higher *n*-3 PUFAs and *n*-9 MUFAs and lower *n*-6 PUFAs and *n*-6:*n*-9 and *n*-6:*n*-3 FA ratios; low levels of pro-oxidants and carcinogens

(e.g., the heme iron from red meat and polycyclic aromatic hydrocarbons from deep grilling) due to the preference for gently grilled and steamed fish and low-fat dairy and poultry; high levels of complex carbohydrates (grains and legumes); and small-to-moderate amounts of alcohol, primarily from antioxidant-rich red wine.

Generally, the diet is known to be a notable risk factor contributing to cancer incidence, including that of melanoma. Nowadays, the idea that such a diet is still the norm in Mediterranean countries is a myth. In fact, these populations eat more Northern European and American foods, and British and Americans now consume more Mediterranean foods than they did 50 years ago. Nevertheless, the people of Greece exhibit the lowest rates of melanoma in Southern Europe, i.e., 2.14–2.99/100,000 for men and women, respectively, followed by people from other sunny regions, including Spain (2.80/4.50) and Italy (4.60/5.50). In contrast, people from the United States (12.70/9.26) and the countries of Northern Europe, including those from Britain (6.81/8.34), Denmark (10.12/11.26), and Sweden (11.70/11.50), display higher rates of melanoma. The worst rates for melanoma are observed in New Zealand (36.70/34.90) and Australia (39.80/31.80) [28]. Although this article only discussed the photoprotective effects of the MD, the diet influences many other healthy aspects. In particular, MD affects food allergy patterns, particularly to fruits and vegetables, including peach, which is considered a major allergen in the Mediterranean area [39–41], grape [42, 43], and tomato [44–46].

In conclusion, the MD may act as a case study for a comprehensive sun-protective nutritional model. This model could be strongly recommended during the early years of life, when the potential for inducing melanoma is highest. It may also be recommended for populations with high risks of skin cancer, including people having a fair skin type, a lifestyle characterized by high levels of UVR exposure, or compromised immunity.

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Chapter 2
Immune-Mediated Disorders of Skin: Role of Dietary Factors and Plant Extracts?

Yashwant Kumar and Alka Bhatia

Key Points

- Since ancient time, many plant products are being used either to accentuate the beauty of skin or as soothing agents in various dermatological ailments.
- Recently certain dietary factors and plant extracts have been found to be responsible for triggering of some of the immune-mediated skin disorders.
- Autoimmune and hypersensitivity reactions are considered to be important mechanisms responsible for these diseases.
- Majority of these conditions can be prevented by knowing and avoiding these substances of plant origin.

Keywords Immune-mediated skin disorders • Diet • Plant extracts • Immunopathogenesis • Prevention

Introduction

The skin, which is the largest organ of the body, constitutes an important barrier between the body and environment. It is one of the most common sites exposed to various infectious and noxious agents. In fact, the skin serves as the first line of defense against the foreign invaders. Therefore, it has long been used as a vehicle for study and manipulation of the immune system. Whereas the role of cutis in the processing and presentation of antigens to the central lymphoid compartments is well recognized, its more complex immune functions remain to be understood. The skin has many characteristics which suggest that it can function as a relatively autonomous immune organ [1]. The cutaneous immunological repertoire involves a wide range of immune cells which act in concert to protect the internal milieu of the body. Any breakdown in cutaneous immunity therefore leads to the development of

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immune-mediated skin disorders (IMSD). A large number of environmental and genetic factors are believed to be important in maintenance of cutaneous health. Out of these the influence of dietary constituents/plant products has been a topic of extensive research in the modern times. This chapter focuses primarily on various IMSD with the possible role of diet or plant extracts in their etiology.

Classification of IMSD

Majority of the IMSD arise as a complex interaction of genetic, environmental, and immunological factors. Broadly they have been classified into those specific to skin and those occurring as a part of systemic disorders with dermatological manifestations (Table 2.1). In many of them the role of diet or plant extract has recently been observed. The diet-related IMSD (drIMSD) can further be divided into those in which the dietary factor has a predominant role in etiology and those in which the disease is a manifestation of deficiency or excess of particular element in diet. Finally, cutaneous disorders exist where the pathogenic interference of dietary factors has repeatedly been advocated, but without a convincing evidence. The literature on drIMSD is sketchy and scattered. However, in recent years enough epidemiologic, clinical, and experimental data has been collected warranting a systematic analysis of role of diet or plant extracts in drIMSD, especially in genetically predisposed individuals.

Skin as an Immune Organ

The common belief that the skin is not an active immune organ has been altered by the identification of an armamentarium of immune competent cells and their cytokines in the various layers of the skin. The epidermis, although not directly accessed by the blood or lymphatic circulation, is equipped with various immune competent cells like Langerhans or dendritic cells, the antigen-presenting cells (APC); Keratinocytes, the epithelial cells with immune properties; Epidermal T lymphocytes; and Melanocytes, the epidermal pigment cells with immune function. The dermis contains a network of

Table 2.1 Immune mediated skin disorders with suggested role of diet or plant extracts

Immune mediated skin disorders		Immune mediated systemic diseases with skin manifestations	
Causative	Preventive/therapeutic	Causative	Preventive/therapeutic/uncertain
Dermatitis hepetiformis	Discoid lupus erythematosus	Gluten-sensitive enterpathy	Systemic lupus erythematosus
Pemphigus	Scleroderma		Rheumatoid arthritis
Atopic dermatitis	Epidermolysis bullosa acquisita		Dermatomyositis
Contact dermatitis	Lichen planus		Sjogren's syndrome
Psoriasis	Vitiligo		Systemic sclerosis
	Psoriasis		Behcet syndrome
			Vasculitis
			Autoimmune endocrinopathies
			Inflammatory bowel disease
			Autoimmune hepatitis
			Autoimmune paraneoplastic dermatosis

lymphatic and blood vessels rich in lymphocytes, other leukocytes, mast cells, tissue macrophages, and cytokines like interleukin 1 (IL-1). All these cells and their cytokines act together to initiate an immune response whenever the cutaneous barrier gets disrupted [2].

The first cell to encounter toxins or antigens in the skin is the APC which presents the antigen to other resident immune cells. The keratinocytes which are the reservoirs of IL-1 release it, which in turn initiates a multitude of local and systemic effects. IL-1 also increases the production of other cytokines by the keratinocytes, induces class II MHC antigen and adhesion molecule expression on keratinocytes and dermal endothelial cells, and thus facilitates leukocyte trafficking into the skin. The cutaneous melanocytes may also promote the immune response by secreting various soluble mediators of inflammation.

Mechanisms of drIMSD

The skin is continuously exposed to a tremendous diversity of antigenic stimuli which may initiate a series of immune responses in both the epidermal and dermal compartments. These immunological reactions may be antibody or T cell mediated in nature. Sometimes, an underlying genetic predisposition may also be present which serves to exaggerate this response resulting in pathological manifestations. The important mechanisms which may play a role in the pathogenesis of drIMSD include:

1. Autoimmunity triggered by dietary antigens
2. Hypersensitivity (HS) to dietary components
3. Idiosyncrasy to commonly used dietary compounds
4. Deficiency or excess of a specific nutrient present in the diet

Autoimmunity Triggered by Dietary Antigens

An autoimmune response to the dietary components has been implicated in etiology of many cutaneous disorders. The diet-related antigen is believed to resemble the skin protein. Therefore the antibody response directed against the diet proteins also damages the skin leading to various cutaneous manifestations. A role of autoimmunity has been suggested in autoimmune bullous disorders like dermatitis herpetiformis (DH) and pemphigus.

Dermatitis Herpetiformis

DH is an autoimmune disease characterized by intensely itchy blisters and hives on an individual's back or buttocks. The DH has been found to be associated with other autoimmune disorders also like celiac disease, rheumatoid arthritis, hypothyroidism, or Sjogren's syndrome. The role of diet in DH is now well established. The gluten proteins present in certain cereals activate the immune system, attacking the skin and proximal intestinal mucosa [3]. In this disease the immunoglobulin A (IgA) autoantibodies formed against gluten proteins are addressed toward an enzyme, type 3 epidermal transglutaminase, which has a marked homology with tissue transglutaminase. This homology is responsible for the cross antibody reaction in patients with gluten enteropathy. Avoidance of gluten in diet is the first and most important therapeutic solution [4]. The gluten free diet requires the strict avoidance of all foods that contain the alcohol soluble fraction of gluten, gliadin. This is present not only in cereals like wheat, rye, barley, buckwheat, and oats but also in thickeners, fillers, and additives contained in a wide variety of foods (Table 2.2) [5].

Table 2.2 Major dietary and plant products implicated in induction of IMSD

Dermatitis herpetiformis				
Barley	Buckwheat	Rye	Oat	Wheat
Pemphigus				
Black pepper	Garlic	Manioca	Radish	Red chilies
Coriander	Horseradish	Mustard	Red pepper	Tea
Cumin seeds	Leek	Onion		
Atopic dermatitis				
Almonds	Carob flour	Eggs	Hazelnuts	Peanuts
Cow's milk	Crustacea	Guar seed flour	Maize	Wheat
Contact dermatitis				
Anise	Carrots	Dill	Mace	Parsnips
Artichoke	Cashew nut oil	Edive	Mango	Parsley
Asparagus	Cassia	Horseradish	Mushroom	Potato
Basil	Cauliflower	Garlic	Mustard	Radish
Bay (Laurel) leaf	Celery	Ginger	Nutmeg	Tomato
Broccoli	Chamomile tea	Ginkgo fruit	Olive oil	Turnip
Brussel sprouts	Chicory	Jamaican pepper	Onion	Rosemary
Cabbage	Cinnamon	Kale greens	Orange	Spearmint
Capsicum	Cloves	Lettuce	Oregano	Turmeric
Caraway oil	Corn	Leek	Paprika	Vanilla
Cardamon	Cucumber	Lime and lemon	Peppermint	
Contact urticaria				
Apple	Chamomile	Flour	Mustard	Seaweed
Apricot	Chicory	Garlic	Oatmeal	Sesame seeds
Almond	Chicken	Grapefruit	Onion	Shallots
Amarith	Chives	Green pepper	Orange	Shellfish
Artichoke	Caraway seed	Honey	Paprika	Soybean
Arugula	Cayenne pepper	Kiwi lamb	Parsley	Spinach
Asparagus	Cinnamon	Lemon	Parsnip	Strawberry
Banana	Coriander	Lettuce	Peach	Sunflower seeds
Barley	Curry	Lime	Peanut	Thyme
Beans	Coffee bean	Litchi	Pear	Tofu
Beef	Corn	Liver	Pickles	Tomato
Beer	Cucumber	Lupin seed	Pineapple	Turkey
Brazil nut	Dill	Maize	Plum	Venison
Buckwheat	Egg	Malt	Pomegranate	Watercress
Cabbage	Endive	Mango	Pork	Watermelon
Carrot	Fennel	Melon	Potato	Wheat
Cauliflower	Fig	Milk	Rice	Wheat bran
Celery	Fish	Mushroom	Rutabaga	Winged bean
Protein contact dermatitis				
Almond	Celery	Fig	Liver (calf, chicken)	Parsley
Banana	Chicory	Fish	Meat (cow, pig, horse, lamb)	Parsnip
Barley Flour	Cheese	Garlic	Mesenteric fat (pig)	Peanuts
Bean	Cress	Gut (pig)	Mushroom	Pineapple
Blood (pig, cow)	Cucumber	Hazelnut	Onion	Potato
Caraway	Curry	Horseradish	Paprika	Rye
Carrot	Dill	Kiwi fruit		Skin (turkey, chicken)
Castor Bean	Eggplant	Lemon		Tomato
Cauliflower	Egg yolk	Lettuce		Wheat
	Endive			

(continued)

Table 2.2 (continued)

Systemic contact dermatitis				
Balsam of Peru	Cinnamon oil	Garlic	Raw cashew nuts	Vanilla
Photo-allergic contact dermatitis				
Bitter	Celery	Fig	Lemon	Parsley
Bergamot	Fennel	Grapefruit	Orange	Parsnip
Carrot				

Pemphigus

Pemphigus is an autoimmune bullous disease of the skin and mucosa. Histologically, it is characterized by acantholysis and immunologically by the presence of specific circulating autoantibodies. These are the immunuglobulin G (IgG) autoantibodies directed against the desmogleins. The desmoglein antigens are the cell–cell adhesion molecules expressed on the keratinocyte cell surface. Binding of circulating autoantibodies to these antigens on skin leads to widening of intercellular space between desmosomal junctions followed by splitting of desmosomes and finally complete epidermal cell detachment (acantholysis) [6]. Involvement of both environmental and dietary triggers in acantholysis has been suggested [7]. The important dietary factors implicated include allelic compounds found in plants of the genus Allium (garlic, onion, leek). The -SH group (thiolic) present in these compounds has been demonstrated to be acantholytic in in vitro experiments. These substances intervene in keratogenesis, modify the assembly of keratinocytes, weaken their mutual cohesion, and alter the biochemical structure of adhesion molecules. In vivo they stimulate the production of B cell clones that specifically generate histolesive autoantibodies.

Role of these food compounds in pathogenesis of pemphigus is supported by the high incidence of pemphigus in countries like India where meals contain lot of garlic and spices such as mustard, red and black pepper, coriander, and cumin seeds which are rich in thiols and isothiocyanates. The disease is especially prevalent in communities with practices of eating betel quid, a package of fresh betel leaves soaked in an infusion of citron and tobacco. Coastal communities with higher consumption of the nutrients like tannins, manioc, and thiocyanates are also predisposed.

Tannins are rich in drinking water in Amazon water basins due to nonstop rotting of huge amounts of tropical vegetation. They are polyphenolic compounds able to release cytokines with cytotoxic and acantholytic properties. Tannins can also be found in guarana, a plant that spontaneously grows in the Amazon area that is employed by indigenous people to prepare a very popular drink. Thus, in genetically predisposed individuals, the use of various foods containing thiols, phenols, or tannins may cause pemphigus, which is a rare disease in some countries but endemic in others. Most common treatment for pemphigus is steroid administration rather than diet free of these substances as seen in DH [4].

Psoriasis

Psoriasis is another autoimmune skin condition indicated by the rapid increase in skin cell proliferation. This leaves what is referred to as “plaque” where patches of raised skin appear. The patches are red and swollen and topped with dead silvery white skin cells. These lesions normally happen on the elbows, knees, lower back, and scalp.

The cause of psoriasis is not fully understood. It is believed that there is a hereditary component and many genes work together and involve immune system. This leads to T cell-mediated inflammatory changes. T cells demonstrating activated memory phenotypes are present within the dermis and epidermis of active psoriatic skin lesions [8]. Activated T cells within psoriatic lesions possibly respond to an autoantigen and elaborate many type 1 and proinflammatory cytokines, including Interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-1, and IL-2. By contrast, cells within the

lesions express relatively low levels of type 2 cytokines such as IL-4 and IL-10. Keratinocyte hyperproliferation is believed to be a secondary biologic phenomenon, driven mainly by proinflammatory and type 1 cytokines produced locally by infiltrating T cells.

There is some evidence of association of disordered arachidonic acid metabolism in the pathogenesis. Studies have found that the skin of people with psoriasis contains high levels of inflammatory compounds called leucotrienes which are by-product of arachidonic acid metabolism [9]. Arachidonic acid is rich in diet containing animal fat.

In severe cases, the disease can also result in an inadequate nutritional status, which may be further compromised by nutrient drug interactions. Protein, folate, and iron deficiencies have been reported in such cases. Fasting periods, vegetarian diets, and diets rich in omega-3 polyunsaturated fatty acids from fish oils have been associated with improvement in the symptoms of the disease in some studies.

Scleroderma, a disease of connective tissue, is characterized by fibrosis and thickening of soft tissue. An association of intake of high fiber diet has been suggested with this condition. Improvement with vitamin E has been reported in these patients [10].

Disorders like discoid lupus erythematosus, dermatomyositis, epidermolysis bullosa, lichen planus, vitiligo, alopecia areata, etc. are few other autoimmune disorders; however the role of diet and plant extracts in these conditions is unclear.

HS to Dietary Components

HS reactions to diet-related antigens constitute the etiological basis for many cutaneous disorders collectively called "eczema" or "dermatitis." The role of immunity has been demonstrated in some of them like atopic dermatitis (AD) and contact dermatitis (CD). Amongst the four types of HS reactions, type I and type IV responses are considered to be more important in diet-related cutaneous HS.

Atopic Dermatitis

AD is a chronic inflammatory skin disease that commonly begins in early infancy, runs a course of exacerbations and remissions, and is associated with a characteristic distribution and morphology of skin lesions. It results from a complex interplay between strong genetic and environmental factors. Genome screens of families with AD have implicated chromosomal regions that overlap with other skin diseases and with inflammatory and autoimmune diseases. This may be one of the reasons that AD is often associated with asthma, allergic rhinitis (hay fever), and food allergy.

Numerous trigger factors for AD have been identified over recent decades, including food allergens, inhalable respiratory allergens, irritative substances, and infectious microorganisms such as *Staphylococcus aureus* and *Malassezia furfur*. AD is regarded as the prototype of spontaneous HS. It manifests itself by means of the heightened capacity of the B lymphocytes to produce IgE antibodies against allergens which trigger off the immune response after contact [11]. This may be due to defective regulation of the T lymphocytes which is associated with inadequate function of the CD8+ lymphocytes in suppressing IgE [12, 13].

Normally the food antigens which are ingested enter the gut and encounter intestinal immune system (Gut-Associated Lymphoid Tissue—GALT). Here they are captured by the APC which then cause apoptosis amongst the antigen-specific T cells or differentiation in the suppressor T cells which produce suppressive Transforming Growth Factor-beta (TGF-β) [14]. A breech in the gut immunity and cross-reactivity of the IgE antibodies with skin antigens or direct interaction with specific IgE, Fc receptors on Langerhans cells, mast cells, monocyte basophilic granulocytes, or skin infiltrating T lymphocytes leads to skin allergy [15].

Cow's milk, eggs, wheat, maize, crustacea, hazelnuts, almonds, and peanuts are the most common allergens implicated in AD and may cause sensitization and an outbreak or worsening of skin changes. Some vegetable gums, carmine red, ethylvanillin, vanilla, and tartrazine can also trigger an IgE-mediated response. In addition to above, the way in which a food is cooked also influences its level of allergenicity. In general, allergens of animal origin continue their activity for longer, whereas vegetable allergens are more easily broken down by cooking or by other processes [16].

Breastfeeding for at least the first 6 months of life is considered to be an important measure in prevention of AD. During breastfeeding, atopic mother's diets should consist of frequently varied organic foods on the basis of their individual food intolerances. As a therapeutic measure also patients of AD should strictly use foods of strictly organic origin, particularly for fruit, vegetables, and whole grains. Frequent use of sunflower oil is also useful due to its high content of -3 and -6 polyunsaturated fatty acids. Topical corticosteroids are still a mainstay of treatment for AD.

Contact Dermatitis

CD occurs when skin comes in direct contact with an allergen. Food handlers, in particular, may acquire dermatoses resulting from occupational exposures. Bakers, chefs, housewives, and spice handlers are at an increased risk. These reactions occur frequently on the hands but may develop around the mouth or on the face. Various types of CD are known: allergic contact dermatitis (ACD), contact urticaria (CU), protein contact dermatitis (PCD), irritant contact dermatitis (ICD), phototoxic contact dermatitis (PTCD), photoallergic contact dermatitis (PACD), and systemic contact dermatitis (SCD). Of these, PTCD and ICD however are nonimmunological responses [17].

Allergic Contact Dermatitis

ACD is characteristically a delayed type of HS, although changes as early as 4–8 h after contact with the allergen can be seen histologically. ACD is a complex immune response, which is a cascade of nonspecific and antigen-specific T cell events. The food allergen that penetrates the stratum corneum after percutaneous contact with the epidermis interacts with APC expressing major histocompatibility complex (MHC) molecules. APC then bind, process, and present the allergen to trafficking lymphocytes in the epidermis. They also acquire the capacity to emigrate and trigger resting T lymphocytes in the lymphoid organs. It was previously thought that only antigen-specific CD4+ T cells and MHC class II restricted T lymphocytes are involved in the immune response, but studies have shown that CD8+ T lymphocytes via MHC class I molecule can also induce the contact HS response. Thus, both CD4+ and CD8+ T lymphocytes mediate the immune response in vivo. Lymphokines released from T cells activate the keratinocytes to up regulate the expression of Class I MHC molecules and expression of Class II MHC molecules along with the release of cytokines that in turn accelerate T cell activation, attract T cell migration into the epidermis, and further potentiate the immune response.

ACD to foods is most often caused by the oleoresins in the fruits and vegetables (Table 2.2). Mango dermatitis is a common reaction seen to the sap, fruit skin, leaf, or stem of the mango tree [18]. Urushiol, the allergen responsible for this reaction, is the same oleoresin present in poison ivy, poison oak, and other members of the Anacardiaceae family. The eruption commonly occurs within hours of contact with the allergen in previously sensitized individuals. The most common presentation is perioral dermatitis resulting from contact with the mango rind. Other members of the Anacardiaceae family, which may also be ingested, include cashew nut shell oil and ginkgo seed. Cashew nut oil is extracted from the cashew nut tree and contains cardol, a phenol similar to urushiol. Seed pulp of the female ginkgo tree (*Ginkgo biloba*) also contains urushiol and may cause perioral or perianal dermatitis upon ingestion. Peeled from its outer coat, the ginkgo seed is added to soups or roasted and eaten. Reportedly, only contact with the pulp will cause a reaction but may occur with the seed kernel as well

[19–21]. *Ginkgo biloba* taken orally for energy and memory is an extract obtained from the leaves. Many spices and their essential oils used in cooking are also reported to cause ACD [22]. Spice allergy usually presents as dermatitis on the palmer sides of the fingers or hands. Paprika, clove, Jamaica pepper, cinnamon, nutmeg, and ginger are the most commonly encountered spice allergens in food workers [23].

Contact Urticaria

CU is a transient wheal and flare reaction occurring in areas of contact with an allergen. This reaction can occur with or without sensitization. An immediate pruritic response develops with erythema and edema at the site of contact which usually subsides within 45 min. Two types of contact urticaria are recognized: nonimmunological contact urticaria (NICU) and immunological contact urticaria (ICU) [24]. The immunological reaction (ICU) occurs in sensitized persons as a type I HS response caused by mast cell degranulation within the skin. The antibodies of IgG1 or IgG3 subclass, directed against alpha subunit of the high-affinity IgE receptor (FcεRIα), effectively fix the complement and induce histamine release from basophils and mast cells that express FcεRIα. The reaction can be local, spread beyond the area of contact, or cause systemic symptoms including rhinitis, asthma, and anaphylactic shock. Food handlers and chefs are in constant contact with meats, fruits, and vegetables and their skin barrier is often compromised from repeated water exposure [25].

Protein Contact Dermatitis

PCD is a rare type of eczematous reaction occurring to large proteins in foods [26]. These proteins are thought to penetrate compromised skin to elicit an immediate urticarial and vesicular reaction. It is considered to be a combination of immediate Type I and delayed Type IV allergic responses. The majority of patients are food handlers. Meats are a well-known cause of PCD; therefore the butchers and slaughterhouse workers are most commonly affected. Different types of flour, including rye, wheat, and barley, as well as their additives such as the enzyme alpha amylase, have also been associated with PCD. Janssens et al. have described four principal groups responsible for PCD: fruits, vegetables, spices, and plants; animal proteins; grains; and enzymes [27].

Systemic Contact Dermatitis

SCD develops after oral or parenteral exposure to an allergen in a topically sensitized individual. This is believed to be resulting from the hematogenous spread of the allergen, provoking a cutaneous reaction. Systemic effects such as rhinitis, conjunctivitis, headache, gastrointestinal complaints, or anaphylaxis are commonly associated. SCD may be mediated by both type III and type IV HS reaction as both immediate and delayed reactions occur upon exposure. The foods that individuals become sensitized to topically and have the potential to cause SCD include flavoring agents such as oil of cinnamon, vanilla, and balsam of Peru, various spices, garlic, propylene glycol, and raw cashew nuts.

Photoallergic Contact Dermatitis

Phytophotodermatitis is the name given to phototoxic reactions occurring to plants, vegetables, or fruits. In PACD, the allergen is photoactivated by either sunlight or artificial light in the UVA range.

Hapten formation between the activated antigen and a skin protein is necessary to incite a delayed HS reaction. Garlic exposure has been suggested as an associated factor [28]. Psoralens (furocoumarins) are the responsible agents found in the Umbelliferae (carrot, celery, fennel, parsley, parsnip), Rutaceae (lemon, lime, bitter and bergamot orange, grapefruit), and Moraceae (fig) families [29].

Avoidance of allergen is the best and the only truly effective treatment for CD. Although exposure of patients to low levels of sensitizing chemicals may be permissible, strict avoidance is preferable. Physical barriers may provide protection; barrier creams are not generally useful. Antihistamines may provide some symptomatic relief and high-potency topical corticosteroids may hasten lesion resolution. Cases of severe CD may require systemic steroid treatment at relatively high doses. Adjunctive phototherapy with ultraviolet B light or PUVA may be indicated for such patients.

Idiosyncratic Susceptibility to Diet

Idiosyncratic susceptibility to dietary factors may sometimes be seen due to interactions with systemic medication, for example, severe alcohol induced flushing in some patients who take chlorpropamide and the reaction to cheese or pickles in patients taking inhibitors of monoamine oxidase [30]. There may be reactions due to anatomical abnormalities such as the flushing that accompanies the post-gastrectomy dumping syndrome. Some idiosyncratic reactions like the urticaria and bronchospasm caused by bisulfites used to keep fruits and vegetables fresh may be mediated by neural activity. The Chinese restaurant syndrome, in which similar symptoms occur after eating sodium glutamate, seems to be mediated by the transient release of compounds similar to acetylcholine [31].

Deficiency or Excess of Specific Dietary Nutrient

The skin functions normally when adequate nutrition is provided. Any dietary imbalance in the form of nutritional deficiency, specific nutrient inadequacy or excess, and toxic components can disturb the equilibrium of the skin. Although the cutaneous manifestations of deficiencies or excess of several vitamins, minerals, and fatty acids are well recognized their effect on immune system is not clear.

A deficiency of minerals like zinc leads to acrodermatitis enteropathica characterized by weeping dermatitis, delayed wound healing, secondary infection, alopecia, and nail defects. Lim et al. have hypothesized the involvement of dietary zinc in activating the nuclear factor-kappa B (NF B), expression of proinflammatory cytokines (IL-1b) and tumor necrosis factor-α, and neutrophil infiltration during the early stages of cutaneous wound healing [32]. Other foodstuffs which can block NF B-mediated activation of inflammatory cytokines include turmeric, red pepper, cloves, ginger, cumin, anise, fennel, basil, rosemary, garlic, and pomegranate that may also play a role in modulation of cutaneous immunity [33].

Vitamin deficiencies may also lead to various cutaneous manifestations, i.e., phrynoderma (vitamin A), scurvy (Vitamin C), pellagra (niacin), etc. Majority of green vegetables and fruits are rich sources of one or other vitamins. Vitamins have been reported to induce increased production and activity of natural killer cells, increase IL-2 production, and stimulate humoral immune responses. Vitamin E has also been seen to decrease prostaglandin E_2 production, as a result of which the T cell proliferation and function may be enhanced. Besides these, some vitamins and minerals like Vitamin C, E, and selenium (found in wheat germ, garlic, Basil nuts, brown rice, whole wheat bread, eggs, and seafoods like Tuna and Salmon) are also required for their antioxidative properties. They prevent free radical damage of collagen and elastin, the fibers that support the skin structure and prevent wrinkles and other signs of ageing.

Carotenoderma, a condition caused by excessive intake of carotene-rich foods like oranges and carrots, manifests as yellowish to orange skin discoloration. Xanthelasma is a condition associated with high intake of fats and lipids. Phytanic acid is found in foodstuffs like dairy products, meat, and fish and its impaired oxidation leads to Refsum's disease causing a rough scaling skin over the extremities [34]. A high glycemic load in food with associated rise in insulin and Insulin Growth Factor-1 (IGF-1) levels has been associated with cutaneous disorders like acne and rosacea which are characterized by a heightened immune response and inflammation [35].

Conclusion

The relationship between diet and skin disorders has gained interest in recent years. There is abundance of literature linking almost every skin disorder with diet in one or other manner. Majority of these explain beneficial role of diet and plant products in dermatology. The literature on diet and plant extracts acting as etiological factors is still scanty and there is a gap in the understanding of their role in causation of IMSD. Futuristic studies may help not only in bridging the gaps in our current knowledge regarding drIMSD but also in developing specific therapies based upon the offending dietary antigens.

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Chapter 3
UV Irradiations, Micronutrient Supplementation, and Cutaneous Health: Overview

Saeed Hosseini and S. Ali Mostafavi

Key Points

- The most probable mechanism of photoprotective effects of vitamin C, vitamin E, β-carotene, lycopene, and omega-3 against UV light damages may be ascribed to their antioxidant functions, in fact they have the capacity to react with free radicals instead of vital skin structures hence protect skin from UV damages.
- Nevertheless, other mechanisms may be involved that are not fully clear so far.
- In addition to antioxidant capacity, at least some aspects of photoprotective effect of dietary nutrients may return to enhancement of cutaneous immune system mainly by enhancing the T-cell-mediated immune responses.
- The micronutrients which probably take part in this mechanism are notably the following: zinc, iron, copper, α-tocopherol, vitamin E, vitamin C, folate, carotenoids, and polyunsaturated fatty acids.

Keywords Skin aging • Sunburns • Fatty acid supplementation • Carotenoids

Introduction

Human skin condition and functioning may be influenced as a result of repeated exposures of edible and nonedible factors. One of these factors is the environmental UV irradiation. Frequent skin exposures to the environmental UV irradiations may cause acute sunburn symptoms and, through the mechanism of photooxidative damage, may lead to long-term damaging effects like photoaging. Photoaging is distinctive by wrinkles[1] and loss of skin flexibility.

[1] Slight lines or folds especially in skin of the face.

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The other frequently exposed factors are different foods ingested at least three times daily. Besides the overall community health benefits, attempts to ensure the sound nutritional habits have additional advantages for skin appearance. In addition to skin color and scent, appearance of the skin can be determined by its surface consistency and physiologic characteristics such as elasticity, sweat, and sebum production.

Some branches of nutritional science deal with how nutrients can affect optimal skin condition,[2] and will be able to reach an accurate and deep intuitive understanding of the relationship between food ingredients and skin health [1, 2].

The ways by which nutritional factors influence skin condition lately have raised a lot of curiosity and interests. At least some part of our knowledge about nutrients and skin relationship come back to the past case reports of nutritional deficiencies and their cutaneous manifestations. Several vitamin and essential fatty acid deficiencies have apparent skin problems as a consequence. Recently, by public health promotions, the incidence of nutritional deficiencies has been decreased, but still imbalance and insufficient diets as a result of disease, aging, and the abuse of alcohol and drugs may affect health status and thus influence skin condition. In other words the most effective diet may not only stop skin disorders but may also promote skin condition.

In the previously published literature, food intake chiefly eating of fat, sugars, and spicy foods is frequently mentioned as influencing skin condition, though the methodical proof of this is limited.

When we go a little more deep into the content, we see that skin structures—lipids and amino acids—help to regulate skin pH. On the other hand, acidity of the skin makes it easier to keep from exogenous pathogens. Skin lipids, amino acids, and consequently its acidity is affected by endogenous and ecological factors, as well as aging, exposure to sunlight, chemicals, and mechanical damage [3].

Furthermore, dietary components, as frequently exposing agent, are declared to be one of the important factors which may influence our skin condition [4, 5]. For example, several cross-sectional studies have shown some associations between dietary components and skin aging. Maeve C Cosgrove and colleagues found that higher intakes of vitamin C and linoleic acid and lower intakes of fats and carbohydrates were associated with better skin-aging appearance [6].

In addition, hydration (existence of an abundant amount of water in the stratum corneum, i.e., outer layer of skin) is crucial for a soft and smooth skin manifestation. Sebum[3] and other epidermal lipids, collectively, supply the skin with a protective surface layer which can prevent skin dehydration [3].

The Role of Ultraviolet Irradiation and Reactive Oxygen Species in Relation to Elastin and Collagen in Skin Aging: Intrinsic Aging vs. Photoaging

Biologically, skin aging happens in two mechanisms: first, the senility, which progresses as years go by and originated from natural inner processes of loosing collagen, dehydration, and disintegration of flexible fiber network, which all together lead to dermis[4] chronic collapse and atrophy. The second is the aging procedure, which originates from environmental exposures. The most prevalent environmental agent is ultraviolet (UV) irradiation which causes photoaging.

In the genetic scale, UV exposure can induce elastin[5] promoter. This procedure facilitates the synthesis of elastin mRNA from the genomic material and leads to enhancement of elastin synthesis and

[2] Skin condition, normally, is defined as a mixture of some typical skin characteristics such as outer layer consistency, color, and physiologic features, such as hydration, sebum content, and surface acidity 1. Boelsma, E., et al., *Human skin condition and its associations with nutrient concentrations in serum and diet.* Am J Clin Nutr, 2003. **77**(2): p. 348–55.

[3] An oily secretion of the sebaceous glands.

[4] The thick layer of living tissue below the epidermis, containing blood capillaries, nerve endings, sweat gland, and other structures.

[5] An elastic, fibrous glycoprotein found in connective tissue.

accumulation of this fibric material in the upper and middle dermis, respectively. All these processes add to the clinical and morphologic changes pragmatic in photoaged skin [7].

Changes of intracellular and extracellular fluid can be seen in photoaged skin cells. Furthermore, dissociated elastin and fibrillin accumulate in the deep dermis, and a severe loss of interstitial collagens occurs in the matrix of the skin connective tissue. One of the pathogenic mechanisms which underlies these changes is the reactive oxygen species (ROS) generated by UV irradiations. The ROS exhaust and impair enzymatic and nonenzymatic antioxidant defense systems in whole body organs as well as in the skin. The ROS may cause everlasting hereditary changes in the genes. Furthermore, ROS can affect the skin cells' growth, development, and aging, and cause connective tissue dissociation by launching cytoplasmic signal transduction pathways in topical fibroblasts [2].

Tanaka and colleagues investigated the effects of ROS on the biosynthesis of connective tissue matrix ingredients, collagen and glucosaminoglycans (GAGs), in cultured human dermal fibroblasts. The ROS decreased collagen production and increased GAGs synthesis. Interestingly, these alterations were associated with the biological changes of connective tissue matrix components observed in photoaging skin. Furthermore, catalase and alpha-tocopherol completely prevented the ROS-induced alterations of collagen and GAGs biosynthesis, whereas superoxide dismutase had no effect on the ROS-induced changes. These findings show that ROS may be one of the factors that cause the biological changes of connective tissue matrix components observed in photoaging skin [8].

Sunburn Caused by Skin Exposure to UV Irradiation

Sunburn is the inflammation of the skin caused by overexposure to UV rays of the sun. Short-term exposure to the sun UV emissions can be abstained by the body defense system and is well tolerated by the skin. But above a specific threshold prolonged topical vasodilatation come into effect. Then the transportation of lymphocytes and macrophages into the tissue and beginning of inflammation process happen. This irritation becomes visible as reddening of the skin and it is clinically manifested as erythema.

One method of measuring the degree of UV radiation-induced erythema is determination of the minimal erythema dose (MED). One MED is the minimum quantity of energy needed to provoke a reliable, visible redness with fixed boundaries 16–24 h after contact with UV irradiation [5].

The Role of Micronutrient Supplementation in Sunburn and Skin Aging

Great amounts of micronutrients such as antioxidant vitamins and carotenoids exist in the skin and are proposed that help keep skin healthy [5]. There are strong evidences that skin damages from sunlight are well protected by dietary antioxidants [9]. Scientists have proven that supplementation with carotenoids, vitamin C and E before UV exposure may avoid the erythema related to sunburn. Reactive oxygen species may be generated in skin following UV exposure. Then, detrimental cascade reactions of photocarcinogenesis, photosensitivity, or early skin aging starts [10]. As a result, one mechanism of action underlying skin protective effects of antioxidants is scavenging reactive oxygen species [11]. But, this is not all a matter. More than a few dietary antioxidants exhibit biological properties other than antioxidant activity. Some fat-soluble nutrients can enter the nucleus and may alter cellular signaling pathways and may influence cell growth, development, and repair systems [12]. The basic aspects of skin aging and how carotenoids, vitamins, essential fatty acids, and trace elements affect this process is not well understood.

Some clinical trials have shown evidences of modulating skin health by oral supplementation with relatively high doses of vitamins, trace minerals, and fatty acids. Some other studies have investigated

the effects of fatty acids on skin condition, modulating (skin) immune function with oral micronutrient supplementation, and the protective effects of antioxidants against photoaging. Though, our knowledge about the effects of nutritional factors on skin condition is still rare.

Vitamins E and C and Selenium Supplementation

A few number of clinical trials carried out throughout the past two decades to inspect the photoprotective effects of dietary supplementation with vitamins C and E and selenium. In a double-blind, parallel, placebo-controlled trial carried out by La Ruche and Cesarini, 200 μg organic form of selenium (plus 16 mg copper) and a vitamin complex (with 14 mg α-tocopherol and 2,700 μg retinol) were examined for their ability to prevent sunburn cell formation in 16 healthy volunteers' skin exposed to ultraviolet radiation. After 3 weeks of supplementation with selenium, especially in association with vitamins, compared with the placebo group, all treatments with active ingredients, especially selenium in conjugation with antioxidant vitamins, provided limited protection against the formation of sunburn cells at a low irradiation dose (suberythemal). In high doses of UV irradiations (supraerythemal) these protective effects were not seen. And supplementation was ineffective in preventing light-induced erythema (skin reddening). It seems that photoprotective effects of these supplements can be attributed to improvement of antioxidant capacity of the skin cells [13].

Heinrich and colleagues showed that antioxidant supplements composed of vitamin E, selenium, and carotenoids promote measurable characteristics associated with overall skin structure as well as skin aging [14].

Karla Werninghaus, Mohsen Meydani, and colleagues investigated the photoprotective effects of 400 IU (295 mg) oral vitamin E (α-tocopherol acetate) or placebo against UV-induced epidermal damage. The results were evaluated at baseline, 1 month, and 6 months after supplementation. Not any significant difference was seen in the number of sunburn cells, produced by a threefold minimal erythema dose exposure, comparing with placebo. Throughout the study in spite of increasing serum α-tocopherol level, not any increases were detected in the skin, where photoprotection procedure must occur. This may logically explain why these investigators have not seen any protection in vitamin E group. Low vitamin E dose, Small sample size (12 subjects) and lack of other antioxidants to recycle α-tocopherol radicals may explain some logical reasons for this lack of success to show photoprotective futures of α-tocopherol [15].

In another trial, 40 healthy volunteers are divided in four trial groups. Two grams of α-tocopherol/day or 3 g ascorbate/day or a mixture of both vitamins (2 g α-tocopherol/day and 3 g ascorbate/day), or placebo was administered for 50 days. MEDs showed an obvious increase after supplementation with α-tocopherol and ascorbate mixture. MEDs also increased in subjects who received either vitamin alone or placebo but more slightly. Comparing with Werninghaus et al.'s study, in this investigation much higher doses of vitamin E were administered in combination with another antioxidant vitamin, and this caused the photoprotective effects to be more distinctive [16].

Vitamin E can modulate arachidonic acid metabolism and can affect eicosanoid system; in addition, it belongs to antioxidant family. All these characteristics may lead to the antiinflammatory properties of vitamin E and by this means harmonize the photoprotective effects of other antioxidants in the skin [5].

In another study by Eberlein-König et al., lower daily dosages of 671 mg vitamins E and 2 g vitamin C were administered for 8 days. In spite of this short study period and lower dose mean MEDs increased significantly compared with the placebo group. These investigators also showed a decrease in cutaneous blood flow in vitamin E plus vitamin C group, while there was an increment in cutaneous blood flow in placebo group.

Accordingly, it is inferable from these studies that short-term supplementation with fairly high doses of vitamin E and C (and maybe with combination of other antioxidant vitamins or minerals) may have photoprotective effects against UV radiations but not necessarily or significantly any effect on reddening of the skin [17].

Carotenoids Supplementation: β-Carotene and Lycopene

About 600 different carotenoids exist in nature, but we consume nearly 40 types of carotenoids in our diet especially by ingesting vegetable foods. Human gastrointestinal tract can absorb approximately 12 of these carotenoids, of which α-carotene, β-carotene, lutein, zeaxanthine, β-cryptoxanthin, and lycopene are among the most common. β-carotene is an important member of our nonenzymatic defense mechanisms against free radicals and potentially can degrade to yield vitamin A [18].

In addition to antioxidant function, carotenoids can modify absorption characteristics of the skin and immunomodulatory effects. β-carotene may have a direct photoprotective effect for the reason that it has the physical capacity to absorb radiance. Furthermore, some investigators reported nonvisible yellowish color of the skin following β-carotene ingestion that caused photoprotection by reflecting fractions of UV irradiations [5].

Photooxidative stress may induce reactive oxygen species, and one of the most efficient antioxidants which act by scavenging these particles are carotenoids. Both β-carotene and lycopene have been identified in the skin, but because of its regularity in our diet the β-carotene supplementation more frequently has been the subject of studies [19, 20].

Following the studies which showed the photoprotective effects of carotenoid supplementation, Stahl et al. studied to investigate whether intervention with a natural dietary source rich in lycopene keeps safe from harms of UV-induced erythema in humans.

Nine volunteers were fed daily with 40 g of tomato paste (containing about 16 mg/day of lycopene) for 10 weeks and were compared with the Control group ($n = 10$). A solar simulator induced Erythema at baseline, after 4 weeks and after 10 weeks. The measurable amount of erythema was evaluated by chromatometry. Serum carotenoid levels were measured by HPLC. Serum levels of lycopene increased in the trial group; the other carotenoids did not change significantly. At the end, in the tomato paste group 40% lower erythema formation was significant comparing with control group.

Therefore, these investigators succeeded to show clearly the protective effects of a commonly consumed dietary source of lycopene against UV light-induced erythema [21, 22]. In vitro investigations showed similar photoprotective effects in cell culture either. Furthermore, each carotenoid provides a level of protection against UV irradiations [23].

Thereafter, Aust et al. carried out another investigation to find whether different available lycopene sources are distinct from one another. In accordance they examined the photoprotective effects of synthetic lycopene in comparison with a tomato extract and a drink containing solubilized tomato extract in a way that all three groups ingested similar amounts of lycopene (about 10 mg/day) for 12 week. All the subjects were exposed by 1.25 minimal erythemal doses (MED) at dorsal skin (scapular region). The photoprotective effect was more noticeable in the two latter groups, which might be ascribed to phytofluene and phytoene, the carotenoids that are abundant in tomato extract and a drink containing solubilized tomato extract as well as lycopene [9].

In an approach to find the most efficient compound to fight against UV irradiations Greul et al. [24] examined the photoprotective effects of a mixture of several fat-soluble and water-soluble antioxidants including carotenoids (β-carotene and lycopene), vitamins C and E, selenium, and proanthocyanidins. To attain a mixture to be consumed safely for long term, the trial provided the antioxidants at near their physiological levels. Not any significant differences were seen between the intervention group and the placebo when minimal erythemal dose and chromametry of the skin were considered.

But matrix metalloproteinases 1 (MMP-1) and MMP-9 levels, two important enzymes that enroll in UV-induced sunburn processes, were significantly different between both groups after 2 weeks of intervention. In fact, supplementation with this antioxidant mixture caused a decrease in the UV-induced expression of MMP-1 and 9.

Wolf et al. investigated the effects of daily supplementation with 150 mg of oral carotenoids (60 mg β-carotene and 90 mg canthaxanthin) for a month. In spite of increments seen in the serum carotene concentrations, MEDs did not change significantly before and after the supplementation [25].

Cho et al. recently carried out a trial to examine the differential effects of low-dose and high-dose β-carotene supplementation on the human skin. Thirty and 90 mg/day of β-carotene were administered to 50 healthy subjects for 90 days. Type I procollagen, matrix metalloproteinase-1, and fibrillin-1 mRNA levels, and UV-induced thymine dimer and 8-hydroxy-2′-deoxyguanosine formation were assessed before and after the trial. Photoaging prevention signs and type I procollagen mRNA levels (4.4 ± 1.6 times increment comparing to the baseline) showed a significant difference just in the low-dose group. Significant decrease in the MED was observed only in the high-dose group. Significant reductions in UV-induced thymine dimer staining and 8-hydroxy-2′-deoxyguanosine staining were observed in the low-dose group in contrast to an increment in the high-dose group. Some investigators ascribe these new conflicting effects to the pro-oxidant effects of β-carotene spatially in high doses [26].

Is Supplementation with β-Carotene Safe?

Supplemental carotenoids are extensively used as skin protective agents against UV-induced erythema, yet little is known about safety and other effects of carotenoids on skin and whole body health [14]. In 1996, the results of an epidemiological cohort study carried out by Omenn et al. [27] raised a lot of worries about β-carotene supplementation safety. They observed the effects of β-carotene (30 mg) and vitamin A (25,000 IU of retinyl palmitate) mixture supplementation or placebo on respiratory organs of 18,314 smokers and asbestos workers. After about 4 years' monitoring, 388 new cases of lung cancer were detected (relative risk compared with placebo group: 1.25). Supplementation also increased the risk of death from lung cancer and cardiovascular disease. Finally the trial was stopped 21 months prior to what had intended to. But the criticizers emphasize that the methodology of this study was confounded by subject selection, since it was performed in high-risk participators (smokers and asbestos exposed workers). The next study (Physicians' Health Study) was performed on 22,071 middle-aged healthy male US physicians. On using 50 mg β-carotene supplementation on alternate days, not any significant differences in cardiovascular diseases, malignant tumor progression, and the overall mortality were seen [18].

So β-carotene supplementation is not a neoplasm or cardiovascular risk factor in healthy subjects, but long-term supplementation might be an additional risk factor for smokers. But the selection bias still remains in this study by attendance of the subjects who are more informed and concerned about their overall health, physicians, and the results cannot be referred to the normal society.

β-carotene is highly reactive and may slow down lipid peroxidation reactions in organic membranes. In fact β-carotene may make photocarcinogenesis worse under certain dietary and lifestyle circumstances. Photocarcinogenesis by β-carotene is diminished as the level of dietary fat decreases; this fact emphasizes on the possible association of lipid peroxidative reactions.

Does β-carotene really exhibit both pro-oxidant and antioxidant capacities? In fact, in photocarcinogenesis, intricate associations exist between the chemical mechanisms and the biological role of antioxidants. Still more studies with more precise design and more focus on mechanism of action are needed to solve this conflicting puzzle [28].

Omega-3 Polyunsaturated Fatty Acid Supplementation

Marine forms of omega-3 polyunsaturated fatty acids (PUFAs) are eicosapentaenoic acid (EPA; 20:5 omega-3) and docosahexaenoic acid (DHA; 22:6 omega-3). The most abundant marine sources are high-fat fishes such as mackerel, salmon, and sardine. However, optimistically, these sources are rare in most people's dietary plan. On the other hand, for reaching the most medical benefits it is important to attain a ratio of omega-6:omega-3 in our diet to about 3:1. This goal is so hard to achieve with mere dietary sources. So supplementation with omega-3 capsules is rational and sometimes indispensible under certain circumstances.

Omega-3 supplementations were the subjects of numerous clinical trials especially for intervening disorders with inflammatory mechanisms. But limited numbers of studies have evaluated the photoprotective effects of omega-3 supplementation against UV-induced erythema.

As discussed earlier, free radicals and lipid peroxidation are involved in the mechanism of erythema induced by Ultraviolet irradiation. Rhodes et al. studied the effects of supplementation with 10 g/day fish oil rich in omega-3 fatty acids (18% eicosapentaenoic acid and 12% docosahexaenoic acid) on UV-induced erythema and epidermal lipid peroxidation. Fifteen subjects participated for 6 months. MED increased significantly at the end but decreased again 10 weeks after the supplementation discontinued. Simultaneously with supplementation progression, skin capacity to lipid peroxidation decreased. In fact omega-3 fatty acids can be oxidized instead of vital inner structures and protect them from free radical damages [29].

To investigate the photoprotective effects of omega-3 fatty acids, Orengo et al. [30] supplemented ten volunteers' diets with 2.8 g EPA and 1.2 g DHA and compared the MEDs with ten subjects in placebo group after 4 weeks. MED increased significantly in the intervention group but prostaglandin E_2 (PGE_2) did not change significantly. These results confirmed the photoprotective effects of omega-3 fatty acids in short term.

One mechanism that could be involved in the photoprotective effects of omega-3 fatty acids is the mediators of vasodilatation, prostaglandins. Rhodes et al. [31] studied the effects of omega-3 fatty acids on UV-induced prostaglandin metabolism as well as potential photoprotective effect of omega-3 fatty acids in light-sensitive patients. Fish oil supplements enriched in omega-3 fatty acids were administered to 13 patients with polymorphic light eruption for 3 months. PGE_2 concentrations in skin fluid were assessed by collecting and analyzing the suction blister fluid. At the end MED increased significantly in the trial group. PGE_2 concentrations in skin fluid decreased after omega-3 supplementation both in irradiated and nonirradiated skin either. It can be inferred, that the inhibition of PGE_2 production in the skin is associated with the photoprotective effects of long-term omega-3 supplementation against UV irradiations.

Enhancement of Cutaneous Immune System by Micronutrient Supplementation

Sound nutritional status and optimal immune system performance are integrated. Sufficient vitamins and trace elements pools are needed for the immune system to work efficiently [32].

Micronutrient deficiencies prevent immune system from being expressed efficiently by the mechanism of attenuating the adaptive antibody and T-cell-mediated immune responses. This predisposes the host to infections especially in elderly, which in a vicious cause-and-effect cycle aggravates the micronutrient deficiency [33].

Lymphocyte count and delayed-type hypersensitivity (DTH) skin tests,[6] for instance, can be used for assessing the nutritional status of a patient. DTH skin responses include proliferation of T cells, interleukin 2, and other lymphokines production, and infiltration of the test position with mononuclear cells. The magnitude of DTH skin responses can be measured by diameter of the reddish area as a response to using a panel of typically facing antigens. But, these tests are complicated and are not routine in nutrition clinics [34].

Marginal nutrient deficiencies such as of zinc; iron; Vitamins B6, B12, folic acid, C, D, and E; and β-carotene are prevalent in older populations. Coexistence of such nutrient deficiencies in the elderly and immune function defects provide evidences that show they may be associated with each other[35, 36]. Scientists are on the verge of reaching an agreement that the supplementation with efficient doses of essential trace elements and vitamins may help to support the immune responses [36].

When we consider skin health as one of the components of immune system we should note that repeated and long-term sun exposures are ascribed to be related with humoral and cell-mediated immune responses and skin aging.

Several researches have been carried out mostly on the elderly to investigate whether supplementation with micronutrients can affect the skin immune function by using DTH test, but studies in healthy young people are scarce.

Researchers assumed that defects in the T cell-mediated immunity functions are responsible for at least some parts of immunosenescence in the elderly. As mentioned before DTH skin responses involve T-cell proliferation and on the other hand the elderly people are unable to respond appropriately to DTH test. So scientists concluded that aging of immune system may be associated with T-cell-mediated immunity defects.

Several cell-mediated immunity mechanisms are responsible for immune-enhancing characteristics of vitamins. Vitamins and β-carotene may enhance production of interleukin 2, increase the number and activity of natural killer cells and may affect DTH skin responses. Furthermore, it has been shown that supplementation with vitamins and β-carotene may provoke humoral immune responses. In fact oxidant-antioxidant balance is crucial for maintaining immune cell functions. It promotes the integration and functions of immune cells. To attain an optimal immune response in all age groups, sufficient amounts of antioxidant nutrients such as vitamin E, β-carotene, and glutathione are needed [37].

Vitamins C and E, selenium, copper, and zinc work against prospective damages of reactive oxygen species and adjust immune cell function. Furthermore, antioxidant vitamins and trace elements modulate cytokines and prostaglandins production. Abundant vitamins B6, folate, B12, C, and E; selenium; zinc; copper; and iron pool supports the proinflammatory Th1 cytokine-mediated immune response and a shift to an anti-inflammatory Th2 cell-mediated immune response is prevented. Through this mechanism, a desired immune response is provided by forenamed micronutrients supplementation. Vitamins A and D have significant roles in the cell-mediated as well as humoral antibody response. They are in favor of Th2-mediated anti-inflammatory cytokines. Vitamin A deficiency weakens both innate and adaptive immune responses. Vitamin D deficiency may increase vulnerability to infections as a result of defected localized innate immunity and impaired the antigen-specific cellular immune response. On the whole, poor status of previously mentioned micronutrients may lead to immunosuppression [33]. In addition to vitamins and minerals, macronutrients (notably, energy and protein) are effective on immune responses especially in the elderly [38].

By measuring DTH, some scientists reported that UV exposure is immunosuppressive. Investigators supplemented the healthy old men with zinc, β-carotene, α-tocopherol, folate, vitamin E, and vitamin C, and then measured DTH responses before and after UV exposures in different randomized double

[6] *Cell-mediated immune memory response.*

blind placebo-controlled trial. The more powerful DTH responses were associated with higher zinc and vitamin concentrations. In another words, higher plasma zinc and vitamin concentrations showed protective effects against UV exposures. These findings are in favor of the zinc and antioxidant vitamin supplementation role for immunomodulation [39–42].

Some investigators declare that dietary fatty acids can influence immune cell function by altering the fatty acid composition of membrane phospholipids in immune cells. Accordingly, the eicosanoids production and activity of membrane-associated enzymes may be influenced.

A reduction in the inflammatory mediator, $PGE_{2,}$ was reported following omega-3 PUFAs as well as vitamin E administration [43–45].

The long-term effects of supplementation in the healthy elderly and the effects of long-term consumption of the amounts of nutrients not above physiological and recommended levels on the immune system are not well understood and further investigations are needed.

Conclusion

Comparing with topical sunscreens which act only locally, supplementing regular diet with antioxidant vitamins, carotenoids, EPA, DHA, or a mixture, may protect the whole body against UV irradiation-induced damage. In spite of the contribution of natural dietary antioxidant nutrients, more amounts are needed above the amounts that exist in regular diets to attain the significant photoprotective effects. However, the level of skin sun-protection attains by antioxidant supplementation is much lower than the level of skin sun-protection can be achieved from the use of topical sunscreens. Up till now little is known about the photoprotective aspects of long-term consumption of physiological amounts of antioxidant vitamins or omega-3, the information which is important in developing functional foods [5, 32].

All the necessity of supplementation with omega-3 fatty acid comes back to omega-3 competition with omega-6 as a substrate for cyclooxygenase and lipoxygenase. If omega-3 fatty acids win the competition the result is production of less-active inflammatory mediators, prostaglandins, and leukotrienes. Lowest ratios of omega-6:omega-3 in the diet and hence in the skin lead to reductions in the synthesis of leukotriene B_4 (LTB_4) and PGE_2 or cytokines, such as interleukin 1 and tumor necrosis factor α. All these alterations lead to the obstruction of inflammatory cascades in the skin. Moreover, omega-3 PUFAs are unstable and may preferably be damaged by free radicals, thereby protecting other structures from attack by free radicals. Nevertheless, to protect against excessive formation of free radicals and lipid peroxidation, appropriate amounts of antioxidants (e.g., vitamin E and C and selenium) should also be consumed.

The most probable mechanism of photoprotective effects of vitamin C, vitamin E, β-carotene, lycopene, and omega-3 against UV light damages may be ascribed to their antioxidant functions; in fact they have the capacity to react with free radicals instead of vital skin structures, hence protect skin from UV damages. Nevertheless, other mechanisms may be involved that are not fully clear so far.

In addition to antioxidant capacity, at least some aspects of photoprotective effect of dietary nutrients may return to enhancement of cutaneous immune system mainly by enhancing the T-cell-mediated immune responses. The micronutrients which probably take part in this mechanism are notably the following: zinc, iron, copper, α-tocopherol, vitamin E, vitamin C, folate, carotenoids, and polyunsaturated fatty acids.

Still the body of our knowledge about the effects of nutrients on skin condition (especially in long-term intakes at recommended levels) and the actual need for supplementation is young and it is worthy of more attention and more long-term researches.

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Chapter 4
Skin Health and Metabolic Complications

Vijaya Juturu

Key Points

- High blood glucose causes feet problems.
- Management of body weight reduces skin problems.
- Control blood pressure and cholesterol for healthy skin.
- Essential fatty acids, vitamins, minerals including antioxidants will prevent skin damage from metabolic complications.
- Cracks allow germs to enter and cause infection.
- Dry skin requires moisturizers to prevent skin cracks and damage to skin. Keep your skin moist by using a lotion or cream after you wash.

Keywords Skin Health • Metabolism • Complications • Diabetes • Cardiovascular disease • Obesity

Prevalence of Metabolic Complications

Diabetes affects 25.8 million people of all ages, 8.3% of the US population (NIH, [1]), and approximately 61 million people in the USA have heart disease. According to the World Health Organization (WHO), 29% of all deaths worldwide are related to heart disease and other metabolic complications. Skin may undergo alterations leading to photo aging, inflammation, immune dysfunction, imbalanced epidermal homeostasis, or other skin disorders. Improving the skin health is important for healthy living. Obesity is responsible for changes in skin barrier function, sebaceous glands and sebum production, sweat glands, lymphatics, collagen structure and function, wound healing, microcirculation and macrocirculation, and subcutaneous fat. Dermatological changes are prominently seen in patients with obesity, including acanthosis nigricans and skin tags (due to insulin resistance), hyperandrogenism, striae due to over extension, stasis pigmentation due to peripheral vascular disease, lymphedema, pathologies associated with augmented folds, morphologic changes in the foot anatomy due to excess load. Circulatory problems due to metabolic complications cause dry skin or spots. Skin condition is based on the pathophysiological changes of diabetes, hypertension, and heart disease.

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Table 4.1 Factors that influence the skin conditions and metabolic complications

Metabolic complications	Dry skin condition	Endogenous factors	Exogenous factors
Diabetes	Fluctuations in glucose levels can lead to dehydration; microvascular changes causes dermopathy often looks like light brown, scaly patches, dry skin and itching of the skin	Genetic predisposition Hormonal Influences Biological skin ageing	Climate and environment Heat Cold Humidity UV radiation Light-induced skin ageing Chemical influences Aggressive cleansing agents Too frequent washing Therapeutic and nutrition measures: medicines and radiotherapy; insufficient fluid intake
Heart disease—atherosclerosis	Thickening of the arteries—atherosclerosis—can affect the skin on the legs; even minor scrapes can result in open sores that heal slowly		
Hypertension	Antihypertensives like diuretics causes dry skin		
Hormonal changes: hypothyroidism	Low levels of thyroid hormone can reduce the amount of oil produced by the skin		
Overweight/obesity	Irritation of the skin with scaling, rough or dry skin, redness, itching, and sometimes oozing, crusts, and erosions. Stripe-like skin marks; are thickenings of the skin composed of keratin; abnormal thickening and darkening of the skin; Increased strain on the leg veins may cause fluid retention, leg swelling, rupture of superficial capillaries (capillaritis), varicose veins, dermatitis, and even ulcers		

Factors Affecting Skin Conditions

The factors affecting skin conditions are aging, duration of diabetes, high blood pressure, heart disease and other metabolic complications of chronic conditions (Table 4.1). Some of them are independent risk factors for dry and fissured skin. The over activity and under activity of the thyroid gland may result in alterations in skin health, hair health or the nails health. The abnormal level of thyroid hormone or a consequence of an underlying condition may lead to dry skin or spots. The common symptoms include intense itching in metabolic complications lead to scratching and rubbing that aggravates dry skin condition. An inflammation may end up in secondary infections such as localized folliculitis (inflammation of the hair follicles on the skin) or even cellulitis. Microvascular impairment may lead to dehydration of the skin and cracks with erythema (redness or inflammation) becoming evident in and around the involved areas of limbs, face, and at intimate areas. Other skin conditions commonly seen in metabolic complications are acanthosis nigricans, scleroderma diabeticorum, vitiligo, necrobiosis lipoidica diabeticorum, psoriasis, pruritus (itching), diabetic dermopathy, digital sclerosis, eruptive xanthomatosis, diabetic blisters, bumps, rashes and disseminated granuloma annulare. Rough, dry, and scaly skin affects at least 75% of people with diabetes over the age of 64. The management of dry skin plays a considerable role in managing skin conditions in diabetes, heart disease, and obesity. Dry skin can be localized such as on the legs, feet, hands, and/or face or intimate areas and it can progress to all the skin.

Table 4.2 Most critical systemic hormones and skin health

Systemic hormones	Effect on skin
Calcium regulating hormones Parathyroid hormone Calcitriol (active vitamin D) Calcitonin	Rate of skin cell reproduction
Sex hormones Estrogen Testosterone	Aid in the prevention of skin aging Prevents a decrease in skin collagen Maintain skin thickness Maintains skin moisture by increasing acid mucopolysaccharides and hyaluronic acid in the skin Possibly maintaining stratum corneum barrier function Increases cutaneous wound healing by regulating the levels of a cytokine Skin wrinkling
Other systemic hormones Growth hormone (GH)/Insulin-like growth factor Thyroid hormone(TH) Cortisol	To instigate the growth of tissue by initiating protein formation, increase feeling of vivacity, vigor, and hardiness. GH aids in keeping the epidermis moist, keeping the skin opaque, thickening the epidermis and aiding in color improvement, flexibility, enhance skin rejuvenation and has anti-aging properties Stimulation of epidermal proliferation, dermal thickening, and hair growth Cortisol-induced collagen loss in the skin is ten times greater than in any other tissue, elevated cortisol reduces skin regeneration, and skin problems such as eczema are associated with gliaden intolerance. Gliaden is a component of gluten grains. Gliaden intolerance is also exacerbated by high copper

Metabolic Complications and Skin Health

Overweight/Obesity

Overweight and obesity are common health conditions and the prevalence of obesity leads to other metabolic complications including hypertension, heart disease, diabetes, and abnormal skin health. The World Health Organization has projected that by 2015 approximately 2.3 billion adults will be overweight and more than 700 million obese. The common skin challenges in obesity are pressure ulcers, candidiasis, delayed wound healing, incontinence dermatitis and irritation in skin folds or any intimate area where skin rubs against skin including acanthosis nigricans, acrochordons, keratosis pilaris, hyperandrogenism and hirsutism, striae distensae, adiposis dolorosa, and fat redistribution, lymphedema, chronic venous insufficiency, plantar hyperkeratosis, cellulitis, skin infections, hidradenitis suppurativa, psoriasis, insulin resistance syndrome, and tophaceous gout. These skin folds harbor moisture and breed bacterial fungal and viral growth resulting in rashes, blisters, and infection which attack the skin integrity. Table 4.2 provides systematic hormones and skin health. Skin thickness, echodensity, changes in transepidermal water loss, blood flow, neuronal responses like pain and irritant stimulus implicated as consequences of hormonal changes. Some of these skin complications in obese people can be managed by improved control of hyperinsulinemia; the vitamin D3 analog calcipitriol, Skin tags can be removed by snipping with curved scissors, by cryotherapy or by electrodesiccation. Hyperandrogenism, a result of increased production of endogenous androgens due to increased volumes of adipose tissue (which synthesizes testosterone) and hyperinsulinemia (which increases the production of ovarian androgens) needs to be carefully assessed to ensure disorders such as virilizing tumors and congenital adrenal hyperplasia. Obesity increases the incidence of cutaneous

infections that include candidiasis, intertigo, candida folliculitis, furunculosis, erythrasma, tinea cruris, and folliculitis. Less common infections include cellulitis, necrotizing fasciitis, and gas gangrene. Leg ulcerations, lymphedema, plantar hyperkeratosis, and striae are more common with obesity. Hormonal abnormalities and genetic syndromes (Prader-Willi) are related to obesity. Changes in hormones may also cause acanthosis nigricans, which are darkened, velvety areas of the neck and body folds, while stretching of the skin may result in stretch marks (striae) in obesity. Increased strain on the leg veins may cause fluid retention, leg swelling, and rupture of superficial capillaries (capillaritis), varicose veins, dermatitis, and even ulcers add additional complications in obesity/overweight. Retention of moisture in body folds enhances the growth of bacteria and fungi, leading to skin rashes and potential breakdown and a variety of infections, such as intertrigo in obesity. Obese people also develop corns and calluses due to the increased weight.

Calluses: A callus is a build-up of hard skin, usually on the underside of the foot. Calluses are caused by an uneven distribution of weight, generally on the bottom of the forefoot or heel. *Corns*: A corn is a build-up of hard skin near a bony area of a toe or between toes.

Diabetes

Diabetes is linked to thickening of the skin. It leads to reduced blood circulation to the skin (microangiopathy) and increased frequency of urination reduces the moisture available for the skin. The first symptoms of compromised skin health are often dry, scaly skin that can appear anywhere on the body and commonly seen at the extremities including: legs, feet, knees, elbows, and hands. Skin cracks increase bacteria, viruses and fungal infections, often leading to open sores and severe infections in diabetes. The four major factors that contribute to the slower healing rate in diabetes are blood flow to the skin, higher blood glucose that supports bacterial growth, slower metabolic rate, and thicker skin. The relationship of diabetic dermopathy to internal complications of diabetes mellitus, such as nephropathy, retinopathy, and neuropathy, is still unknown. The common skin problems in diabetes are the following:

Scleroderma diabeticorum. Thickening of the skin on the back of the neck and upper back is seen in DM.
Vitiligo. Vitiligo affects skin coloration in type 1 diabetes than type 2 diabetes. The special cells that make pigment are destroyed, resulting in patches of discolored skin. Vitiligo often affects the chest and abdomen but may be found on the face around the mouth, nostrils, and eyes.
Acanthosis nigricans. This results in the darkening and thickening of certain areas of the skin especially in the skin folds.
Necrobiosis lipoidica diabeticorum. Necrobiosis lipoidica diabeticorum (NLD) is caused by changes in the collagen and fat content underneath the skin. The overlying skin area becomes thinned and reddened.
Diabetic dermopathy. Dermopathy appears as a shiny round or oval lesion of thin skin over the front lower parts of the lower legs. Itching and burning of lower legs are common in DM.
Digital sclerosis. Digital sclerosis is a health condition in which the skin on toes, fingers, and hands become thick, waxy, and tight. Stiffness of the finger joints also may occur.
Eruptive xanthomatosis. Eruptive xanthomas appear as firm, yellow, waxy pea-like bumps on the skin in prediabetes and DM conditions. They are commonly found on the face, buttocks, back side of the arms and legs.
Rashes and bumps. Allergic reactions to foods, bug bites, and medicines can cause rashes, depressions, or bumps on the skin in the areas where they inject their insulin.
Diabetic blisters (bullosis diabeticorum). Blisters can occur on the fingers, hands, toes, feet, legs, or forearms commonly found in DM and diabetic neuropathy.

Disseminated granuloma annulare. Ring or arc-shaped areas on the skin, rashes most often occur on the fingers, ears, chest, and abdomen.

Atherosclerosis. Atherosclerosis is a serious health condition caused by the narrowing of blood vessels from a thickening of the vessel walls due to plaque buildup. This condition is often associated with blood vessels including those that supply blood to the skin. The narrowing of blood vessels supplying blood to the skin reduces the circulation of blood and lack of oxygen may cause hair loss, thinning and shiny skin especially on the shins, thickened and discolored toenails, and cold skin. Legs and feet affected by atherosclerosis increase bacterial infections and heal more slowly when they are injured.

Hypertension

Psoriasis was independently associated with an increased risk of diabetes and hypertension [2]. Psoriasis affects about 4% of the US population and causes patches of itchy, thickened, dry, reddened skin. People with psoriasis may be more prone to developing constricted blood vessels and increase blood pressure. Psoriasis is an inflammatory disease [3]. In two cross-sectional studies, individuals with psoriasis have a higher prevalence of obesity, diabetes, and hypertension. Individuals with psoriasis were more likely to have cardiovascular risk factors including hypertension and myocardial infarctions at a younger age [4, 5].

Skin Infections and Metabolic Conditions

Bacterial infections. There are different kinds of bacterial infections commonly affecting the skin of those with diabetes, obesity, and CVD. Staphylococcus is more common and more serious in people with DM. Boils, an inflamed nodule, occur in areas of hair follicles, infections of the glands of the eyelids, and bacterial nail infections in DM.

Fungal infections. Candida albicans and angular cheilitis are common fungal infections in DM. Fungus also can occur in the corners of the mouth, between the toes and fingers and in the nails (onychomycosis). Three common fungal infections are jock itch (red, itchy area on the genitals and the inside of the thighs), athlete's foot (affects the skin between the toes), and ringworm (ring-shaped, scaly patches that can itch or blister and appear on the feet, groin, chest and abdomen, scalp, or nails). A potentially fatal fungal infection with Mucormycosis is seen in people with diabetes usually starts in the nasal cavities and can spread to the eyes and brain. Nails that are infected with a fungus may become discolored (yellowish-brown or opaque), thick and brittle, and may separate from the bed of the nail. Foot problems can possibly lead to infection and serious complications, such as amputation.

Yeast Infections. Yeast infections are common in diabetes, obesity, and cardiovascular disease.

Balanced nutrition and certain nutrients were identified to play a critical role in the normal functioning of the skin. Several studies have observed improved protection of the skin against sun damage (photoprotection) by dietary supplementation with vitamins E, vitamin D and C, carotenoids (β-carotene and lycopene), and polyunsaturated fatty acids (PUFAs), carotenoids, flavonoids, and minerals [6–10]. There is great interest in anti-aging substances derived from food, and the most popular ingredients are antioxidants, especially coenzyme Q10, phytoestrogens, probiotics and omega-3 fatty acids [6, 11–16]. The challenge in the future will be strategic combining of cosmetics and functional nutrients in order to intervene in biological aging processes and degenerative skin changes in normal healthy skin conditions and in chronic conditions such as diabetes, obesity, hypertension, and heart disease.

Antioxidant activity is provided by a number of naturally occurring substances including alpha-tocopherol (vitamin E) and beta-carotene, polyphenols and essential fatty acids, whose effects are mediated by their capacity to quench singlet oxygen, scavenge free radicals [17–19]. Skin-aging appearances were defined as having a wrinkled appearance, senile dryness, and skin atrophy and prevent the formation of free radicals [13].

Dietary Factors, Metabolic Complications, and Skin Health

A diet high in fat and carbohydrates promotes skin aging. The effects of food ingredients on skin conditions may prove to be biologically relevant for optimal skin health. Low-fat dairy products, blackberries, blueberries, strawberries, plums, salmon, walnuts, canola oil, flax seeds, whole-wheat bread, muffins, cereals turkey, tuna and brazil nuts are the important sources of fiber, essential fatty acids, vitamins and minerals may reduce metabolic complications and maintain skin integrity (Table 4.3).

Many reports suggest that the intake of *n*-3 PUFAs, particularly eicosapentaenoic acid (EPA; 20:5*n*-3), may provide considerable health benefits in relation to inflammatory diseases and reduces triglycerides [20–25]. Dietary plant stanols and sterols have been found to inhibit the absorption of cholesterol [26]. Based on the current evidence, 25 g of soy protein or a food providing at least 0.65 g sterol ester or 1.7 g stanol esters per serving can be recommended to improve coronary risk lipids and lipoproteins [27]. Flavanol-rich cocoa and chocolate have the potential to augment an individual's antioxidant defense system; improvements in inflammation, platelet aggregation, and nitric oxide-mediated endothelial changes are additional factors that can be achieved by flavanols [28, 29].

The Hawthorn berry comes from a large genus of shrubs and trees in the family Rosaceae. Preliminary evidence indicates that Hawthorn (*Crataegus* spp.) may have some potential benefits in congestive heart failure [30]. Hawthorn decreased serum TC, LDL-C, and TG in hyperlipidemic subjects [31, 32]. Lycopene is the most common carotenoid in the human body and is one of the most potent carotenoid antioxidants [33, 34]. Anthocyanins in blueberries increase insulin release from pancreatic cells, improve insulin sensitivity, and reduce food intake [35]. Polyphenols have demonstrated several effects, including promoting scavenging of free radicals, regulating NO, decreasing leukocyte mobilization, inducing apoptosis, inhibiting cell proliferation and angiogenesis, and phytoestrogenic activity [36]. Dietary flavonoids found primarily in green tea and red wine [37] may protect against cardiovascular disease. The four major catechins found in green tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). Catechins can increase the antioxidant capacity of human plasma, which could help reduce cardiovascular disease risk [36]. Preclinical and clinical studies suggest that cranberries are more effective in improving endothelial function, inhibiting LDL oxidation, decrease lipid oxidation and protein glycosylation, may decrease the side effects of diabetes, hypertension-related ACE-I inhibitory activities, and have antiatherogenic properties [38].

Summary

The common clinical recommendations for metabolic complications are lifestyle, individual risk factor interventions, and drug intervention including smoking cessation, physical activity, a heart-healthy diet, and weight maintenance. They are important for all people regardless of risk level, even if only to reinforce established healthy behaviors. Management of metabolic complications under

Table 4.3 Functional nutrients, biological effects, and skin health

Nutrients/nutritional supplements/food sources	Biological effects on metabolic health	Skin health
Minerals, vitamins, essential fatty acids, essential amino acids, polyphenols, plant stanols/soy protein	Improves lipids, insulin sensitivity; increases insulin production and protects beta cell; improves glucose utilization and metabolism; reduces diabetic complications Anti-lipidemic effects; binds bile acids; may inhibit key enzymes in cholesterol biosynthesis Antihypertensive effects: diuretics; central alpha agonists; direct vasodilators; calcium channel blockers; angiotensin-converting enzyme inhibitors; angiotensin receptor blockers	Immune systems, and modulate inflammatory and degenerative processes Antioxidants normally delay or prevent oxidation of a substrate Improves cutaneous barrier function Decrease lipid peroxidation and to enhance concentrations of antioxidants and antioxidant enzymes in the circulation Antioxidants improves pigmentation of the skin Significant increase of melanin concentrations in skin Improves basal skin properties, including hydration, sebum production, and elasticity Protection against ultraviolet light May boost cell-mediated immunity Modulate the balance of lipid inflammatory mediators
Conjugated linoleic acid	Decreases body fat and improves lean body mass	
L-arginine; soy; calcium; magnesium; monounsaturated fatty acids; omega-3 fatty acids	Improves endothelial function; inhibits the transmembrane influx of calcium ions into cardiac and vascular smooth muscle; improves coronary vascular circulation and reduces coronary constriction, resulting in reduction in systolic and diastolic blood pressure	
Zinc; selenium; folic acid; coenzyme Q10; phytoestrogens; probiotics; monounsaturated fatty acids; omega-3 fatty acids; L-arginine; pycnogenol; milk and milk products (source of conjugated linoleic acid)	Anti-inflammatory effects: lowers cytokines, C-reactive protein, homocysteine; reduces eNOS transcription; inhibits IL-6 release; ET1 release; decrease macrophage LDL uptake; decrease plaque rupture; reduced activation of transcription factors such as, NF-κB; decreases PAI-1	
Omega-3 fatty acids; polyphenols; antioxidants	Decreases clotting effects; protects from thrombosis	
Selenium; zinc; omega-3 fatty acids; fruits and vegetables; coenzyme Q10; thiols (glutathione, lipoic acid); ubiquinols; flavonoids; polyphenols	Decreases free radicals; decreases the damage caused by oxidative stress; helps protect tissues	

GLUT-4 glucose transporter 4, *IL* interleukin, *PAI-1* plasminogen activator inhibitor 1, *PI3* phosphoinositide 3-kinases, *PTB1B* protein tyrosine phosphatase 1B, *eNOS* endothelial nitric oxide synthase, *ET1* endothelin, *NF-B* nuclear factor B

control is the most important factor in preventing the skin-related complications in chronic conditions. Proper skin care can help reduce the risk of skin-related problems such as

- Changes in skin color
- Changes in skin temperature
- Swelling in the foot or ankle
- Pain in the legs
- Open sores on the feet that are slow to heal or are draining
- Ingrown toenails or toenails infected with fungus
- Corns or calluses
- Dry cracks in the skin, especially around the heel
- Unusual and/or persistent foot odor

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Chapter 5
Probiotics and Skin Health

Audrey Guéniche, J. Benyacoub, S. Blum, L. Breton, and I. Castiel

Key Points

- Certain probiotic strains alive exert their effects beyond the gut to the skin level and contribute to the reinforcement of the barrier function and the modulation of immune system.

Keywords Probiotic • Lactic acid bacteria • Skin • Stress • UV • Reactive skin

Probiotics: Definition and General Health Benefits

The term probiotics, popularized by R. Fuller in 1989 [1], was recently defined by an expert committee as "living microorganisms, which, when consumed in adequate amounts, confer a health effect on the host" [2].

Specific strains of probiotic lactic acid bacteria (LAB) have been shown to beneficially influence the composition and/or metabolic activity of the endogenous microbiota [3–7], and some of these have been shown to inhibit the growth of a wide range of enteropathogens [8, 9]. Competition for essential nutrients, aggregation with pathogenic microorganisms [10], competition for receptor sites [11], and production of anti-microbial metabolites [8, 9] have all been reported to play a role.

Probiotics can be consumed in various forms of fermented or non-fermented food products. As a common feature, after ingestion, probiotics become transient constituents of the gut microbiota capable of exerting their biologic effects, thus giving a rationale for their use as a component of functional foods. Weaning, stress, dietary changes, use of antibiotics, and intestinal infections are all conditions that affect the natural balance of the intestinal microbiota for which the application of probiotics might be beneficial.

The most often used probiotic genera in humans and animals are enterococci, lactobacilli, and bifidobacteria, which are natural residents of the intestinal tract.

Multiple criteria have been defined for the selection of probiotic strains (reviewed 12). Obviously, the most important criterion is that the selected strains must be safe for use to the host and for the environment. One of the most commonly reported selection criteria is the ability to survive during

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passage through the gastrointestinal tract (GIT) of the host for which the capacity of a strain to remain unaltered over conditions prevailing in the stomach (acidity) and intestinal tract (bile acids, pancreatic and other digestive enzymes) is crucial. Adhesion to intestinal epithelial cells is considered important for immune modulation, pathogen exclusion, and prolonged residence time in the GIT. Viability of the probiotic strain is assumed to be important and metabolic activity may be crucial for the expression of anti-pathogenic activity. There is increasing evidence that bacterial compounds such as DNA (some CpG motifs) or cell-wall fragments and/or dead bacteria can elicit certain immune responses [13–16].

Although species-specific origin is thought to be important for host-specific interactions with the probiotics, there are examples of efficiency of non-species-specific probiotic strains. Indeed, health benefits have been shown using the yeast *Saccharomyces boulardii* in humans [17]. Finally, probiotics need to have good technological properties to achieve high cell counts after fermentation at an industrial scale and to survive downstream processing, drying, food manufacturing, and long-term storage under adverse environmental conditions (e.g., humidity, high temperature, and presence of oxygen).

The first aim of using probiotics has been to improve the composition of the intestinal microbiota from a potentially harmful composition toward a composition that would be beneficial to the host. Indeed, this approach is particularly relevant since the intestinal microbiota is known to play a major role in the physiological balance, the intestinal development and maturation of the host immune system [4–6, 18]. An adequate balance of the microbiota is crucial to maintain good health conditions. In that sense, a decrease in clostridia and coliforms and an increase in lactobacilli and/or bifidobacteria have often been seen as evidence of healthy gut conditions [19]. In contrast, changes of the intestinal microbiota composition are associated with certain types of pathologies, in particular gastrointestinal infections, inflammations, and allergies [2, 19].

Different studies showed that specific probiotic strains are able to positively influence the microbiota composition [20]. In that respect, some probiotic strains have been successfully used to improve the outcome of gastrointestinal diseases, in particular diarrhea or *Helicobacter pylori* infections and related gastritis [21–25].

Beyond their capacity to influence positively the composition of the intestinal microbiota, several lines of evidence suggest that some probiotic bacteria can modulate the immune system both at the local and systemic levels [5, 18] thereby improving immune defense mechanisms and/or down-regulate immune disorders such as allergies or intestinal inflammation [6, 26–28].

Indeed, several strains of lactic acid bacteria were shown to modulate cytokine and growth factor production in vitro and in vivo [29–32]. Moreover, results from different pre-clinical and clinical trials have shown the capacity of various probiotic strains to enhance non-specific and specific immunity [33–38].

Probiotics and Skin

Different human trials widely suggest that probiotic supplementation might be useful in the management of atopic dermatitis [26, 28, 39, 40]. Based on these properties it appears that, beyond the gut, probiotics might exert their benefit at the skin level. Thus, it is assumed that some specific probiotic strains may be useful for the maintenance of cutaneous homeostasis and regulation of the skin immune system.

The skin plays a crucial role to protect against dehydration and damage or insults from external aggressions, e.g., chemical (pollution, tobacco, xenobiotics), mechanical, physical (UV radiations, changes in temperature and hygrometry), or infections. It is composed of a stratified epithelium with various cell types, including keratinocytes whose differentiation results in building barrier function and, in a lower proportion, dendritic cells, melanocytes, and Langerhans cells. Each of these cell types contributes to skin protection. Moreover, the underlying dermal compartment harbors leukocytes, mastocytes, and macrophages that are key actors of cell defense.

Skin reflects the general health status and age of the host. Although skin aging is genetically programmed, the health and functions of the skin are also influenced by environmental factors, especially in exposed areas such as the face. Indeed, lifestyle, food, climate conditions, the extent and frequency of UV exposure, free radicals, toxins and allergens, xenobiotics, and mechanical damage are all exogenous factors suspected to alter skin health. Furthermore, hormonal status, immunological status, and psychological stress are endogenous factors that can alter skin quality and biological functions.

In this context, the skin can undergo various changes including immune dysfunction, inflammation, photoaging, dryness, wrinkles, dyschromia, and a variety of hyperplasia [41].

Skin Allergic Reactions

The capacity of certain probiotic strains to modulate immune functions was a rationale for using probiotics to prevent and/or improve clinical outcome of diseases related to immune disorders such as allergies.

Recently, several researchers have focused on the suppressive effect of probiotic agents on allergic response and have evaluated their prophylactic and/or therapeutic efficacy on atopic dermatitis, asthma, and food allergies [5, 6, 28, 42, 43]. Especially, perinatal administration of the probiotic *Lactobacillus rhamnosus* GG (LGG) has been shown to reduce the incidence of atopic dermatitis in children at risk during infancy [26, 39, 40]. It is postulated that a boost in Th1 response, mainly IFN-γ production, may be associated and/or responsible for the beneficial effect of LGG on atopic dermatitis [44, 45].

Moreover, another study showed that a *Lactobacillus casei* strain decreased specific-allergen contact hypersensitivity reaction in mice [46].

Skin Aggression: Environmental Stress

Apart from pathological cutaneous disorders such as atopic dermatitis, the skin is continuously challenged by diverse environmental stress which can later induce important alterations of the cutaneous homeostasis.

Indeed, the skin is known to be an immune-competent tissue and thus it is important for the protection of the host against infections and the control of cell malignancies [47]. Several epidemiological studies demonstrated that UV radiations induce dramatic change in immune functions. This UV-induced alteration of the immune system is considered as one of the major risk factors in the development of certain skin cancers associated with sun overexposure [48–50]. Among these changes, a decrease in number and morphological modifications of the Langerhans cells as well as an alteration of their capacity to present antigens have been proved [50–52]. An increase in immune-suppressive cytokine levels such as IL-10 was also reported [53].

These skin disorders associated with dysregulation of immunological and/or neurosensitive mechanisms could be modulated or prevented by nutritional support and in particular by the use of certain probiotics [54].

In this context, pre-clinical studies were performed to evaluate the effect of a diet supplemented with *Lactobacillus johnsonii* on the cutaneous immune system. Supplementation with this probiotic modulated the production of IL-10 in the serum and maintained Langerhans cell density at the site of UV exposure. Overall, the results showed that supplementation with *L. johnsonii* could prevent the deleterious effects of UV radiation on the skin immune system and reinforce host skin defense against antigenic challenges [55].

The same probiotic was tested in a randomized, double-blind, placebo-controlled study to evaluate its capacity to maintain skin homeostasis under UV exposure. Fifty four volunteers were randomized

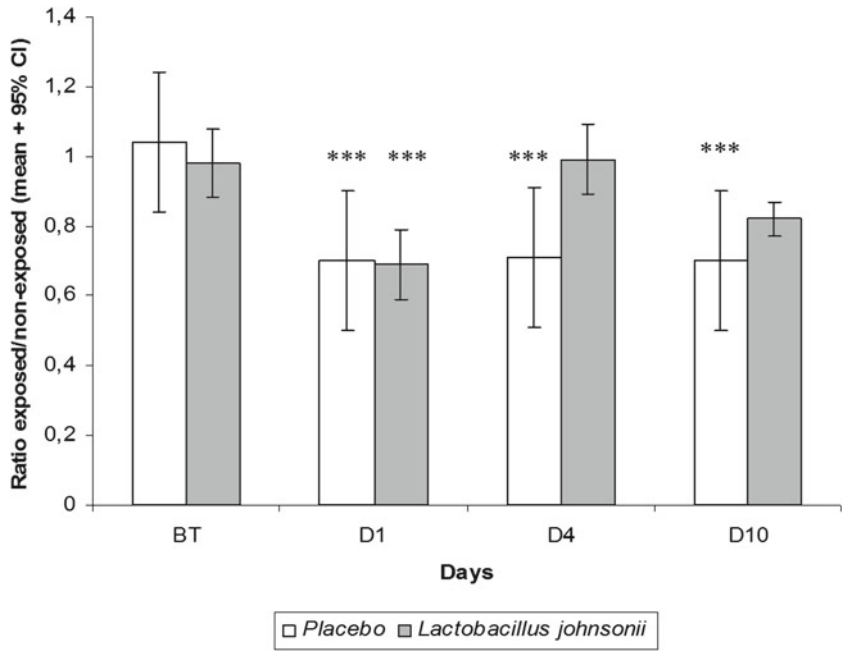

Fig. 5.1 Results from MECLR:cpm ratios from exposed versus non-exposed skin samples. The ratios were calculated from 54 volunteers distributed among the *Lactobacillus johnsonii* and placebo groups. ***Statistically significant differences at $p < 0.001$ between exposed and non-exposed sides. *BT* before treatment

in two groups ($n = 27$ per group) taking either *L. johnsonii* or a placebo daily for 6 weeks before UV exposure to 2×1.5 MED. Biopsies of skin were analyzed to investigate the effects on the phenotype of skin immune cells and the mixed epidermal cell lymphocyte reaction (MECLR).

L. johnsonii supplementation did not prevent UV-induced phenotypic maturation of dendritic Langerhans cells (LCs) or the decrease in MECLR in irradiated skin samples, 1 day post-irradiation. On day 4 after UV exposure, however, MECLR was still decreased in the placebo group with parallel reduction in the CD1a LC marker in irradiated epidermis whereas the allostimulatory capacity of epidermal cells was totally recovered in the La1 group correlating with normalization of CD1a expression within epidermis (Fig. 5.1). Moreover, CD36+ monocytic cells colonized the epidermis 1 day post-irradiation in all subjects but disappeared faster in the La1 group, which may suggest that they differentiated into CD1a+ dendritic cells [56].

These results show for the first time that ingested probiotic bacteria can accelerate the recovery of cutaneous immune homeostasis after UV exposure in humans and may therefore play a role in UV-induced skin damage prevention and photoprotection [47–51, 53].

Reactive Skin

Another indication for which the probiotics could be beneficial is to improve reactive skin symptoms. Reactive skin is characterized by marked sensitivity of the skin to physical (heat, cold, wind) or chemical (topical product application) stimuli and impaired ability to rebuild skin barrier function. Clinically, reactive skins are generally associated with important skin dryness [57, 58].

Several epidemiological studies conducted in Europe and the USA reported that about half of women and a third of men showed sensitive skin [58–63]. Subjects with sensitive skin primarily

complain of cutaneous discomfort. The main manifestations of this "cutaneous discomfort" are neurosensory signs such as feelings of heat, burning, stinging, or itching [57–64]. The signs may remain isolated or be associated with fleeting erythema. In most cases, the symptoms are limited to the face. Sometimes, other areas of the body (most often the scalp) could also be affected by a hyper-reactivity.

Several factors are implicated in the onset of reactive skin symptoms. These include, environmental factors (temperature changes, heat, cold, wind, sun, air pollution, etc.), contact with certain products such as "hard" water, or internal factors (emotional factors, menstrual cycle, dietary factors, etc.) [57–64]. In most cases, skin hyper-reactivity is constitutional. In certain situations, a lowering of the cutaneous tolerance threshold may be acquired (sensitive skin induced by application of irritant products) or concomitant with an episode of skin disease.

Even though the skin of a person suffering from eczema is hyper-reactive (episodes of seborrheic dermatitis, rosacea, atopic dermatitis, or contact eczema), many cases of sensitive skin are not all related to allergic skin diseases. The etymological relationship between the terms "sensitive" and "sensitization" is without doubt responsible for the confusion that still exists between "allergic skin" and "sensitive skin".

Two types of mechanism underline skin sensitivity: (a) the exacerbated reactivity of sensory nerves and/or (b) impaired barrier function which contribute to a better accessibility of nervous fiber by exogenous potentially irritant compounds [65, 66].

During acute phases of skin sensitivity, neurogenic inflammation may be triggered [67–69]. This could have long-term consequences as it may contribute to the maintenance of inflammatory conditions leading to chronicity.

Different studies confirm that there is an association between sensitive skin, the propensity to erythema, and dry skin [51, 58, 60–63]. Several authors have demonstrated that an impaired barrier function is associated with the onset of sensitive skin [60, 70, 71].

Recently, reactive or sensitive skin was classified into three different types according to physiological characteristics [61]. Type I was defined as the group with low barrier function. Type II was defined as the inflammatory group with a normal barrier function but an increased inflammatory status. Type III was defined as a pseudo-normal group in terms of barrier function and inflammatory status. It is interesting to note that the three types of reactive skin exhibit much slower restoration of barrier function following a skin lesion than that observed in subjects with non-reactive skin [61].

Epidermal sensory nerves whose endings are in the *stratum corneum* play a key physiological role in sensitive skin symptoms development. Thus, sensitive skin is considered as reflecting cutaneous sensory hyper-reactivity. Subjects with sensitive skin have an elevated neuropeptide level in the *stratum corneum* compared to the levels found in subjects with normal skin. Moreover, subjects of types I, II, and III reactive skin present a high sensitivity to electrical stimuli [61].

Homeostatic hydration level of the epidermis is related to the status of the skin barrier and the interrelationships between the cells and their lipid environment. Lipids are involved in the rate of trans-epidermal water loss (TEWL). Disruption of skin barrier primarily gives rise to an increase in TEWL [72–77].

Impairment of skin barrier is most frequently associated with "dry" skin, or xerosis. The skin exhibits a dull color and appears fragile with visible scaling. The smoothness of the skin is impaired. The feelings of tightness and tension may eventually result in pruritus. Skin penetration of various compounds is increased compared to normal skin and the topical risks of infection and allergy are enhanced.

A mixed preparation of *Lactobacillus paracaseï* CNCM I-2116 and *Bifidobacterium lactis* CNCM I-3446 was tested in a randomized, double-blind, placebo-controlled trial. Sixty female volunteers (18–35 years old) with reactive skin ingested daily either probiotic (10^{10} cfu of each probiotic strain) ($n=33$) or placebo ($n=33$) powder suspended in drinking water for 8 weeks. Skin reactivity was assessed by a stinging test performed at start of supplementation, middle, and end of the study. The results showed a significant decrease in cutaneous neurosensitivity ($p=0.02$) in volunteers receiving

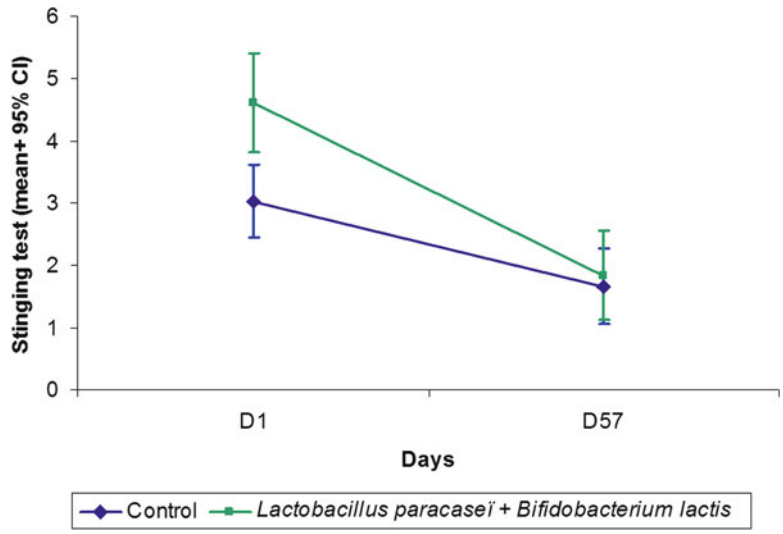

Fig. 5.2 Results from the skin sensitivity evaluated by stinging test between volunteers distributed among the combination *Lactobacillus paracaseï* and *Bifidobacterium lactis* and placebo groups

probiotic mix compared to those taking control powder at the end of the treatment (Fig. 5.2). These results provide the first clinical evidence that specific probiotic strains can have beneficial effects on skin reactivity and afford new opportunity to devise strategies to improve sensitive skin [78, 79].

Mechanisms

Indirect Action

The maintenance and protection of the gastrointestinal tract contributes to the overall host equilibrium. Although a direct relationship between probiotics and the bioavailability of nutrients has not yet been established, probiotics nonetheless positively influence the gastrointestinal homeostasis which contributes to promote the absorption of dietary nutrients at the intestinal mucosal level. This may help to provide essential nutrients for cell metabolism and the synthesis of the various functional and structural components of the skin.

Direct Action

The mechanisms whereby probiotics may play a role on skin physiology are not fully elucidated. However, it is proposed that, as shown for other commensal bacteria, probiotics could be directly sampled in the lumen by mucosal dendritic cells, which express tight junction proteins and penetrate gut epithelial monolayer (reviewed 80). It is postulated that upon interaction of the probiotic bacteria (or their components) with the intestinal epithelium and/or direct interaction with dendritic cells, other immune cells, such as B and T lymphocytes may be activated (primed) and immune mediators, including cytokines may subsequently be released. These cytokines, bacterial fractions, and primed

immune cells may be transported via the blood to other organs, including the skin, where they could modulate the immune status.

In addition, the improvement of reactive skin after probiotic supplementation could also result from a direct activity of the ingredient on neurosensitve mechanisms. On the one hand, immunoregulating properties of probiotics at skin level could modulate the inflammatory reactions generated by the release of neuromediators involved in skin neurosensitivity [70, 81, 82]. On the other hand, the capacity of certain probiotics to modulate the production of regulating cytokines and growth factors may play a role in the proliferation and differentiation of skin keratinocytes which is important for skin barrier repair [31]. Such possible effects on the process of generating the stratum corneum allow the quality of the cutaneous barrier function and skin dryness to be improved [82].

Conclusions and Perspectives

In conclusion, the current experimental and clinical data strengthen the assumption that certain probiotic strains exert their effects beyond the gut and confer benefits at the skin level. There are indeed emerging evidences that such probiotics alive can contribute to the reinforcement of skin barrier function and modulate skin immune system leading to the preservation of the skin homeostasis.

Altogether the data affords the possibility of designing new strategies based on a nutritional approach for the treatment and/or prevention of UV-induced damaging effects, and of symptoms related to reactive skin or atopic skin or changes in skin homeostasis.

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Part II
Dietary Nutrients and Skin

Chapter 6
Vitamin C (L-Ascorbic Acid): Antioxidant Involved in Skin Care

Rashmi Saini, Sachin L. Badole, and Anand A. Zanwar

Key Points

- Vitamin C, also called L-ascorbic acid, is derived from glucose in many animals.
- Vitamin C is a potent antioxidant.
- It is an essential component for collagen synthesis.
- Ascorbic acid is an effective depigmenting agent.
- It inhibits synthesis of melanin.
- It is used in repairing past damage to skin by age and sun.

Keywords Vitamin C • Ascorbic acid • Antioxidant • Skin cancer • Depigmenting agent

Introduction

Vitamin C has received a great deal of attention being considered one of the safest and most effective nutrients. Over a hundred studies from the previous 10 years revealed a growing list of benefits of vitamin C as published in Preventive and Alternative Medicine recently. The benefits of vitamin C include protection against immune system deficiencies, cardiovascular disease, prenatal health problems, eye disease, cancer, and even *skin* wrinkling. Higher blood levels of vitamin C may be the ideal *nutrition* marker for overall health, says study researcher Mark Moyad, MD, MPH, of the University of Michigan. The more we study vitamin C, the better our understanding of how diverse it is in protecting our health, from cardiovascular, *cancer*, *stroke*, *eye health,* and immunity to living longer.

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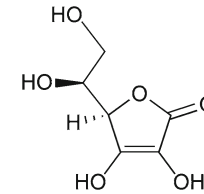

Fig. 6.1 Ascorbic acid (reduced form)

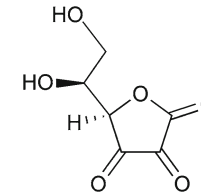

Fig. 6.2 Dehydroascorbic acid (oxidized form)

L-Ascorbic Acid (Vitamin C)

Vitamin C, also called L-ascorbic acid, is derived from glucose and many animals can make it starting from glucose. Primates like humans cannot synthesize it (we lack an enzyme in that pathway), making ascorbic acid a vitamin because we cannot make it but need it, so we have to ingest it from a source. It is water-soluble, which means it is easily absorbed through the water in your body. Our body does not store vitamin C, so we must replace our supply every day and excess amounts are flushed out through your kidneys. Vitamin C is needed for the growth and repair of tissues in all parts of our body. It helps the body make collagen, an important protein used to make skin, cartilage, tendons, ligaments, and blood vessels. Vitamin C is essential for healing wounds, and for repairing and maintaining bones and teeth. Due to its beneficial effects on skin, it has become a popular natural ingredient in skin care cosmetics.

Structure

Vitamin C is purely the l-*enantiomer* of ascorbate; the opposite d-*enantiomer* has no physiological significance. Both forms are *mirror images* of the same molecular structure. When L-ascorbate, which is a strong *reducing agent* (Fig. 6.1), carries out its reducing function, it is converted to its *oxidized* form, l-*dehydroascorbate* (Fig. 6.2). L-Dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by *enzymes* and *glutathione*. Its IUPAC name is 2-Oxo-L-threo-hexono-1,4-lactone-2,3-enediol.

Dietary Sources

Some excellent sources of vitamin C are oranges, green peppers, watermelon, papaya, grapefruit, cantaloupe, strawberries, kiwi, mango, broccoli, tomatoes, Brussels sprouts, cauliflower, cabbage, and citrus juices or juices fortified with vitamin C. Raw and cooked leafy greens (turnip greens, spinach), red and green peppers, canned and fresh tomatoes, potatoes, winter squash, raspberries, blueberries, cranberries, and pineapple are also rich sources of vitamin C. Vitamin C is sensitive to light, air, and heat, so its advisable to get the most vitamin C by eating fruits and vegetables raw or lightly cooked.

Role in Skin Care

Potent Antioxidant

Vitamin C is a potent antioxidant. Antioxidants block some of the damage caused by free radicals, which occur naturally when our bodies transform food into energy. The build-up of free radicals over time may be largely responsible for the aging process and can contribute to the development of health conditions such as cancer, heart disease, and arthritis. Free radicals caused by exposure to sunlight and pollutants cause premature skin aging. Vitamin C being highly effective antioxidant counters these highly damaging compounds and reduces skin damage, wrinkles by soaking up harmful free radicals [1].

Augments Collagen Production

Vitamin C is an essential component for collagen synthesis [2]. Collagen is a part of normal cartilage. Cartilage is destroyed in osteoarthritis (OA), putting pressure on bones and joints. In addition, some researchers think free radicals—molecules produced by the body that can damage cells and DNA, may also be involved in the destruction of cartilage. Antioxidants such as vitamin C appear to limit the damage caused by free radicals. Collagen in the skin is malformed and skin and gums would not heal properly without adequate vitamin C. This is obvious in patients who are clinically deficient of vitamin C, a condition called scurvy. Among other problems, scurvy patients have bleeding from their gums and poorly healing wounds. Topical ascorbic acid accelerates the healing of wound as it aids in stabilizing and generation of collagen. This vitamin stimulates collagen synthesis, production of stable collagen, and triggers the production of enzymes that is necessary for the cross-linking of collagen molecule which in turn gives better tissue strength. Moreover, it helps to thicken the skin and diminishes fine lines and wrinkles.

Effective Depigmenting Agent

Ascorbic acid is an effective depigmenting agent and is known to inhibit synthesis of melanin, probably because melanin is made by our skin in response to stress, and ascorbic acid is in the first line of defense, preventing the damage before melanin synthesis can be initiated.

Skin Protection

Ascorbic acid and its derivatives promote wound healing, controls inflammation, and reduces erythema. In short, ascorbic acid is a safe, active that will bring multiple benefits to your skin, by improving blood flow, preventing future damage but also repairing past damage to skin by age and sun. The exposure to solar ultraviolet radiation increases in summer, often resulting in a higher incidence of skin lesions. Ultraviolet radiation is also a genotoxic agent responsible for skin cancer, through the formation of free radicals and DNA damage. Studies analyzing the effect of sustained exposure to a vitamin C derivative, ascorbic acid 2-phosphate (AA2P), in human dermal fibroblasts revealed that genes responsible for skin regeneration are activated in these cells [3].

It has been also demonstrated that vitamin C improve wound healing by stimulating quiescent fibroblasts to divide and by promoting their migration into the wounded area. Vitamin C could also protect the skin by increasing the capacity of fibroblasts to repair potentially mutagenic DNA lesions. Thus vitamin C contributes significantly to the maintenance of a healthy skin by promoting wound healing and by protecting cellular DNA against damage caused by oxidation. Free radicals are associated with premature skin aging, and antioxidants, such as vitamin C, are known to counter these highly damaging compounds. This new evidence suggest that, in addition to "mopping up" free radicals, vitamin C can help remove the DNA damage they form, if they get past the cell's defences. Topical Vitamin C increases the immune function of skin cells and keeps a control over acne. Vitamin C increases skin hydration by preventing moisture loss and makes aged dull and dry skin radiant, and glowing. Improvement in skin texture and skin tone, reduction in fine lines and wrinkles is easily noticeable after several days of its use. Vitamin C also protects and lessens the effects of sunburns. Ultrastructural evidence of elastic-tissue repair confirmed the clinical improvement in the skin associated with vitamin C. It also has the potential to enhance the density of dermal papillae, perhaps through the mechanism of angiogenesis. Topical vitamin C thus has therapeutical effects for partial corrections of the regressive structural changes associated with the aging process.

Boosts Effectiveness of Vitamin E

Ascorbic acid boosts the effectiveness of Vitamin E, which is important in protecting our cell's membranes. The regeneration of vitamin E from tocopheryl radical by vitamin C via the donation of a hydrogen atom has been well characterized by in vitro studies [4, 5]. Vitamin C works with other antioxidants, including vitamin E to protect the eyes against developing macular degeneration (AMD), the leading cause of legal blindness in people over 55 in the USA. The people who seem to benefit are those with advanced AMD. It is not known whether this combination of nutrients helps prevent AMD or is beneficial for people with less advanced AMD. Some studies suggest that taking vitamin C along with vitamin E may help prevent pre-eclampsia in women who are at high risk. Pre-eclampsia, characterized by high blood pressure and too much protein in the urine, is a common cause of pre-term births [6].

Treatment of Cancer

The most common form of skin cancer, basal cell carcinoma, often responds to a remarkably simple, safe, at-home treatment with vitamin C. Physicians and patients report that vitamin C, applied directly to basal cell skin cancers, causes them to scab over and drop off. Basal cell carcinomas are slow growing and it is rare for them to metastasize. This provides an opportunity for a therapeutic trial of vitamin C [7], other forms of skin cancer, such as melanoma, are faster growing and more dangerous. Successful use involves a highly concentrated vitamin C solution, directly applied to the blemish two or three times a day. Vitamin C is selectively toxic to cancer cells, but does not harm healthy skin cells. This is also the basis for high-dose intravenous vitamin therapy for cancer [8]. Ascorbate protects against cancer by increasing collagen synthesis. It has been hypothesized that ascorbate has anti-cancer action by inhibiting hyaluronidase and thereby preventing cancer spread. Ascorbate is toxic to a variety of cancer cell lines [9–11]. Extracellular concentrations as low 100–200 mM are toxic to some cell lines, but many types of malignant cells are killed only at concentrations approaching the mM range. Although ascorbate toxicity to cancer cells appears to be a result of high extracellular, rather than high intracellular concentrations, the mechanism of toxicity is unknown. Possibilities

include stimulatory effects on apoptotic pathways, accelerated pro-oxidant damage that cannot be repaired by tumor cells and increased oxidation of ascorbate at high concentrations in plasma to the unstable metabolite dehydroascorbic acid, which in turn can be toxic [12].

Vitamin C Deficiency

Many people may be mildly deficient in vitamin C, although serious deficiencies are rare in industrialized countries. Smoking cigarettes lowers the amount of vitamin C in the body, so smokers are at a higher risk of deficiency. Signs of vitamin deficiency include dry and splitting hair; gingivitis (inflammation of the gums) and bleeding gums; rough, dry, scaly skin; decreased wound-healing rate, easy bruising; nosebleeds; and a decreased ability to ward off infection. A severe form of vitamin C deficiency is known as scurvy. Low levels of vitamin C have been associated with a number of conditions, including high blood pressure, gallbladder disease, stroke, some cancers, and atherosclerosis (the build-up plaque in blood vessels that can lead to heart attack and stroke). Getting enough vitamin C from your diet (by eating lots of fruit and vegetables) may help reduce the risk of developing some of these conditions.

Vitamin C Supplements: Good, Bad, or Ugly

Vitamin C needs to be in acidic environment and in a high concentration in order to penetrate the skin. In addition, topical vitamin C is highly degradable. When exposed to air it oxidizes and its free radical soaking capabilities are muted, i.e., it becomes inert. To improve the practicability of vitamin C in skin care, scientists have been looking for its relatives with comparable or superior skin benefits. An ideal vitamin C derivative should be able to easily penetrate into skin cells and release L-ascorbic acid in amounts sufficient to boost collagen synthesis. Also, it should be more stable and less irritating than vitamin C. So far, two compounds have found their way into the broad skin care market: ascorbyl palmitate and magnesium ascorbyl phosphate. A few other highly promising derivatives are on the horizon. We need only 90 mg of vitamin C daily which can be found in a couple of orange slices. Eating a whole orange or other citrus fruit will easily give many times the amount of vitamin C we need. There is no evidence that taking vitamin C supplements or consuming huge amounts of vitamin C will have any impact on your skin. Once you have an adequate supply of vitamin C to make collagen, having a huge oversupply is not likely to lead to more collagen production. But it certainly will lead to lots more vitamin C in your urine, it is simply eliminated by kidneys. American Dietetic Association spokeswoman Dee Sandquist, RD, suggests do your best to work more fruits and vegetables into your *diet* before taking vitamin C *supplements*.

Vitamin C: Side Effects

Vitamin C should not be used by people with very sensitive skin. Topical vitamin C produces stinging sensation on areas where it is applied. Products with very low concentration of vitamin C do not produce the familiar sting. Magnesium ascorbyl phosphate and ascorbyl palmitate do not produce the characteristic sting. The practical use of vitamin C in skin care presents some difficulties due to its lack of stability. When exposed to air, vitamin C solution undergoes oxidation and becomes not only ineffective but also potentially harmful (oxidized vitamin C may increase the formation of free radicals).

Ascorbic acid interacts with several medications like aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen (tylenol), aluminum-containing antacids, barbiturates, nitrate medications for heart disease, Oral contraceptives, protease inhibitors, tetracycline, warfarin (coumadin), and thus should be taken only after consulting a doctor.

Conclusion

Vitamin C is widely used by the people, probably with little harm. Vitamin C protects the skin by promoting fibroblast proliferation, migration and replication-associated base excision repair of potentially mutagenic DNA lesions. Genome-wide effects of vitamin C on gene expression in primary dermal fibroblasts helps to gain new insights in the participation of vitamin C in important processes in human skin, such as wound healing or the repair of oxidative DNA lesions in skin cells. In summary, while the activation of specific signalling pathways remains to be elucidated, recent researches reveal that vitamin C repletion in skin cells is required for efficient wound healing and replication-associated repair of potentially mutagenic products of DNA oxidation. Vitamin C is thus being effectively used for skin care and is recognized as a major component in cosmetics used for skin treatment.

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Chapter 7
Omega 3 Fatty Acids in Psoriasis

Aman B. Upaganlawar and Sachin L. Badole

Key Points

- Certain omega-3 fatty acids are essential fatty acids and present in various natural sources. Important omega-3 fatty acids include α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid.
- Various research studies report the use of omega-3 fatty acids in the management of inflammation, heart diseases, skin disorders, cancer, and arthritis.
- Research also shows the effect of omega-3 fatty acids in the treatment of psoriasis which is one of the common skin disorders. So, in this chapter we have reported in brief the use of different omega-3 fatty acids in the management of psoriasis.

Keywords Omega-3 • Psoriasis • Skin disorders • Arthritis • Fish oil

Abbreviations

AA	Arachidonic acid
ALA	α-Linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
PUFA	Polyunsaturated fatty acids

Introduction

Omega-3 fatty acids are considered as essential fatty acids. They are necessary and very important for maintaining human health but the body cannot make them and one has to get them through food source externally. In nutrition, important omega-3 fatty acids include ALA, EPA, and DHA. Omega-3

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fatty acids are present in various sources. Fish, plant, and nut oils are the primary dietary source of omega-3 fatty acids. EPA and DHA are found in cold-water fish such as salmon, mackerel, halibut, sardines, tuna, and herring. ALA is found in flaxseeds, flaxseed oil, canola (rapeseed) oil, soybeans, soybean oil, pumpkin seeds, pumpkin seed oil, purslane, perilla seed oil, walnuts, and walnut oil. Other sources of omega-3 fatty acids include sea life such as krill and algae. Both EPA and DHA can be taken in the form of fish oil capsules. Flaxseed, flaxseed oil, fish, and krill oils should be kept refrigerated. Whole flaxseeds must be ground within 24 h of use, so the ingredients stay active. Flaxseeds are also available in ground form in a special mylar package so that the components in the flaxseeds stay active [1].

Omega-3 fatty acids are also known as polyunsaturated fatty acids (PUFAs), it plays a crucial role in brain function as well as normal growth and development. They have also become popular because they may reduce the risk of heart disease. The American Heart Association recommends eating fish (particularly fatty fish such as mackerel, lake trout, herring, sardines, albacore tuna, and salmon) at least two times a week. Research shows that omega-3 fatty acids reduce inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. Omega-3 fatty acids are highly concentrated in the brain and appear to be important for cognitive (brain memory and performance) and behavioral function. In fact, infants who do not get enough omega-3 fatty acids from their mothers during pregnancy are at risk for developing vision and nerve problems. Symptoms of omega-3 fatty acid deficiency include fatigue, poor memory, dry skin, heart problems, mood swings or depression, and poor circulation [2].

The supplementation of fish oil is based on the amount of EPA and DHA, not on the total amount of fish oil. A common amount of omega-3 fatty acids in fish oil capsules is 0.18 g (180 mg) of EPA and 0.12 g (120 mg) of DHA. Five grams of fish oil contains approximately 0.17–0.56 g (170–560 mg) of EPA and 0.072–0.31 g (72–310 mg) of DHA. Fish oils contain approximately 9 cal per gram of oil.

Psoriasis

Psoriasis is a fairly common skin disease which is regarded as immunologically based disease which combines dermal inflammation with secondary epidermal hyperplasia. It is characterized by thick, silvery white scales surrounded by a red, inflamed border. Psoriasis is accompanied by high concentrations of arachidonic acid in the plaques and profound changes in the metabolism of eicosanoids leading to an increase in proinflammatory agents. It is known that EPA counteracts the formation of these proinflammatory agents and some studies have shown that oral supplementation with fish oils benefits psoriasis patients. Increased concentrations of free AA and its proinflammatory metabolites have been observed in psoriatic lesions. Replacement of arachidonic acid by alternative precursor PUFA, especially EPA, which can be metabolized via the same enzymatic pathways as AA, might be a therapeutic option in psoriasis [3].

Omega 3 Fatty Acid in Psoriasis

The use of omega 3 fatty acids was studied by various authors. Three studies were carried out to evaluate the efficacy and safety fish oil derived lipid emulsion administered intravenously on different forms of psoriasis. Patients received daily infusions of an *n*-3 fatty acid-based lipid emulsion (Omegaven) or a conventional *n*-6 lipid emulsion (Lipoven) 50 ml for 10 days in different time and dose regimens. EPA and AA-derived neutrophil 5-lipoxygenase (LO) products, thromboxane (TX) B2/B3, PAF and plasma-free fatty acids were investigated. Treatment with *n*-3 fatty acids resulted in

a considerably higher response rate than infusion of *n*-6 lipids. A more than tenfold increase in neutrophil EPA-derived 5-LO product formation was noted in the *n*-3 group, accompanied by a rapid increase in plasma-free EPA within the first days. This study showed that administration of *n*-3-fatty acid intravenously causes reduction of psoriasis, which may be related to changes in inflammatory eicosanoid generation [4].

Wolter [1], carried out four uncontrolled crossover studies to compare the consumption of fish or oil fish in the cure of psoriasis. It was found that consumption of 170 g of white fish or oily fish for 4 weeks showed increase in the plasma EPA concentration; however, the oily fish group showed only modest significant clinical improvement [1]. Other four uncontrolled studies supplementation with EPA/DHA daily with intake ranging from 2 to 12 g omega-3 fatty acids also showed improvement in psoriasis severity. However, the results was found to be less promising in randomized controlled trial, with only one of the four studies reporting positive results with daily omega-3 supplementation.

A study was designed to evaluate the efficacy of a nutritional complement rich in omega-3 fatty acids in patients with mild or moderate plaque psoriasis. Thirty patients were selected, control group containing 15 patients and were treated with topical tacalcitol. Other 15 patients were given topical tacalcitol and two capsules of Oravex® daily (composition: 280 mg of eicosapentaenoic acid, 40 mg of docosahexaenoic acid, 50 mg of thyme extract, 50 mg of olive leaf extract, 20 mg of green tea extract, 7.5 mg of zinc, 27.5 μg of selenium per capsule, TheaLaboratories, Barcelona, Spain). Baseline, intermediate (week 4), and final (week 8) visits, were held over an 8-week period. The main efficacy end points were the Psoriasis Area and Severity Index (PASI), Nail Psoriasis Severity Index (NAPSI), and Dermatological Life Quality Index (DLQI). A clear and significant improvement was observed in all the efficacy end points in both groups between the baseline visit and the end visit. This improvement was significantly greater in the group treated additionally with Oravex® than in the control group. This study showed that supplementary treatment with omega-3 fatty acids complements topical treatment in psoriasis, and makes a significant contribution to reducing PASI and NAPSI and improving DLQI and reduced scalp lesion and pruritus, erythema, scaling, and infiltration of the treated areas [3].

One study from Austria, reported that intravenous infusions of a fish oil emulsion is quite effective in ameliorating the symptoms of chronic plaque-type psoriasis. This multicenter trial involved 54 men and 29 women between the ages of 18 and 80 years who had been hospitalized with severe psoriasis. The patients were divided into two groups. Group 1 composed of 43 patients and received fish oil emulsion (100 ml of a 10% emulsion infused over a period of 90 min) twice daily. Group 2 composed of 40 patients and received twice daily infusions of a placebo emulsion based on linoleic acid. The severity of the psoriasis was assessed by physicians on days 0, 4, 7, 11, and 15 of the 2-week trial. Sixteen of the 43 patients (37%) receiving fish oil showed at least a 50% improvement in their condition at the end of the trial as compared to 9 out of 40 patients (23%) in the placebo group. The researchers showed that intravenous administration of a fish oil emulsion is safe and effective in the treatment of chronic plaque-type psoriasis [4].

Researchers from the Shiga University of Medical Science reported that a combination of EPA and etretinate at a lower dose (0.3–0.5 mg/kg per day) works as well as the pure, high-dose and has significantly fewer side effects. Etretinate is a powerful drug used to treat psoriasis but it can cause serious adverse effects when used in the regularly prescribed dose of about 1 mg/kg per day. Forty psoriasis patients were selected for the trial. The patients were randomly assigned to receive 20 mg etretinate capsule daily or 20 mg etretinate plus 1,800 mg of EPA ethyl ester (in capsules). After 12 weeks 45% of the patients from the combination group showed excellent improvement (greater than 75%) as compared to 15% in the pure etretinate group. Adverse reactions such as inflammation of the lips, dry mouth and eyes, and scaling were observed in both groups, but were mild and tolerable. This study showed that the combination regimen of eicosapentaenoic acid and etretinate was effective in the treatment of psoriasis without marked adverse reactions [5]. Psoriasis is a relatively common skin disorder that affects between 1 and 2% of the population. Itching, scaling, and erythema

(abnormal flushing of the skin) are common features. Abnormal levels of leukotrienes (metabolites of arachidonic acid) are believed to be involved in the development and progression of the disorder. It is well-established that fish oils suppress the formation of leukotriene B4.

Researchers at the University of Buenos Aires Faculty of Medicine investigated the effect of topical application of fish oil on skin areas affected by psoriasis. In their clinical trial, 25 patients having psoriasis were randomly assigned and applied either fish oil or liquid paraffin to their psoriatic plaques and left them covered for 6 h overnight under an occlusive dressing. The treatment was repeated daily for a 4-week period. Fish oil proved highly effective in reducing scaling, plaque thickness, and erythema. Itching was not relieved by the fish oil treatment. The 4-week liquid paraffin treatment was also effective in reducing erythema, but was significantly lower to the fish oil treatment in reducing scaling and had no significant effect on itching or plaque thickness. This study concluded that fish oil treatment being superior to the paraffin treatment [6]. EPA, a major component of fish oils, is known to dampen the adverse effects of leukotrienes and has been proven to have significant anti-inflammatory effects. A clinical trial was designed by medical doctors at the Royal Hallamshire Hospital to evaluate the effects of oral supplementation with fish oils in the treatment of psoriasis. Twenty-eight patients were selected in the trial and were diagnosed with chronic psoriasis. They were randomized into two groups with one group receiving ten fish oil capsules (containing 1.8 g of EPA) and the other group receiving ten olive oil capsules every day for the duration of the 12-week trial. After 8 weeks of treatment there was a significant reduction in itching, erythema, and scaling in the fish oil group and a trend toward a decrease in the surface area of skin affected by the disease [7].

A recent German study highlighted the anti-inflammatory benefits of omega-3 fatty acids. These oils were found to reduce inflammation in 20 patients suffering from psoriasis. 10% of psoriasis patients also develop psoriatic arthritis. Studies showed that 1–3 g daily use of omega-3 fish oils can help both conditions.

EPA also proved beneficial when nine psoriasis patients were treated with 3.6 g EPA for up to a year [8]. Recently, a double-blind, placebo-controlled study was carried out on the effects of the addition of a maximum of 6 g/day of omega-3 fatty acids containing eicosapentaenoic acid (EPA) ethyl esters to patients with lithium-induced bipolar disorder [9]. Two patients from this study reported a spontaneous reduction of psoriasis after taking omega-3 fatty acids [10].

Summary Points

- Certain omega-3 fatty acids are essential fatty acids and it is important for maintaining human health.
- Omega-3 fatty acids reduced psoriasis severity.
- Intravenous administration of a fish oil emulsion is safe and effective in the treatment of chronic plaque-type psoriasis.
- Topical application of fish oil on skin areas affected by psoriasis.
- Fish oil proved highly effective in reducing scaling, plaque thickness, and erythema.

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Chapter 8
Arginine Derived Nitric Oxide: Key to Healthy Skin

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Key Points

- Nitric oxide synthases (NOS) metabolize arginine and molecular oxygen to citrulline and nitric oxide.
- Arginine is a nonessential α-amino acid.
- Arginine is manufactured by the human body and does not need to be obtained directly through the diet.
- NO's role in the progression of skin cancer is continually evolving.
- Arginine derived NO is important when one is concerned with overall healthy skin.

Keywords Nitric oxide • Arginine • Skin care

Introduction

The gaseous free radical nitric oxide (NO), once considered a noxious byproduct of combustion, has now been shown to be an endogenous messenger that plays various physiological and pathophysiological roles in nearly every organ system. The tremendous impact NO has had on research in biology and medicine was reflected by the nomination of NO as the "molecule of the year 1992," by the prestigious journal "Science" [1] and in 1998, three American researchers received the Nobel Prize for Medicine for their work with NO. Nitric oxide is generated in biologic tissues by specific nitric oxide synthases (NOS) that metabolize arginine and molecular oxygen to citrulline and nitric oxide. Besides its function as a diffusible messenger in the vasculature and in neurons, nitric oxide also plays a key role in innate immunity and inflammation. Recent progress has allowed the identification of the nitric oxide pathway in several cell types that reside in the skin, including keratinocytes, melanocytes,

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Langerhans cells, fibroblasts, and endothelial cells. Despite role of NO in various normal physiological processes of skin, it is also involved in pathological conditions related to skin which include psoriasis and other immune-mediated skin diseases as well as skin cancer. Arginine is the immediate precursor of NO and Arginine-Derived Nitric Oxide (ADNO) has been shown to provide a wide range of life-enhancing benefits, including repairing and preventing damage in blood vessels and stimulating regeneration in the skin. Arginine intake leads to a healthier, smoother, tighter, and wrinkle-free skin, and thus it has been named as "magic anti-aging bullet."

L-Arginine: Semi-Essential Amino Acid

Arginine, an amino acid, which is abundant in protamines and histones (both proteins associated with nucleic acids) was first isolated from a lupin seedling extract in 1886 by the Swiss chemist Ernst Schultze. Amino acids are generally classified as essential or nonessential. Essential amino acids are those that the body cannot synthesize; a steady supply of amino acids must be provided through the diet. The body can manufacture nonessential amino acids, so an exogenous supply of them in the diet is unnecessary. Arginine is a unique amino acid, generally referred to as semi-essential. This noncommittal label indicates that although the body can manufacture arginine, at times it does so in an amount that is insufficient to meet physiological needs and dietary supplementation may be required. This often occurs during the periods of growth, illness, and metabolic stress. In other words, arginine is a nonessential amino acid during the periods of maintenance, but is an essential amino acid during the periods of growth and healing. In addition, newborns are not able to make their own supply of this substance, so arginine is considered essential in the first months of life.

Structure

Arginine (abbreviated as Arg or R) is a α-amino acid. The L-form is one of the 20 most common natural amino acids. The amino acid side chain of arginine consists of a 3-carbon aliphatic straight chain, the distal end of which is capped by a complex guanidinium group (Fig. 8.1). The guanidinium group is positively charged in neutral, acidic, and even most basic environments, and thus imparts basic chemical properties to arginine. Its IUPAC name is (*S*)-2-Amino-5-guanidinopentanoic acid.

Biosynthesis of NO

NO is synthesized from the guanidino nitrogen in the L-arginine molecule, converting L-arginine to NO and L-citrulline in a two-step reaction that requires cofactors including FAD, FMN, NADPH, calmodulin (CaM), and tetrahydrobiopterin (BH_4) (Fig. 8.2) [2, 3] NOS isoforms are classified as

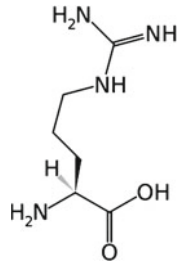

Fig. 8.1 L-Arginine

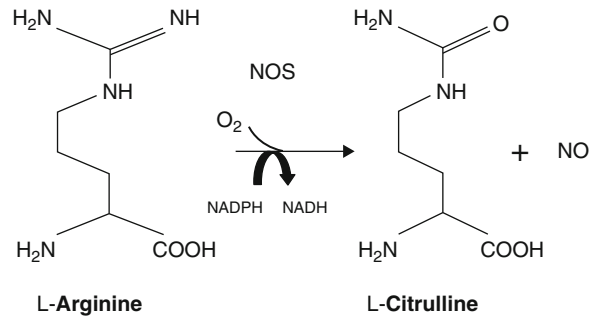

Fig. 8.2 Biosynthesis of NO from L-Arginine. NO is synthesized from the guanidino nitrogen in the L-arginine molecule by nitric oxide synthase (NOS) enzyme-converting L-arginine to NO and L-citrulline, requiring cofactors including FAD, FMN, NADPH, Calmodulin, and Tetrahydrobiopterin (BH4)

constitutive neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) nitric oxide synthase. Drs. Marie-madeleine Cals-Grierson and Anthony Ormerod report in the June 2004 issue of "Nitric Oxide" that skin cells contain three different types of nitric oxide synthase. Most cells in the skin contain inducible nitric oxide synthase. The cells that maintain skin's elasticity contain endothelial nitric oxide synthase. Cells making up the majority of the epidermis release neuronal nitric oxide synthase. Stem cells in the skin help maintain and repair damage to the skin's outer layers, the epidermis and the dermis. In the July 2010 issue of "Archives of Dermatological Research," researchers at Polytechnic University of Marche in Ancoma, Italy, reported the presence of three types of nitric oxide synthase enzymes in skin stem cells (Fig. 8.2)

Dietary Sources of Arginine

Arginine, being a nonessential amino acid, can be manufactured by the human body and does not need to be obtained directly through the diet. The biosynthetic pathway, however, does not produce sufficient arginine, and some must still be consumed through diet. Individuals who have poor nutrition or certain physical conditions may be advised to increase their intake of foods containing arginine. Arginine is found in a wide variety of foods, including

- Animal sources: dairy products (e.g., cottage cheese, ricotta, milk, yogurt, whey protein drinks), beef, pork (e.g., bacon, ham), gelatin, poultry (e.g. chicken and turkey light meat), wild game (e.g., pheasant, quail), seafood (e.g., halibut, lobster, salmon, shrimp, snails, tuna)
- Plant sources: wheat germ and flour, buckwheat, granola, oatmeal, peanuts, nuts (coconut, pecans, cashews, walnuts, almonds, Brazil nuts, hazelnuts, pinenuts), seeds (pumpkin, sesame, sunflower), chick peas, cooked soybeans, chocolate, *Phalaris canariensis* (canaryseed)

L-Arginine-Derived Nitric Oxide (ADNO) in Skin

The body needs arginine to produce nitric oxide, a chemical that causes blood vessel relaxation (vasodilation). Preliminary studies indicate that arginine may be useful in the treatment of angina, atherosclerosis, coronary artery disease, intermittent claudication, erectile dysfunction, female impotence, migraine, and other conditions that are linked to reduced blood flow throughout the body. ADNO (L-Arginine derived Nitric Oxide) is a multifaceted molecular marvel that has been shown to provide a wide range of life-enhancing benefits, including repairing and preventing damage in blood vessels and stimulating regeneration in the skin as well as the heart, thymus gland, liver, kidneys, and

other internal organs. L-Arginine derived nitric oxide promotes the production of collagen. Nitric oxide is said to dilate the capillaries and increases healthy blood circulation to the skin. The enhanced circulation helps to bring a flood of nutrients saturating the malnourished skin with new life. It improves skin texture, elasticity, thickness, stimulating cell regeneration, and restores moisture. It creates tighter, smoother skin, reducing wrinkles as well as dark circles under the eyes. Arginine is used for speeding up wound healing and increasing blood flow to cold hands and feet, especially in people with diabetes. When the body is not functioning at optimum, L-arginine production suffers. As it regulates blood pressure, reduces plaque, lowers cholesterol, and prevents blood clots, it is not unusual for cardiovascular problems to follow. L-Arginine may help circulatory problems and the resultant dry skin. Large concentrations of arginine are found in the skin, and this amino acid plays a key role in the health of all the body's connective tissues, particularly the muscles. Arginine helps the body process both creatine, a natural substance that helps build muscle mass, and nitrogen, a chemical needed for muscle metabolism. Laboratory research suggests that arginine may help reduce body fat and speed up weight loss. Arginine has also been shown to help heal and repair damaged tissues, and thus may be beneficial to both athletes and those suffering from arthritis.

NO and Skin Biology

Practically every cutaneous relevant cell type expresses some isoform of NOS and is therefore able to generate and release NO for a broad spectrum of physiologic processes. Keratinocytes, the major constituent of the epidermis, express both constitutive and inducible NOS and produce NO and hydrogen peroxide in response to inflammatory stimuli. This may act as one of the cardinal broad protective mechanisms of the skin, as the epidermis is constantly exposed to foreign matter and organisms. In addition, finely regulated responses are also exhibited by NOS species, such as in wound healing. Fibroblasts, found in the dermis, regulate the structural framework of the skin by synthesizing extracellular matrix, collagen, and fibrin, while orchestrating the complex steps of wound healing. Fibroblasts demonstrate NOS expression, but this expression is inconsistent across different cells, possibly dependent on cell maturation [4]. Additionally, eccrine glands express eNOS, melanocytes have shown eNOS and iNOS expression [5]. Due to its widespread distribution, NO is able to participate in basic physiological roles such as establishing and maintaining circulation, forming a protective barrier against microorganisms, and UV-induced melanogenesis [6, 7] and development of erythema [8].

It has become apparent that NO is also produced by other cell types residing in the skin. Expression of both the constitutive and the inducible pathway has been demonstrated in dermal fibroblasts and endothelial cells [9]. It will be of interest therefore to further elucidate the physiologic and/or pathophysiologic roles of NO production in these cells with particular reference to cutaneous inflammatory and immune responses. It has been discovered that NO is produced in Langerhans cells of human skin [10]. From these studies it has been concluded that NO may affect Langerhans cell functions such as microbicidal activity, antigen presentation, and cytotoxicity, and may also affect adjacent keratinocytes and melanocytes. Dermal papilla cells play an important role in hair growth and have been shown to produce NO after exposure to bacterial endotoxin [11]. Modulation of Ca^2-activated K channels by NO has been identified in these cells, although the biologic function of this particular activity in human skin is not yet known.

NO is known to have role in normal physiological as well as pathophysiological processes of skin. Biological role of NO in the regulation of vascular homeostasis [12, 13] is well known where the NO produced from endothelial cells causes vascular smooth muscle relaxation. NO also has an important role to play in immunological activities involved in skin which acts as a significant barrier against many pathogens and thus contributing in first line of defense [14]. Moreover, recent studies emphasize the role of NO in ultraviolet-induced melanogenesis [15] apart from its significant role in wound repairs and skin cancers.

NO in Normal Physiological Processes of Skin

Vascular Homeostasis

Endothelial cells produce via eNOS activity small pulses of NO resulting in a basal level of vascular smooth muscle relaxation. In addition to the regulation of systemic blood pressure, however, NO has recently been reported to control the local blood flow to specific vascular beds, in the brain, heart, lung, gastrointestinal tract, and the skin [16, 17]. A local deficiency of NO therefore could cause vasospasm in selected organs. Nitric oxide helps control the blood flow to the skin thus restores some of the youthful vibrancy to the skin. Reduced blood flow in the area under the eyes results in dark circles. A study conducted by AGI Dermatics in New York published in "Nitric Oxide" in August 2006 shows increasing the release of nitric oxide in blood vessels under the eyes improves blood flow in those vessels and decreases the appearance of dark circles under the eyes. The microvascular endothelial cells have been shown to release NO in response to the vasodilatory neuropeptides calcitonin gene-related peptide and substance P, suggesting that NO provides a molecular link between the nervous system and the skin [18]. Eczema flare-ups often associated with dysfunction of the nervous system; and, some hormonal imbalances (hypothyroidism being a very common example) are so often associated with dry, itchy, inflamed skin.

Immune System

The skin is a site of significant immunologic activity because of its constant exposure to environmental challenges such as physical stress, trauma, chemical irritants, and infectious micro-organisms. In consequence, a complex set of immune reactions can be observed in the skin, providing an appropriate defense under a variety of circumstances. The first indication that NO might be an integral part of the immune response in human skin came from in vitro experiments showing that inflammatory stimuli induce keratinocytes to produce NO as well as hydrogen peroxide [19]. Numerous studies have demonstrated that NO synthesis is a necessary component of nonspecific defense mechanisms for several pathogens, including bacteria, viruses, parasites, and fungi [20]. In particular, NO synthesized at high concentrations eliminates intracellular pathogens, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*, *Leishmania species*, *Trypanosoma cruzi*, and *Plasmodium falciparum*, and is thought to block viral replication. Initially, this type of NO-mediated cytotoxicity was presumed to be restricted to macrophages; however, it has now become apparent that other cell types that express iNOS, such as keratinocytes and endothelial cells, may also contribute to this innate immunity. Specifically, the skin, acting as immunologic barrier, appears to be well equipped for this first line of defense. NO is able to regulate skin flora through this non-NOS-dependent synthetic pathway. Nitrite is a known constituent of both blood and sweat, and in the case of sweat, the acidity of the skin surface allows for the reduction of nitrite to NO [21]. The acidified nitrite functions as a moat, so to speak, of protective antimicrobial NO, preventing pathogen access to the body.

Melanogenesis: Reponses to Ultraviolet Irradiation

Melanin pigments produced in human melanocytes are classified into two categories; black coloured eumelanin and reddish-yellow pheomelanin. Nitric oxide (NO) is melanogenesis-stimulating factor. The ratio of eumelanin and pheomelanin increased significantly with the addition of NO and thus contribute to UV-induced hyperpigmentation by enhancing eumelanogenesis [22]. Within the epidermal-melanin unit, melanocytes synthesize and transfer melanin to the surrounding keratinocytes. Keratinocytes produce paracrine factors that affect melanocyte proliferation, dendricity, and melanin

synthesis. It has been demonstrated that normal human keratinocytes secrete nitric oxide (NO) in response to UVA and UVB radiation and this involves constitutive isoform of keratinocyte NO synthase [23]. Melanogenic effect of NO by keratinocytes in response to UV radiation was investigated using melanocyte and keratinocyte cocultures. Conditioned media from UV-exposed keratinocytes stimulate tyrosinase activity of melanocytes. This effect is reversed by NO scavengers, suggesting an important role for NO in UV-induced melanogenesis. These observations suggest that NO plays an important role in the paracrine mediation of UV-induced melanogenesis [24].

Several melanogenic factors released from keratinocytes and other cells surrounding melanocytes in the skin following UV radiation are reported to up-regulate tyrosinase gene expression through a different pathway, but most regulate tyrosinase via microphthalmia-associated transcription factor (MITF). NO donors increase tyrosinase activity and melanin synthesis in human melanocytes. This effect is positively correlated with an increased amount of both tyrosinase and tyrosinase-related protein 1 (TRP-1), two enzymes involved in melanogenesis [23, 24]. Recent research provide exciting new evidence that NO can enhance melanogenesis in alpaca skin melanocytes by stimulating MITF phosphorylation [25].

Ultraviolet irradiation is one of the major assailants to the skin and it is constantly exposed to this stressor capable of inducing oxidative cellular damage. As one of the skin's primary defense mechanisms, keratinocytes produce sustained concentrations of NO upon exposure to both UVA and UVB irradiation, which only declines after 3 days and coincides with the time course of sun-induced erythema. In this regard, NO may quench free radical damage that can result from UV radiation exposure if generated photoproducts are allowed to propagate unhindered. This proposed role of NO is supported by reports that endothelial cells are protected from UVA-induced apoptosis by NO [21] and that UV-induced lesions in cutaneous lupus erythematosus demonstrate reduced expression of iNOS. More recently, a non-enzymatic pathway of NO production was elucidated, where NO is derived from biologically relevant NO-related products in the human epidermis, superficial vascular dermis and sweat. In the setting of acute UVA exposure, these products are quickly mobilized within 30 min to generate NO, resulting in keratinocyte cytoprotection from ultraviolet radiation-induced apoptosis. These studies suggests that intake of external sources of NO precursors, such as, arginine may influence the innate and acute cutaneous response to ultraviolet radiation [21]

NO in Pathophysiological Processes of Skin

Skin Cancer

Our understanding of NO's role in the progression of skin cancer is continually evolving. Over the past two decades, its precise role in tumor pathophysiology has been a matter of great debate. There is extensive evidence that tumor expressed NOS and subsequent NO production can be both pro- and anti-carcinogenic, depending on the concentration of NO produced. NO may function in an anti-carcinogenic role via the induced apoptosis of mutated cells or through modulation of growth responses and gene expression patterns. Dong et al. [25] demonstrated the potential anti-carcinogenic features seen with increased expression of NOS2. In a comparative study between several nonmetastatic and highly metastatic melanoma clones, it was demonstrated that the nonmetastatic clones expressed much higher levels of endogenous NO. Since then, a multitude of projects designed to generate high intratumoral levels of NO have been pursued, including the use of NO donor drugs and the transfection of a functional NOS2 gene [26]. Additionally, NO is able to further exert its anti-carcinogenic effects through the regulation of MMP levels [27] and by increasing the release of cytochrome c, which activates caspases and induces the release of massive quantities of cellular calcium both of which result in apoptosis.

It is clear that NO is involved in a multitude of signaling pathways that vary based on cell type and the level of NO produced. Therefore, it is not surprising that a myriad of effects have been observed

following the modification of NO levels in different tumor cell lines. It appears that at modest concentrations, the effects of NO could be characterized as pro-malignant, whereas, at highly elevated concentrations, NO acts as a potent anticancer agent, promoting apoptosis and inhibiting metastasis.

Wound Repair

Wound healing of the skin represents a highly ordered process of important tissue movements that aims for a rapid closure of the wound site and a subsequent regeneration of the injured tissue. The factors ensuring the intercellular communication during repair are only known in part. However, although protein-type mediators are well-established players in this process, it has become evident that the diffusible, gaseous molecule nitric oxide (NO) participates in the orchestration of wound healing. NO also accelerates wound healing which is greatly needed in the case of major burns or injuries. The role of wound-derived NO critically influences macrophage, fibroblast, and keratinocyte behaviour within the intercellular communication network during repair [28, 29]. NO synthesis in human fibroblasts has been shown to attenuate the pathophysiologic sequelae in wound healing early during the inflammatory stages and later during stages of proliferation and tissue remodeling [30]. Additionally, reduced NO synthesis has been demonstrated in fibroblasts of hypertrophic scar tissue [31]. These findings suggest that changes in NO levels can lead to significant alterations of cellular responses to wounding. Several cytokines and growth factors that are known regulators of iNOS expression control critical aspects of wound healing, suggesting a complex network of autocrine and paracrine cellular responses. The inducible isoform (iNOS) is synthesized in the early phase of wound healing by inflammatory cells, mainly macrophages. However many cells participate in NO synthesis during the proliferative phase after wounding. NO released through iNOS regulates collagen formation, cell proliferation, and wound contraction in distinct ways [32].

Mode of Action of NO in Skin

In multicellular organisms tissue homeostasis is maintained through a delicate balance between cell proliferation and cell death. Arrest of cell division is a prerequisite for cells to enter a program of terminal differentiation. Mitogenesis and cytostasis can be induced by diverse intrinsic and extrinsic stimuli, and convincing evidence suggests that alterations in this process contribute to the pathogenesis of several human skin diseases, including psoriasis and other hyperproliferative diseases [18]. NO could mediate cessation of growth and synchronize commitment for differentiation in epidermal keratinocytes. In human skin, NO has important role to play in normal development as well as host responses to infection and tissue injury, which is orchestrated through an intricate and ordered series of interactions between cells resident to the skin as well as cytokines, growth factors, and extracellular matrix proteins. Pertubations in these cell–cell and cell–matrix interactions have been shown to result in a loss of skin integrity, as characterized, for example, during wound healing processes. Reduced blood flow causes dead and dry skin, NO dilates the blood vessels, increases the blood flow, and results in rejuvenated healthier skin [16, 17].

Arginine–Skin Connection: Liver, Hormones, and Blood Sugar Levels

Arginine is known to improve insulin sensitivity. One of the main physiological problems in type 2 diabetes is that the body's cells become increasingly resistant to the action of insulin. This is the hormone that helps cells take in glucose (the "fuel" the body needs to stay alive) from the blood. If insulin

resistance develops, glucose is not transported into the cells as efficiently as it should be, and it builds up in the blood. That is why people with diabetes are often said to have high blood sugar—and it must be controlled. Research study showed that arginine supplementation may help people with type 2 diabetes utilize glucose more efficiently by improving their insulin sensitivity [33]. Regulation of blood sugar levels is essential for healthy skin. Both high and low blood sugar levels dry the skin, and high blood sugar can also lead to the cracked, split skin sometimes seen in diabetics. People with arginine deficiency show fat build ups in their liver. Arginine helps eliminate toxins. Poor liver function, results in a buildup of toxins which then get excreted through the skin causing skin dryness and inflammation. When liver function is poor, metabolism slows down which causes dry skin, but as we gain weight from improper metabolism of fats our circulation slows down and the skin dries even further.

L-Arginine Deficiency

Arginine is a nonessential amino acid during the periods of maintenance, but is an essential amino acid during the periods of growth and healing. L-Arginine helps the body get rid of waste and synthesize proteins. When the body is not functioning at optimum, L-arginine production suffers and arginine deficiency occurs in those fighting infection, severe burns, undergoing dialysis, experiencing rapid growth, or those with trouble processing urea. Certain conditions such as protein deficiencies and malnutrition also affect ability to produce L-arginine. People with L-arginine deficiency may have fat build ups in their liver. Its deficiency is accompanied by symptoms such as alopecia (hair loss), skin rashes, poor wound healing, and other skin problems.

L-Arginine Supplements: Side Effects?

L-arginine supplements can cause some side effects such as abdominal pain, bloating, diarrhea, gout, blood abnormalities, allergies, airway inflammation, worsening of asthma, and low blood pressure. L-arginine should not be taken by pregnant or nursing women as it stimulates growth hormone in young children. Persons having herpes should also avoid taking arginine as it can stimulate the herpes infection. People taking medication for high blood pressure, impotence, migraines, or any other problem, or having a history of kidney or liver disease, should check with their doctors before taking L-arginine supplements. Long-term use of arginine supplements is not recommended, as it may result in thickening and coarsening of the skin and/or nitrogen imbalance in the body. Arginine has also been shown to increase or decrease the effects of certain medications, including lysine, NSAIDS (non-steroidal anti-inflammatories), ACE inhibitors, or potassium sparing diuretics. People with herpes or schizophrenia should avoid arginine supplementation altogether as it may aggravate these conditions.

Conclusion

NO responses known from other biologic systems, such as vasodilation, neurotransmission, as well as cytotoxicity and immunoregulation, are also of significant importance in human skin. Characterization of the role of NO in cutaneous disease will not only provide an important addition to our understanding of cutaneous biology but also is likely to be the foundation for the development of new therapeutic approaches that can modify, arrest, or reverse the course of human skin disease. The demonstrable and

potential roles of nitric oxide in skin disease pathogenesis and treatment has led to the use of arginine as a source of NO in diet. More recent modalities that have been evaluated and developed include continuous horizontal-flow delivery of gaseous NO, and its local skin effects like quick healing wounds [34] but arginine-derived NO is important when one is concerned with overall healthy skin.

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Chapter 9
Taurine (2-Aminoethanesulfonic Acid): Useful in Skin Diseases

Sachin L. Badole and Swapnil M. Chaudhari

Key Points

- Taurine is an organic acid widely distributed in animal tissues.
- It is used in skin ulcers, psoriasis, atopic eczema, etc.
- Taurine as a new therapeutic option in inflammatory acne.

Keywords Taurine • Skin cancer • Psoriasis • Atopic eczema

Introduction

Taurine, or 2-aminoethanesulfonic acid, is an organic acid widely distributed in animal tissues. It is a major constituent of bile and can be found in the large intestine and accounts for approximately 0.1% of total human body weight. Taurine has many fundamental biological roles such as conjugation of bile acids, antioxidation, osmoregulation, membrane stabilization, and modulation of calcium signaling. Taurine is used clinically in the treatment of cardiovascular diseases, hypercholesterolemia, seizure disorders, ocular disorders, diabetes, alzheimer's disease, hepatic disorders, cystic fibrosis, alcoholism, and various skin diseases.

Taurine (2-Aminoethanesulfonic Acid)

Taurine is named after the Latin *taurus* (a cognate of the Greek $\tau\alpha\,\rho o\varsigma$) which means bull or ox, as it was first isolated from ox bile in 1827 by German scientists Friedrich Tiedemann and Leopold Gmelin. Taurine (2-aminoethanesulfonic acid) is different from other amino acids in that it contains a sulfonic acid group in place of the carboxylic acid group, and it is not incorporated into proteins. Therefore, it is not an amino acid in the true sense of the word. It is synthesized in human liver tissue and in pancreas from cysteine and methionine via three known pathways, all of which require pyridoxal-5′-phosphate, the active coenzyme form of vitamin B6. The highest concentrations of taurine are found

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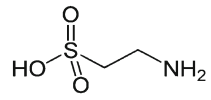
in the neutrophil and the retina, and the largest pools of taurine are found in skeletal and cardiac muscles. Taurine excretion is via the urine or in the bile as bile salts [1]. Due to its beneficial effects on skin, it has become a popular natural ingredient in skin care cosmetics.

Structure



2-aminoethanesulfonic acid (Taurine)

Taurine is a derivative of cysteine, an amino acid which contains a sulfhydryl group. Taurine is one of the few known naturally occurring sulfonic acids. In the strict sense, it is not an amino acid, as it lacks a carboxyl group, but it is often called one, even in scientific literature. It does contain a sulfonate group and may be called an amino sulfonic acid. Small polypeptides have been identified which contain taurine, but to date no aminoacyl tRNA synthetase has been identified as specifically recognizing taurine and capable of incorporating it into a tRNA [2].

Dietary Sources

Taurine occurs naturally in food, especially in seafood and meat. Some excellent sources of taurine are beef meat, chicken meat, turkey meat, lamb meat, pork, seafood like tuna, white fish, mussels, oysters, cod, clams. Apart from the above sources taurine is also present in pasteurized milk, cheese, yogurt, fruit, vegetables, seeds, nuts, grain, beans peanuts, cereals [3]. The mean daily intake from omnivore diets is 58 mg (range from 9 to 372 mg) and to be low or negligible from a strict vegetarian diet.

Application of Taurine in Skin Diseases

Acne

Acne vulgaris (or cystic acne) is a common human skin disease, characterized by areas of skin with seborrhea, comedones papules (pinheads), pustules (pimples), Nodules, and possibly scarring. Acne affects mostly skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back. Severe acne is inflammatory, but acne can also manifest in noninflammatory forms. The lesions are caused by changes in pilosebaceous units, skin structures consisting of a hair follicle and its associated sebaceous gland, changes that require androgen stimulation. *Propionibacterium acnes* and *Staphylococcus epidermidis* are common pus-forming microbes responsible for the development of various forms of acne vulgaris [4].

Taurine bromamine (TauBr), the physiological product of hypobromous acid reaction with taurine, shows antioxidant, anti-inflammatory, and anti-bacterial properties. Importantly, *P. acnes*, a potential pathogenic agent for acne vulgaris, is extremely sensitive to TauBr. In addition, TauBr inhibits the generation of H_2O_2 by activated neutrophils, which seems to be crucial for reducing the number and severity of inflammatory acne lesions. All these data strongly support the concept of using TauBr for topical anti-acne therapy. In a pilot clinical study, comparison of the efficacy of TauBr cream with

clindamycin gel, one of the most common topical agents used in the treatment of acne was performed. After 6 weeks, both treatments produced comparable, beneficial results. More than 90% of patients improved clinically with similar reductions in a number of acne lesions (~65%). Therefore, the results from clinical studies are consistent with previous in vitro data and strongly suggest the use of taurine as a new therapeutic option in inflammatory acne [5].

Skin Carcinogenesis

Skin neoplasms (also known as "skin cancer") are skin growths with differing causes and varying degrees of malignancy. The three most common malignant skin cancers are basal cell cancer, squamous cell cancer, and melanoma, each of which is named after the type of skin cell from which it arises. Skin cancer generally develops in the epidermis (the outermost layer of skin), so a tumor can usually be seen. This means that it is often possible to detect skin cancers at an early stage.

Taurolidine an analogue of taurine was used to explore anti-tumor promoting activity in a skin carcinogenesis model (in vitro, in vivo). The study evaluates whether taurolidine, a novel antibiotic agent, induces murine melanoma cell apoptosis in vitro and in vivo. For this purpose murine melanoma cells (B16 4A5 and B16 F10) were treated with taurolidine (0–100 μM) for 12 and 24 h. Cell viability and apoptosis were assessed by MTT assay and FACScan analysis. Expression of the Bcl-2 family proteins was detected by Western blot analysis. In vivo, taurolidine-induced anti-tumor cytotoxicity was assessed in C57BL/6 mice. Therapeutic effectiveness, by intraperitoneal injection of taurolidine (15 mg/mouse) on alternate days for 2 weeks, was evaluated in mice bearing B16 4A5 tumor xenografts. Primary and metastatic tumor growth and intra-tumor apoptotic index were measured, which showed positive results. Hence as a consequence taurolidine significantly attenuates melanoma tumor growth, which results from taurolidine-induced apoptosis by modulation of the Bcl-2 family proteins [6]. Further investigations are needed to be carried out to test the potency of taurolidine in humans, which may prove beneficial in treating various skin neoplasms.

Melanogenesis

Melanocytes beneath the skin produce melanin, which is a pigment found in the skin, eyes, and hair, and the process by which it is formed is termed as melanogenesis. Pigmentation in human skin is an important defense mechanism against sunlight or oxidative stress. Despite the protective role of melanin, abnormal hyperpigmentation such as freckles and chloasma sometimes can be serious aesthetic problems. Because of these effects of hyperpigmentation, people have considered the effect of depigmentation.

In a study, antimelanogenic activity of combination of azelaic acid and taurine in B16F10 mouse melanoma cells was performed. Melanin contents and tyrosinase activity were measured. To gain the change of protein expression, western blotting was performed. These findings indicated that azelaic acid with taurine might play an important role in the regulation of melanin formation and be useful as effective ingredients in antimelanogesis. Hence taurine is beneficial as antimelanotic agent [7].

The use of taurine thus has gained increase as a depigmenting agent in cosmetology.

Wound Healing

Wound healing, or cicatrisation, is an intricate process in which the skin repairs itself after injury. In normal skin, the epidermis and dermis exists in a steady-state equilibrium, forming a protective

barrier against the external environment. Once the protective barrier is broken, the process of wound healing is immediately set in motion. Wounds normally heal in a very orderly efficient and highly controlled process of repair [8].

Taurine plays various important roles in large number of physiological and pathological conditions in human body, such as cytoprotective functions, anti-inflammatory, anti-apoptosis effects. Taurine exhibits an antioxidant effect, and effects on cell proliferation, inflammation, collagenogenesis. Many antioxidants have been used to eliminate the negative effects of oxygen free radicals on wound healing. Furthermore taurine has been documented to have anti-apoptosis effect. The decrease of apoptosis of wound repair cells involving fibroblasts, keratinocytes, and endothelial cells may contribute to promotion of repair phases. So the wound healing process is accelerated due to synergistic effect of both antioxidant and anti-apoptosis activity. Hence taurine combined with cooper is a new therapeutic candidate for infected wound healing [9].

Antiseptic

Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit their growth.

N-chlorotaurine (NCT), the *N*-chloro derivative of the amino acid taurine, is a long-lived oxidant produced by activated human granulocytes and monocytes. The successful synthesis of the crystalline sodium salt ($Cl–HN–CH_2–CH_2–SO_3Na$) facilitated its development as an endogenous antiseptic. NCT has killing activity against bacteria, fungi, viruses, and parasites. Transfer of the active chlorine to amino groups of molecules of both the pathogens and the human body (transhalogenation) enhances its activity, mainly because of the formation of monochloramine. Furthermore, surface chlorination after sublethal incubation times in NCT leads to a post-antibiotic effect and loss of virulence of pathogens. Being a mild oxidant, NCT proves to be very well tolerated by human tissue in Phase I and II clinical studies. A 1% aqueous solution was used in skin ulcerations, outer ear canal, nasal and paranasal sinuses, and oral cavity. Therapeutic efficacy in Phase II studies has been shown in external otitis, purulently coated crural ulcerations and keratoconjunctivitis. Based upon all the above data, NCT seems to be an antiseptic with a very good relation between tolerability and activity [10].

Skin Ulcers

An ulcer is a sore on the skin or a mucous membrane; Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract. Cutaneous (crural) ulcers are characterized by oval shape, rolled borders, and sticky, suppurative coating with abundant granulation tissue. Skin ulcers may take a very long time to heal. Treatment is typically to avoid the ulcer getting infected, remove any excess discharge, maintain a moist wound environment, control the edema, and ease pain caused by nerve and tissue damage.

According to a study, particularly, patients with leg perfusion problems develop crural ulcerations, which frequently become infected and inflamed. A Phase IIb clinical study was performed comparing the effect of 1% *N*-chlorotaurine (NCT) and 1% Chloramine-T (CAT) in patients suffering from crural ulcers. Twice daily irrigations and bandages soaked with these antiseptics for 5 days were found sufficient to remove the clinical signs of infection in both groups. However, pain was significantly

lower, and granulation and re-epithelialization occurred significantly earlier in the NCT group. Thus proving the role of taurine in skin ulcers/cural ulcers [11].

Irritant Dermatitis

Irritant dermatitis is inflammation of the skin typically manifested by erythema, mild edema, and scaling. Irritant dermatitis is a nonspecific response of the skin to direct chemical damage that releases mediators of inflammation predominately from epidermal cells. A corrosive agent causes the immediate death of epidermal cells, manifested by chemical burns and cutaneous ulcers. Anti-irritants are used in cosmetic products to prevent or to treat skin irritations that arise during daily life. A study was designed to examine the effects of different polyols (including glycerol, xylitol, and mannitol) and the amino acids taurine and glycine on sodium lauryl sulfate (SLS)-induced skin irritation. For this, healthy adult volunteers were patch-tested with 0.1% SLS in the presence or absence of one or another polyol or amino acid. Skin reactions were evaluated via measurements of transepidermal water loss (TEWL). Taurine showed inhibition of irritation with SLS-induced increase in TEWL. Similar to the action of the well-known anti-irritant glycerol, SLS-induced skin irritation is suppressed by xylitol and taurine. These results suggest that taurine is effective in preventing irritative dermatitis [12].

Antiwrinkle

A wrinkle is a fold, ridge, or crease in the skin. Skin wrinkles typically appear as a result of aging processes such as glycation or, temporarily, as the result of prolonged (more than a few minutes) immersion in water. Wrinkling in the skin is caused by habitual facial expressions, aging, sun damage, smoking, poor hydration, and various other factors. Wrinkles now have a greater social impact because people live longer. Functional agents currently include alpha hydroxy acids, retinoids, fish polysaccharides, anti-enzymatic agents, antioxidants (including ascorbic acid, vitamin E, taurine, L-carnosine). Most are topical, some can be given by mouth, even as food supplements. Thus, taurine due to its antioxidant, anti-apoptotic properties exert an antiwrinkle effect on the skin [13].

Skin Hydration

Dehydration is defined as the excessive loss of body fluid. It is literally the removal of water within an organism. Dehydration of skin and mucous membranes can be called medical dryness. Epidermal keratinocytes are exposed to a low water concentration at the stratum corneum–stratum granulosum interface. When epithelial tissues are osmotically perturbed, cellular protection and cell volume regulation is mediated by accumulation of organic osmolytes such as taurine. Previous studies reported the presence of taurine in the epidermis of several animal species.

Therefore, analysis of human skin for the presence of the taurine transporter (TAUT) and accumulation of taurine as one potential mechanism protecting epidermal keratinocytes from dehydration was studied. Keratinocyte accumulation of taurine was induced by experimental induction of skin dryness via application of silica gel to human skin. Cultured human keratinocytes accumulated taurine in a concentration and osmolarity-dependent manner. TAUT mRNA levels were increased after exposure of human keratinocytes to hyperosmotic culture medium, indicating osmosensitive TAUT mRNA expression as part of the adaptation of keratinocytes to hyperosmotic stress. Accumulation of taurine

protected cultured human keratinocytes from both osmotically induced and ultraviolet-induced apoptosis. The above data strongly indicates that taurine is an important epidermal osmolyte required to maintain keratinocyte hydration in a dry environment [14].

Psoriasis

Psoriasis is a fairly common skin disease which is regarded as immunologically based disease which combines dermal inflammation with secondary epidermal hyperplasia. It is characterized by thick, silvery white scales surrounded by a red, inflamed border. In a study, neutrophil taurine was measured in 30 subjects presenting with chronic stable plaque-type psoriasis. The taurine concentration expressed per 5×10^6 cells was significantly lower ($p < 0.002$) in these subjects compared to neutrophil taurine measured in 20 control subjects. Thus indicating possible roles of taurine in the aetiology of psoriasis. Also various studies propose taurine in maintaining normal neutrophil function coupled with anti-inflammatory effects. Hence taurine dosing of 0.05–0.15 g/day is found effective in treating the underlying causes of psoriasis [15].

Atopic Eczema

Atopic eczema (atopic dermatitis) is an allergic disease believed to have a hereditary component and often runs in families whose members also have asthma. Itchy rash is particularly noticeable on head and scalp, neck, inside of elbows, behind knees, and buttocks. A case study of a 43 year old woman with impetiginous atopical eczema on hands and feet was observed. The patient was initially treated with various topical ointments like Dermatop cream, prednisolone, cefpodoxim, etc. Since no improvement could be recorded, the hands of the patient were treated three times daily with taurolidine-salve 2%. After 2 days, improvement was seen and after 1 week of treatment, a skin normal condition was recorded. Thus indicating the role of taurine in atopic eczema [16].

Rosacea

Rosacea is a chronic condition characterized by facial erythema (redness). Rosacea typically begins as redness on the central face across the cheeks, nose, or forehead, but can also less commonly affect the neck, chest, ears, and scalp. In some cases, additional symptoms, such as semi-permanent redness, telangiectasia, red domed papules and pustules, red gritty eyes, burning and stinging sensations, red lobulated nose may develop. A case study of three patients with rosacea in face was demonstrated. All were treated two times per day with 2% taurolidine cream. Immediately after a first treatment period of only 1 day, a clear reduction of erythema and inflammatory process was observed. After 2 days a reduction of papules and pustules was observed. Consequently, pointing the role of taurine in treating rosacea [16].

Taurine Deficiency

Given taurine's considerable biological significance, deficiency clearly has the potential to cause clinical consequences. Deficiency may be due to lack of molecules like cysteine and methionine, pyridoxal-5-phosphate, cysteinsulfinic decarboxylase, vitamin A, and zinc, also due to candida infection,

anerobic bacteria, and monosodium glutamate. Individuals suffering from taurine deficiency show the following signs and symptoms like impaired vision, anxiety, depression, skin disorders, hypertension, weight gain, kidney problems, heart problems, muscle problems, and reduced endurance. Parentral, local taurine supplementation is necessary in all the above cases for immediate relief from the underlying causes of these disorders [3].

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Part III
Plant and Plant Components and Skin Care

Chapter 10
Turmeric (*Curcuma longa* L.) the Indian Golden Curry Spice as a Skin Care Agent: Validation of the Traditional Uses

Manjeshwar Shrinath Baliga, Sunitha Venkatesh, Shilpa Mrinal, Nandakishore Bala, and Princy Louis Palatty

Key Points

- *Curcuma longa* L. colloquially known as turmeric or Indian saffron in English is an important spice and a medicinal plant in the various traditional and folk systems of medicine in India.
- Turmeric is widely used to treat biliary disorders, jaundice, anorexia, cough, hepatic disorders, rheumatism, inflammation, hematuria, and hemorrhage.
- Turmeric is arguably the most commonly used medicinal and homemade remedy for skin ailments and is used as an antiseptic, analgesic, anti-inflammatory, wound healing agent and scientific studies have validated many of the ethnomedicinal claims and observations.
- Preclinical studies have shown that turmeric and its principle compound curcumin are effective as skin care agent and to be effective in the treatment of psoriasis, wound healing, to retard ageing, prevent UV-induced skin damage, and chemical carcinogenesis.
- The current review summarizes the observations for the skin care effects and the mechanisms responsible for this property.

Keywords *Curcuma longa* • Psoriasis • Wound healing • Ageing • UV-induced skin damage • Chemical carcinogenesis

Introduction

Turmeric (*Curcuma longa* L), a tropical plant originally native to India is today considered to be a very important herb. The name turmeric is known to have originated from the medieval Latin name terramerita, which then became terremerite in French, meaning deserved earth or meritorious earth [1, 2].

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It belongs to the family Zingiberaceae that also includes other important plants like ginger (*Zingiber officinale*), galangal or Thai ginger (*Alpinia galanga*), melegueta pepper (*Aframomum melegueta*), myoga (*Zingiber mioga*), and cardamom (Amomum, Elettaria) [1, 2]. Turmeric is cultivated most extensively in India, Sri Lanka, Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Philippines and also in tropical regions of Africa, America, and Pacific Ocean Islands. India is the largest producer, consumer, and exporter of turmeric [1, 2].

The plant is a perennial herb with short stem and large simple oblong leaves. The tubers (rhizomes) are oblong or ovate or pyriform and are often branched. The rhizomes are yellowish brown externally while internally they are orange in color. The rhizomes possess the characteristic odor and are slightly pungent bitter to taste. The rhizomes are also boiled for several hours, dried in hot ovens, and then finely powdered to be used to impart organoleptic properties to curries and as a food preservative in many Indian, Persian, Sri Lankan, Nepali, Pakistani, and Thai dishes. In India, turmeric has strong associations with the socio-cultural life and is used extensively in various religious and auspicious ceremonies [1, 2].

Turmeric in Traditional Medicine

Turmeric is extensively used in the various traditional systems of medicine like Ayurveda, Chinese, Unani, and Siddha, the various folk medicines and as a household remedy for various diseases and ailments [1]. Turmeric has been used extensively in these systems of medicine to treat phlegmatic, digestive, and metabolic disorders. Turmeric is known to be effective against sore throat, dyspepsia, stomach ulcers, fibroids, cysts, mastitis, endometriosis, dysmenorrhoea, amenorrhoea, leucorrhoea, colitis, asthma, rheumatoid arthritis, osteoarthritis, gout, broken bones, conjunctivitis, styes, anemia, diabetes, liver, cardiac, and skin ailments [2, 3].

Phytochemistry

Turmeric contains a wide variety of phytochemicals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols. Of these curcumin, which is water insoluble but soluble in dimethylsulfoxide, acetone, ethanol, and oils, is one of the highly studied phytochemical for its myriad biological and pharmacological effects [2] (Fig. 10.1).

Turmeric in Skin Care

Ancient texts of Indian medicine describe the use of turmeric in treating various skin ailments and also as a wound healing agent either alone or in combination with other medicinal plants (like sandal wood). In Ayurveda, turmeric is also addressed as Varna datri (that which gives color and an enhancer of body complexion) and accordingly is found to be useful in treating various skin diseases, inflammations, abscess, eczema, leucoderma, bruises, wounds, eczema, urticaria, psoriasis, and acne [2]. Scientific studies carried out in the past three decades have validated the ethnomedicinal uses and turmeric is shown to possess wound healing, anti-ageing, and anti-psoriatic properties and also shown to be effective against the UV-induced skin damage and chemical carcinogenesis. In the following section the validated properties will be accordingly addressed.

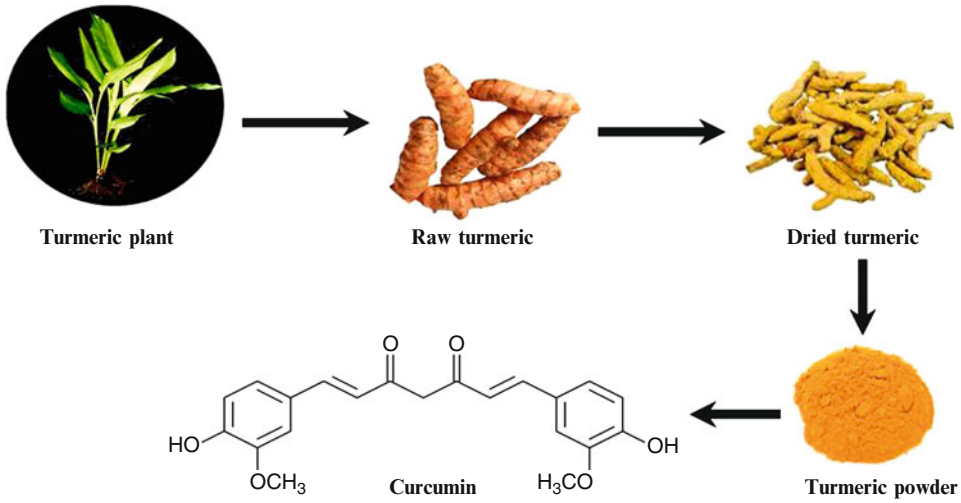

Fig. 10.1 Pictorial depiction of obtaining curcumin from the turmeric (*Curcuma longa*) plant

Turmeric May Be Effective Against Psoriasis

Psoriasis a chronic inflammatory skin disease characterized by rapid proliferation of keratinocytes and incomplete keratinization affects about 3% of the population worldwide. The precise cause of disease remains unknown, but numerous factors including genetics, immune system, and environmental stimuli can induce the disease. The treatment options for severe psoriasis are either time-consuming (e.g., ultraviolet B or psoralen plus UVA therapy) or have the potential for organ toxicity with chronic use (methotrexate, acitretin, cyclosporine). Newer biologic therapies (infliximab, etanercept, adalimumab, efalizumab, and alefacept) are immunosuppressive and could increase the risk of infections and malignancies with long-term use, and are limited by their high cost. Given the chronic nature of psoriasis and the need for long-term treatment, there exists an unmet need for effective, nontoxic therapies that are also convenient and affordable [4–8].

Human studies with psoriatic patients has shown that topical application of an alcoholic gel preparation containing 1% curcumin caused better resolution of the disease within a shorter period of time when compared to the calsipotriol ointment (Dovonexw, 0.005% calcipotriol). Studies have shown curcumin to inhibit proliferation of keratinocyte [6]. Mechanistic studies showed that curcumin decreased phosphorylase kinase activity in curcumin-treated psoriasis and concomitantly also caused a decrease in the levels of keratinocyte transferrin receptor (TRR) expression, severity of parakeratosis, and density of epidermal CD8+ T cells [5]. Cell culture studies have shown turmeric extract to decrease the expression of CSF-1, IL-8, NF-κB2, NF-κB1, and RelA in the keratinocyte cell line (HaCaT) [8]. TNF-α is known to contribute to various inflammatory skin diseases like psoriasis and in vitro studies with HaCaT cells have shown curcumin to inhibit the expression of TNF-α-induced IL-1b, IL-6, expression cyclin E through inhibition of NF-κB and MAPK pathways [7].

Turmeric in Wound Healing

Wound, which in simple terms means disruption of the cellular and anatomic continuity of a tissue, is a commonly encountered condition. Wound may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue [9]. Tissue repair and wound healing are complex processes

that involve inflammation, granulation, and remodeling of the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. However, the process of wound healing is affected in certain clinical conditions (e.g., diabetes) and on exposure to xenobiotic stress (corticosteroids, anti-neoplastic, radiation, etc.) thereby resulting in non-healing, under-healing, or delayed wound healing. Turmeric has a long history of being useful in healing open wounds and burns in the Ayurvedic system of medicine and scientific experiments have validated the ethnomedicinal uses. In the following sections, the scientifically validated skin care properties of turmeric will be addressed.

Turmeric Enhances Healing of Open Wounds

Scientific studies have shown turmeric to be effective in enhancing the excision wound healing in rabbits [10] and rats [11]. The principle phytochemical curcumin is also reported to be effective in healing excision wounds [12, 13]. Administration of curcumin caused faster wound closure of punch wounds in animals [13]. The histopathological studies showed reepithelialization of the epidermis; increased migration of myofibroblasts, fibroblasts, and macrophages in the wound bed; increased neovascularization and collagen deposition in curcumin-treated wounds [13]. Curcumin also increased TGF-β1 and fibronectin in curcumin-treated wounds [13]. Studies have also shown that curcumin-incorporated collagen matrix were also effective in healing open wounds in normal rats, fastening the wound reduction, and enhancing cell proliferation [14]. Cell culture studies have also shown that curcumin modulates wound healing in a biphasic dose–response manner. At low concentrations (between 1 and 5 μM) it stimulates cell migration and cell proliferation while at the higher concentrations (<5 μM) it possess inhibitory effects [15]. In vitro studies have also shown that curcumin at high doses (>25 μM) induces apoptosis in the fibroblasts, thereby offering a novel way to modulate pathological scar formation [16].

Turmeric Enhances Healing of Burn Wounds

Cutaneous burns consisting of a central zone of necrosis surrounded by a zone of ischemia is a dynamic injury whose severity/complications are dependent on the depth of the wound and the proportion of the body affected. A superficial burn is mostly not life threatening as it involves only the epidermal layer of the skin, while partial thickness burns involving damage to more structures within the skin and full thickness burns involving all layers of the skin. The extent of the injury is usually expressed in percent of total body surface area which is burnt. Animal studies have shown that oral administration of curcumin 30 min before induction of burns and then for 3 consecutive days reduced the percentage of unburned skin interspaces that progressed to full necrosis indicating its usefulness [17].

Turmeric Enhances Healing of Wounds in Diabetic Rats

Wound healing is impaired in diabetes and may be complicated by infection. Hyperglycemia and oxidative stress impairs wound healing and extends the process. Additionally, the formation of advanced glycation end products (AGEs) has also been shown to contribute toward diabetic complications. Increased accumulation of various glycosylation products in the skin alters the physicochemical structure of the skin and consequently leads to various skin disorders and to delay wound

healing in diabetes. Animal studies have shown that curcumin is effective in enhancing the wound healing in chemically induced diabetic rats [18]. Oral administration of curcumin (200 mg/kg body wt) for 8 consecutive weeks reduced the oxidative stress and levels of serum lipid peroxidation. Curcumin reduced the glycation and cross-linking of collagen in the tail tendon and skin, indicating its usefulness [18].

Turmeric Enhances Healing in Dexamethasone-Treated Rats

Dexamethasone, a potent glucocorticoid class of steroid drugs, impairs wound healing. Preclinical studies have shown curcumin to be effective in healing the dexamethasone-impaired cutaneous healing in a full-thickness punch-wound model in rats. Topical application of curcumin accelerated the healing of wounds with or without dexamethasone treatment, as revealed by a reduction in the wound width and gap length compared with controls. Curcumin treatment enhanced expression of TGF-β1 and TGF-β tIIrc in both normal and impaired healing wounds. The macrophages in the wound bed showed enhanced expression of TGF-β1 mRNA in curcumin-treated wounds, as evidenced by in situ hybridization. iNOS levels were increased following curcumin treatment in unimpaired wounds, but not so in the dexamethasone-impaired wounds. These observations suggest that topical curcumin had a differential regulatory effect on TGF-β1, its receptors, and iNOS in this cutaneous wound-healing model [19].

Turmeric Enhances Healing in Mice Exposed to Ionizing Radiation

Exposure to ionizing radiation affects the normal wound healing process and may cause severe morbidity and even mortality of the affected individuals. Animal studies have shown that curcumin was effective in ameliorating radiation-induced delay in wound repair and enhanced the rate of wound contraction, decreased mean wound healing time [20–22] and to decrease acute and chronic radiation skin toxicity [23]. Pretreatment with curcumin increased synthesis of collagen, hexosamine, DNA, nitric oxide and improved fibroblast and vascular densities [20–22]. Mechanistic studies have also shown that administering curcumin before or after radiation decreased mRNA expression of early responding cytokines (IL-1 IL-6, IL-18, TNF-α, and lymphotoxin-β) and the fibrogenic cytokine, TGF-β, in cutaneous tissues at 21 days postradiation, clearly indicating that curcumin mediates its protective effects by downregulation of both inflammatory and fibrogenic cytokines in irradiated skin and muscle, particularly in the early phase after radiation [23].

Turmeric Retard Ageing of Skin

Aging or senescence often viewed as a random process arising from the accumulation of both genetic and epigenetic changes is an inevitable process. Free radical stress is postulated to be an important hypothesis and to substantiate this; reports indicate that, treatment with exogenous hydrogen peroxide can trigger certain primary cells to rapidly enter senescence [24]. Cell culture studies have shown curcumin to be effective in preventing H_2O_2-induced damage to cultured human keratinocytes and fibroblasts indicating its usefulness [25]. Cell culture studies with early passage young human skin fibroblasts have shown curcumin to initially induce oxidative stress and impair GSH redox state in the cells. Subsequently, a time- and concentration-dependent induction of heme oxygenase-1 (HO-1),

increase in glutathione-*S*-transferase activity, GSH levels, and GSH/GSSG ratio occurs. Together all these observations indicate curcumin to exhibit a hormetic stimulation of cellular antioxidant defenses in these cells, and be of use in preventing aging [26].

Turmeric/Curcumin Is Effective in Preventing UV-Induced Skin Damage

Ultraviolet radiations (UV) consisting of the short wave (UVC 200–280 nm), mid wave (UVB 280–315 nm), and long wave (UVA 315–400 nm) is an important dermatoxic agent and are proved to cause skin cancer, photoaging, actinic keratoses, lupus vulgaris (tuberculosis of the skin), psoriasis or vitiligo. Preclinical studies have also shown that curcumin was effective in preventing the UVA-induced damage and ill effects. Pretreatment with curcumin reduced TPA + UVA-induced ODC levels, decreased thickness of epidermis and dermis and the number of dermal infiltrating inflammatory cells in mice [27]. Cell culture studies have also shown that combination of low concentrations of curcumin with UVA or visible light decreases proliferation and induces apoptosis in human skin keratinocytes by the release of cytochrome c from mitochondria, activation of caspases-9 and -8, inhibition of NF-κB activity, and inhibition of extracellular regulated kinases 1/2 and protein kinase B [28].

With respect to UVB, studies have also shown that the topical application of turmeric extract (at 300 or 1,000 mg/kg, twice daily) was effective in preventing chronic UVB-induced increase in skin thickness, formation of wrinkles and melanin, reduction in skin elasticity, and increase in MMP-2 expression [29]. Recent studies have also shown that encapsulated curcumin was effective in attenuating the UV-induced photoaging in mice [30].

Topical application of curcumin to mouse skin (twice a day for 5 days) immediately after UVB exposure marginally inhibited the expression of c-Fos and c-Jun and epidermal hyperplasia [31]. Cell culture studies with human keratinocytes (HaCaT cells) have shown that treatment with curcumin strongly inhibited UVB-induced COX-2 mRNA and protein expressions by affecting the activations of p38 MAPK and JNK, and decreasing the DNA binding activity of AP-1 transcription factor [32]. Studies have also shown that topical tetrahydrocurcuminoid cream and targeted narrowband UVB phototherapy was marginally more effective than targeted narrowband UVB monotherapy for vitiligo [33].

Turmeric Is Effective in Preventing Skin Carcinogenesis

Globally, skin cancers, comprising of basal cell carcinoma, squamous cell carcinoma, and melanoma, are the leading form of cancer and are a major health problem in many countries [34]. Seminal studies by Azuine and Bhide [35] have shown that dietary turmeric (2%) was effective in preventing DMBA-induced skin tumorigenesis. Since then other investigators have also observed turmeric to be effective in preventing skin cancer in experimental animals by various mutagens and carcinogens [36]. Mechanistic studies showed that turmeric modulated the hepatic cytochrome b5, cytochrome P-450, glutathione, and glutathione *S*-transferase thereby mediating the protective effects [35]. In addition to the preclinical observations, studies with cancer patients have also shown that the ethanolic extract of turmeric as well as an ointment of curcumin produced remarkable symptomatic relief in patients with external cancerous lesions, clearly demonstrating its use in clinics [37]. With regard to the phytochemicals studies have also shown that both dietary feeding [38, 39] and topical application of curcumin [40, 41] was effective in preventing chemically induced skin carcinogenesis in mice.

Mechanistic studies have shown that topical application of curcumin inhibits B[a]P-mediated formation of DNA-B[a]P adducts in the epidermis, epidermal DNA synthesis and the oxidized DNA base 5-hydroxymethyl-2′-deoxyuridine [41]. Curcumin also inhibits arachidonic acid-induced edema of

mouse skin and ears in vivo and epidermal cyclooxygenase and lipoxygenase activities in vitro [41, 42]. In vitro studies with cytosol prepared from the homogenates of mouse epidermis have also shown that curcumin inhibited the metabolism of arachidonic acid to 5-hydroxyeicosatetraenoic acid (5-HETE) and the metabolism of arachidonic acid to 8-HETE [43]. Studies with cultured NIH 3T3 cells have shown curcumin to be effective in inhibiting TPA-induced protein kinase C activity [44] and to suppress the expression of c-jun in TPA-treated NIH3T3 cells [45]. Curcumin is also reported to inhibit PKC activity in vitro by competing with phosphatidylserine [45]. Dietary feeding of curcumin is also shown to cause a significant decrease in the expression of ras and fos proto-oncogenes in the tumorous skin [39]. Studies with mouse fibroblast cells have shown curcumin to inhibit TPA-induced PKC activity, tyrosine protein kinase activity, and arachidonic acid metabolism [46].

Topical application of curcumin reduced TPA-induced increase in hydrogen peroxide, skin inflammation, mRNA level of ODC [44], activity of ODC [46], hyperplasia, formation of c-Fos, and c-Jun proteins [41]. At the molecular level, curcumin decreased the TPA-induced translocation of PKC isozymes (alpha, beta, gamma, epsilon, eta) from the cytosol thereby altering its activity. Curcumin pre-treatment reduced the TPA-induced levels of mitogen-activated protein kinases and transcription factors (c-jun, c-fos, and nuclear factor-κB) and downstream target proteins associated with cell proliferation (cyclin D1 and ODC), cell death (Bax and Bcl2), inflammation (cyclooxygenase-2 and prostaglandin E2) and oxidative stress (8-hydroxy-2′-deoxyguanosine) in mouse skin [47, 48].

Studies have also shown that the topical application of curcumin, pure curcumin, or demethoxycurcumin were equally effective in decreasing TPA-induced increase in ODC activity and TPA-induced tumor promotion in DMBA-initiated mouse skin, while bisdemethoxycurcumin and tetrahydrocurcumin were less active [49]. Additionally, curcumin, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin were also equally effective in inhibiting the TPA-induced inflammation of mouse ears, as well as TPA-induced transformation of cultured JB6 (P+) cells, while tetrahydrocurcumin was less active [49]. Together these observations clearly indicate that the pure curcumin and demethoxycurcumin have the same potent inhibitory effects as commercial grade curcumin in inhibitiing TPA-induced tumor promotion, but bisdemethoxycurcumin and tetrahydrocurcumin are less active [49].

In vitro studies have also shown that curcuminoids protected normal human keratinocytes from free oxygen radical stress [50] and to induce apoptosis in immortalized NIH 3T3 [46], malignant cancer cell lines [46], human basal cell carcinoma cells [51], and mouse melanoma cells [52]. Mechanistic studies showed that the curcumin-mediated apoptosis in basal cell carcinoma cells was mediated by p53, p21 (CIP1/WAF1), and Gadd45, and that treatment with p53 antisense oligonucleotide reversed the effect [51]. Studies have also shown that curcumin caused a concentration-dependent apoptosis in melanoma cells by activating caspase-3, inversion of membrane phosphatidyl serine and arresting the cells in the sub-G1 phase. Curcumin treatment inhibited NF-κB-driven reporter activity, decreased phospho-IkappaBalpha and concomitantly reduced the expression of downstream target genes like the COX-2 and cyclin D1 [52].

Studies with C57BL6 mice have shown that curcumin decreased lung metastases of B16F10 melanoma by modifying the cell receptor binding proteins. Mechanistic studies showed a concentration-dependent reduction in their binding to the extracellular matrix proteins (fibronectin, vitronectin, and collagen IV), decrease in the expression of alpha5beta1 and alpha(v)beta3 integrin receptors, to inhibit pp125 focal adhesion kinase (FAK), tyrosine phosphorylation of a 120 kDa protein, and collagenase activity. Concomitantly, curcumin also increased the expression of the antimetastatic proteins like tissue inhibitor metalloproteinase (TIMP)-2, nonmetastatic gene 23 (Nm23), and E-cadherin indicating its usefulness as an antimetastatic agent in melanoma [53]. Studies with the mouse epidermal keratinocytes have shown that curcumin reduced the TGF-β1 stimulated cell migration and invasiveness in a concentration-dependent manner [54].

With regard to humans, phase I clinical trial with Bowen's disease (squamous cell carcinoma in situ) have shown that curcumin did not possess any treatment-related toxicity (up to 8,000 mg/day for

up to 3 months) and caused histologic improvement of precancerous lesions in around 33% of patients [55]. Together all these observations clearly indicate that turmeric/curcumin is effective in preventing/progression of skin cancer.

Conclusions

Since antiquity, turmeric has been used in the various traditional systems of medicine for various skin ailments and preclinical studies have validated many of the ethnomedical uses. A few of the human clinical trials conducted so far showed the protection rendered by curcumin from skin diseases like psoriasis. Turmeric/curcumin-based creams have been used extensively in India and are considered to be effective in preventing acne, ageing, and to promote good skin. However, detailed studies are required to validate the accepted beliefs. Curcumin is nontoxic in large doses even at 8 g/day. Turmeric and its principle compound curcumin appear to protect skin by quenching free radicals and reducing inflammation which it mediates at least in part by inhibiting the NF-κB. Although supportive the preclinical observations are not conclusive suggesting the need for well-designed clinical trials, supported by better formulations of curcumin or novel routes of administration be conducted in the near future.

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Chapter 11
Ginger (*Zingiber officinale* Roscoe) the Dietary Agent in Skin Care: A Review

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Key Points

- The rhizome of *Zingiber officinale* Roscoe [family: Zingiberaceae], commonly known as "ginger," is one of the regularly used spices in many parts of the world. In addition to its dietary use, ginger has a long history of medicinal use in various folk systems of medicine and is also an integral part in many traditional medicines.
- It is extensively used in Chinese, Ayurvedic, Tibetan, Unani, Sri Lankan, Arabic, and African traditional medicines for many unrelated human ailments including common colds, fever, sore throats, vomiting, motion sickness, gastrointestinal complications, indigestion, constipation, arthritis, rheumatism, sprains, muscular aches, pains, cramps, hypertension, dementia, fever, infectious diseases, and helminthiasis. Scientific studies have shown that ginger possess antimicrobial, anti-inflammatory, gastroprotective, hypoglycemic, hepatoprotective, diuretic, hypocholesterolemic, antipyretic, and antiemetic effects.
- The observed myriad beneficial effects are supposed to be due to the presence of bioactive phytochemicals like gingerols, shogaols, paradols, gingerdiols, and zingerone. Experimental studies performed in accordance to the principles of modern medicine have shown that ginger or its phytochemicals possess skin care properties and prevents UV-induced skin damage and photoageing, inhibits melanogenesis and prevents chemically induced skin cancer.
- The current review summarizes the scientific observations on the skin care effect of ginger and emphasizes aspects that need further investigations for it to be of use in clinics in the future.

Keywords *Zingiber officinale* • Ginger • Skin cancer • Photo damage • Melanogenesis

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Fig. 11.1 Photograph of a ginger plant with rhizome

Introduction

Ginger the rhizome of *Zingiber officinale* Roscoe (family Zingiberaceae) is globally one of the most common spices and has been used as a culinary agent for over 1,000 years in Asia (Fig. 11.1). Historical evidences and reports suggest that ginger plants were originally found growing in South-East Asia (today's Northeast India). In Sanskrit, ginger is known as Sringavera and it is speculated that this term may have given way to Zingiberi in Greek and then to the Latin term Zingiber [1]. Ginger belongs to the family Zingiberaceae that also includes other important plants like turmeric (*Curcuma longa*), galangal or Thai ginger (*Alpinia galanga*), melegueta pepper (*Aframomum melegueta*), myoga (*Zingiber mioga*), and cardamom (Amomum, Elettaria).

Ginger has been cultivated for thousands of years as a spice and also for its medicinal purposes. Currently, India and China are the major suppliers to the world market [1]. Ginger also has a long history of being cultivated in other countries as during the medieval years, ginger plants were carried on ships from the Indian subcontinent and were introduced to different parts of the world [1]. Ginger is today also grown in the other tropical countries like Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica, Nepal, Haiti Mexico, and Hawaii [1–4].

In India ginger is also used in various traditional cooking and estimates are that the average daily consumption is about 8–10 g [4, 5]. Ginger is normally made in to paste and added in to the curries. However, dried powder is also used and is an indispensable component of curry powder and sauces [4–7].

In India a special tea is also prepared with ginger and is commonly called as the *Masala Chai* or *ginger tea*. Recently, it is also used in some products like ginger candy, ginger jams, ginger bread, biscuits, pickles, and ginger-flavored carbonated drinks [7, 8].

Chemistry of Ginger

Innumerable phytochemical studies have shown that ginger rhizome contains a wide variety of biologically active compounds and that their ratio and concentration vary with the season, place, and time of harvest. The characteristic organoleptic properties of ginger are due to steam volatile oil and the non-volatile pungent compounds [1–3, 7]. The volatile oil consists mainly of the mono and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol, and α-farmesene [1–3, 7]. The essential oil isolated from ginger rhizomes grown is Ghana is also reported to contain zerumbone and that its concentration varies in the different varieties [8]. The sesquiterpene hydrocarbon α zingiberene predominates and accounts for 20–30% of the oil obtained from dry ginger [1–3, 7, 9].

The non-volatile pungent phytochemicals of ginger consists of the biologically active components, predominated by gingerols, shogaols, paradols, and zingerone [1] (Fig. 11.2). These compounds are responsible for the warm pungent sensation in the mouth and are also reported to account for many of its pharmacological effects [2, 3]. The quantity of [6]-gingerol in the fresh ginger rhizome is reported to be 104–965 μg/g in common varieties of ginger available in Indian market [10]. In fresh ginger, the gingerols, a series of chemical homologs differentiated by the length of their unbranched alkyl chains; [3–6]-, [8]-, [10]-, and [12]-gingerols; and having a side-chain with 7–10, 12, 14, or 16 carbon atoms, respectively, are the major active components. Of all the gingerols, the compound 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant [1–3, 7, 9].

Gingerols are thermally labile and readily undergoes dehydration to form the corresponding shogaols, which may be further converted to paradols by hydrogenation [2, 3]. The extent of this conversion is likely to have a significant impact on the medicinal benefits of ginger, as the two classes of compounds vary in their bioavailability, pharmacokinetics, and pharmacological properties [1–3, 7, 9]. The other constituents include ginger protease, zingerone, dehydrozingerone, capsaicin, gingediol, galanolactone, neral, gingesulfonic acid, galactosylglycerols, gingerglycolipids, diarylheptanoids, and phytosterols [9, 11]. The major pharmacological activity of ginger appears to be due to gingerol and shogaol and the relative proportions of gingerols, shogaols, and paradols in ginger extracts are determined by a number of factors, including the geographic origin, the maturity of the rhizomes at the time of harvest, and the method by which the extracts are prepared [1–3, 7, 9].

Ginger in Traditional Medicines

Since ancient times, the rhizome of ginger has been used in Greek, Roman, Asian, Indian, Sri Lankan, Tibetan, Mediterranean, and Arabic systems of alternative medicines. In these systems of medicine ginger is used to treat cold, headaches, nausea, stomach upset, diarrhea, indigestion, arthritis, rheumatological conditions, and muscular discomfort [4, 7, 9]. It has been recommended for use as carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic, and digestive aid [4, 7, 9] (Table 11.1).

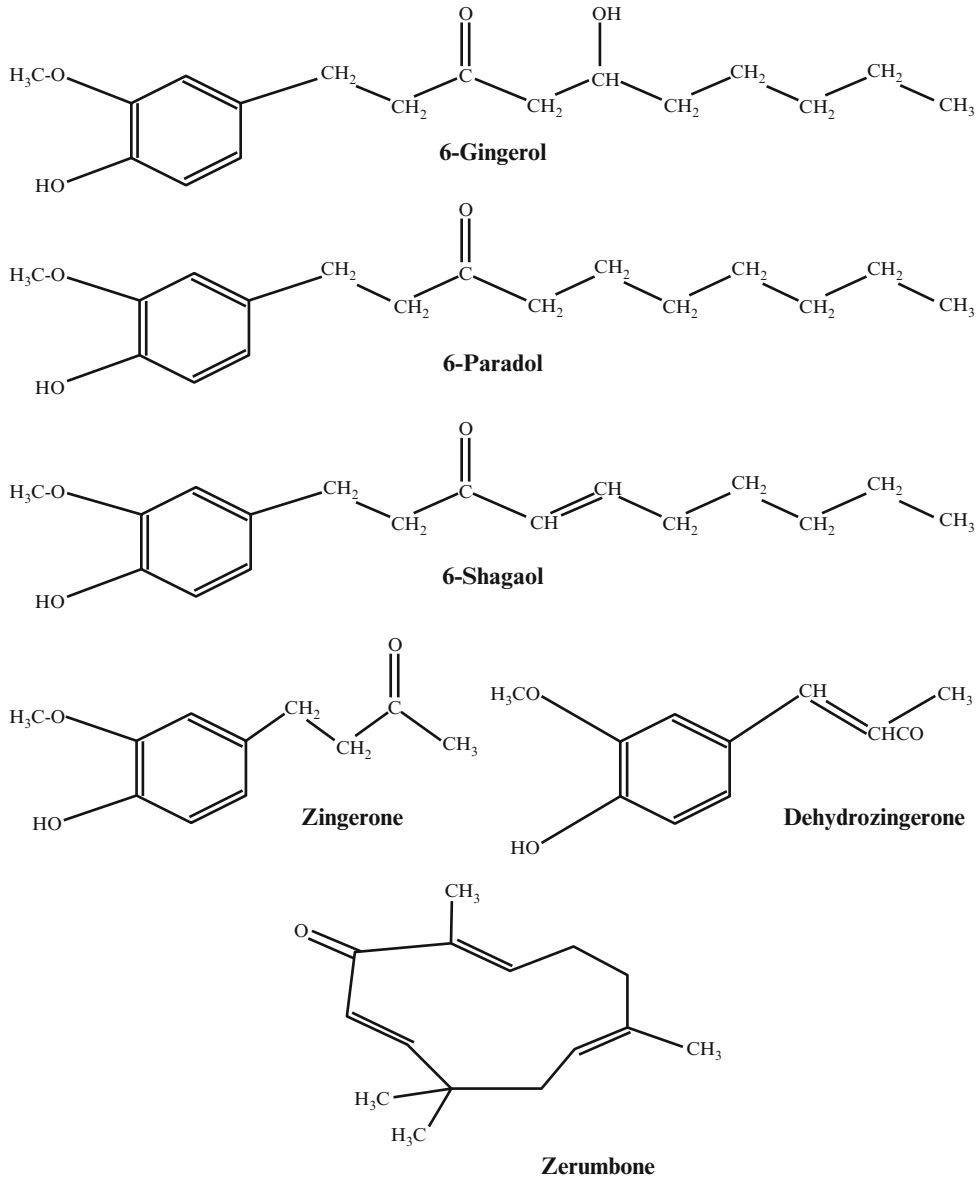

Fig. 11.2 Some important phytochemicals present in ginger rhizome

Ginger has a long history of use in South-East Asia, both in dried and fresh form. It is an integral part of several medicinal formulations in Ayurveda, Siddha, Unani systems of medicine. The Chinese considered it a tonic root for all ailments and consumed ginger for a wide variety of medical problems such as stomachache, diarrhea, nausea, cholera, asthma, heart conditions, respiratory disorders, toothache, and rheumatic complaints [9]. In India, ginger has been used as medicine from vedic period and is called *"maha aushadhi,"* meaning the great medicine [1–4, 7]. The use of ginger in various cultures is enlisted in Table 11.1.

Table 11.1 Traditional uses of ginger rhizome in different countries [1–3, 9, 12]

Country	Pharmacological property
Arabian nations	Aphrodisiac, antiemetic, stomachic, carminative, cold, headaches, nausea, stomach upset, against motion sickness and morning sickness, diarrhea, help digestion, treat arthritis, rheumatological conditions, muscular discomfort, carminative, and antiflatulent
Burma	Anti-flu agent, antiemetic, rheumatological conditions, carminative, cold, nausea, against motion sickness and morning sickness, and stomach upset
China	Antiemetic, antitussive, expectorant, diaphoretic, antihypertensive, arthritis, rheumatological conditions, muscular discomfort, against motion sickness and morning sickness, carminative, and antiflatulent
Congo	Against common cold, antiemetic, arthritis, rheumatological conditions, carminative, antiflatulent, cold, nausea, and stomach upset
Europe	Antiemetic, digestive aid, carminative, antiflatulent, cold, nausea
Germany	Antiemetic, digestive aid, preventing motion sickness
Greece	Digestive aid, antiemetic, rheumatological conditions, against motion sickness and morning sickness
India	Antispasmodic, anti-inflammatory, antiemetic, aphrodisiac, astringent, digestive aid, against motion sickness and morning sickness, antithrombotic, and antiarthritic
Indonesia	Improving fatigue, antihypertensive, digestive aid, antirheumatic, carminative, antiflatulent, cold, nausea
Japan	Antiemetic, antitussive, expectorant, diaphoretic, carminative, antiflatulent, cold, nausea
Sri Lanka	Carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic, and digestive aid
Tibetan	Carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic, and digestive aid
United States of America	Carminative, stomachic, antispasmodic, diaphoretic, against motion sickness and morning sickness

Pharmacological Properties

Scientific studies have shown that ginger possess antimicrobial, antischistosomal, anti-inflammatory, antipyretic, antioxidative, hypoglycemic, hepatoprotective, diuretic, and hypocholesterolemic effects [9, 12]. Ginger is reported to possess myriad benefits to the gastrointestinal tract. It increases the bile secretion; prevents occurrence of gastric ulcers; and enhances pancreatic lipase, intestinal lipase, disaccharidases, sucrase, and maltase activities in animals [9, 12]. Preclinical studies performed in the past 15 years have shown ginger and or some of its phytochemicals to prevent melanogenesis, to reduce ultraviolet-B-induced wrinkle formation and photoageing, and prevent chemical-induced carcinogenesis in mouse skin. In the following section the beneficial effects of ginger and its phytochemicals will be addressed by emphasizing on the mechanism of action.

Ginger Prevents UV-Induced Skin Damage and Photoageing

UV rays consisting of the short wave (UVC 200–280 nm), mid wave (UVB 280–315 nm), and long wave (UVA 315–400 nm) is a very important carcinogen as chronic exposure to it causes skin cancer, immune suppression, photoageing, and actinic keratoses. Sun-protection products reduce the risk of erythema and

DNA damage. However, even products with a high sun protection factor (SPF) may not prevent UVR-induced ill effects and immune suppression. Therefore, the development of effective methods which not only complement sunscreens but can also decrease the incidence, morbidity, and mortality of skin cancer as well as also prevent the photoageing and other dermal problems is a pressing public health issue. Animal studies have shown that the topical application of ginger extract to rat or hairless mouse skin significantly inhibited the wrinkle formation by chronic UVB irradiation at a suberythemal dose. Ginger halted the decrease in skin elasticity in both rats and mice [13]. Additionally in rats it was also observed that in addition to the inhibition of reduction in skin elasticity, ginger also prevented wrinkle formation and decreased the curling and three-dimensional tortuosity of dermal elastic fibers [13].

Inflammatory cytokines play an important role in mediating the UVB-induced skin damage and studies have shown that the aqueous extract of ginger and the phytochemicals gingerol, and shogaol inhibited the production of cytokines in UVB-irradiated HaCaT cells [14]. Animal studies have also shown that these agents when applied topically to the backs of C57BL/6 mice attenuated UVB-induced hyperplasia, infiltration of leukocytes, and dilation of blood vessels in the dermis of mice [14]. Pre-treatment with [6]-gingerol is also shown to reduce UVB-induced intracellular reactive oxygen species levels, activation of caspase-3, -8, -9, and Fas expression in HaCaT cells. It also reduced UVB-induced expression and transactivation of COX-2, inhibited translocation of NF-κB from cytosol to nucleus by suppressing phosphorylation of IκB (ser-32). Topical application of [6]-gingerol prior to UVB irradiation also inhibited the induction of COX-2 mRNA and protein, as well as NF-κB translocation in mice thereby indicating that the in vitro observations extended to the in vivo system [15]. Cumulatively all these observations clearly indicate the usefulness of ginger and its phytochemicals in preventing UVB-induced skin damage.

[6]-Gingerol Inhibits Melanogenesis

The melanin pigment, produced inside the melanosomes and synthesized from the amino acid L-tyrosine that is converted by the enzyme tyrosinase to dopaquinone is important in imparting the color to the skin. However, hyperpigmentary conditions, like melasma, café au lait spot and solar lentigo, postinflammatory hyperpigmentation, freckles or lentigines although not dangerous, affect the quality of life of the individuals. Skin-lightening products containing hydroxyl acids possess unwanted side effects thereby negating the beneficial effects [16]. This has necessitated the need for a safe and efficacious agent that is devoid of any deleterious effects and is safe. In vitro studies with mushroom tyrosinase and B16F10 murine melanoma cells have shown that [6]-gingerol inhibited the activity of tyrosinase, suppressed murine tyrosinase activity and to decrease the amount of melanin in a concentration-dependent manner. Additionally, it also decreased the intracellular reactive oxygen species (ROS) level in a dose-dependent pattern (25–100 μM). Together both these observations clearly indicate the possible usefulness of [6]-gingerol in inhibiting melanogenesis and as a possible skin whitening agent [17].

Ginger in Prevention of Chemical-Induced Skin Cancer

Animal studies by Katiyar et al. [18] showed for the first time that the topical applications of the ethanolic extract of ginger (1, 2, or 4 mg/animal) was effective in inhibiting the DMBA- and TPA-induced carcinogenesis in SKH1 hairless mice. The investigators observed that at the termination of the experiment (20 weeks) when compared to the carcinogen treated (no ginger) cohort's, the application of the ginger 1, 2 and 4 mg of ginger had 10%, 35% and 50% reduction in the animals with skin tumors.

Additionally there was a significant decrease in the number, multiplicity, and size/volume (of each tumor) in the ginger-treated groups indicating the effectiveness of ginger as a chemopreventive agent [18]. Mechanistic studies showed that the administration of ginger decreased the TPA-induced ornithine decarboxylase (ODC), cyclooxygenase, and lipoxygenase activities, which consequentially lead toward a concentration-dependent decrease in both edema and hyperplasia [18]. Recently, Tjeerdsma et al. [19] have reported that the topical application of a gel containing green tea, algae, mustard oil, ginger oil, and calendula oil as a body wrap in a spa once a month caused a temporary arrest of basal cell carcinoma formation in a patient with basal cell naevus syndrome.

With regard to phytochemicals, studies have also shown that topical application of [6]-gingerol or [6]-paradol prior to TPA is shown to attenuate DMBA-induced skin papillomagenesis in female ICR mice [20]. Recently, [6]-gingerol has also been shown to be effective in preventing benzo[a]pyrene (B[a]P)-induced mouse skin tumorigenesis [21] thereby clearly indicating its effectiveness in preventing skin tumorigenesis by another carcinogen. Mechanistic studies have also shown that both [6]-gingerol or [6]-paradol to be effective in inhibiting the tumor-promoter-stimulated inflammation, TNF-α production, activation of epidermal ornithine decarboxylase in mice [21] and to inhibit epidermal growth factor-induced cell transformation and activation of activator protein 1 [22].

Detailed studies with [6]-gingerol have shown that prevents TPA-induced inflammation, and epidermal ornithine decarboxylase (ODC) activity in mouse skin [23], to inhibit anchorage-independent growth of cultured mouse epidermal cells stimulated with epidermal growth factor [24], to inhibit TPA-induced COX-2 expression in mouse skin [23, 24], to suppress NF-κB-DNA binding activity in mouse skin [24, 25], and to inhibit phoshorylation of p38 mitogen-activated protein kinase in mouse skin [24, 25].

Studies have also shown that the topical treatment of [6]-gingerol 30 min prior and post to B[a]P for 32 weeks was effective in delaying the onset of tumorigenesis, to reduce the cumulative number of tumors, and reduce tumor volume [21]. Cell cycle analysis showed that [6]-gingerol-treated animals had more sub-G1 peak cells than the B[a]P alone cohorts indicating that the observed chemopreventive effects were mediated by the induction of apoptosis in the preneoplastic and neoplastic cells [21].

[6]-gingerol treatment increased the B[a]P suppressed p53 levels and Bax while concomitantly decreasing the expression of Bcl-2 and Survivin. Further, [6]-gingerol treatment resulted in release of cytochrome-c, caspases activation and also increased apoptotic protease-activating factor-1 (Apaf-1) [21]. To further substantiate these observations, studies with cultured human epidermoid carcinoma A431 cells have also shown [6]-gingerol to induce apoptosis by generating ROS and decreasing mitochondrial membrane potential, which consequentially caused down-regulation of Bax/Bcl-2 ratio, up-regulation of cytochrome-c and Apaf-1, and to trigger caspase-mediated apoptosis [21].

Recently Wu et al. [26] have also observed that 6-shogaol inhibited DMBA/TPA-induced skin tumor formation and reduced the tumor multiplicity of papillomas. Topical application of 6-shogaol reduced the TPA-induced inflammatory response in mouse skin [27] and to inhibit TPA-stimulated transcription of iNOS and COX-2 mRNA expression and the effect was better than curcumin and 6-gingerol [26]. 6-shogaol treatment reduced TPA-induced nuclear translocation of the nuclear factor-κB subunits, TPA-induced phosphorylation of IkappaBalpha and p65, and caused subsequent degradation of IkappaBalpha [26].

6-shogaol markedly suppressed TPA-induced activation of extracellular signal-regulator kinase 1/2, p38 mitogen-activated protein kinase, JNK1/2, and phosphatidylinositol 3-kinase/Akt, the upstream of nuclear factor-κB, and AP-1 [26]. Studies with the murine RAW 264.7 cells have also shown that 6-shogaol inhibited LPS-induced induction of nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) and that this effect was mediated by inhibiting the activation of NF-κB by interfering with the activation PI3K/Akt/IkappaB kinases IKK and MAPK [27].

Animal studies have shown zerumbone (a minor constituent of *Z. officinale* Roscoe but a principle phytochemical of tropical ginger (*Zingiber zerumbet* Smith) to be also effective in preventing the DMBA-induced TPA-promoted skin carcinogenesis [28]. Zerumbone enhanced the mRNA expression level of manganese superoxide dismutase, glutathione peroxidase-1, glutathione *S*-transferase-P1,

and NAD(P)H quinone oxidoreductase in the epidermis, but not that of cytochrome p450 1A1 or 1B1. It also decreased the TPA-induced cyclooxygenase-2 protein expression and phosphorylation of extracellular signal-regulated kinase 1/2, decreased TPA-induced hydrogen peroxide formation and edema induction. Histologic studies showed zerumbone to suppress leukocyte infiltration and reduced proliferating cell nuclear antigen-labeling indices [28].

Topical application of zerumbone onto dorsal skin of hairless mice induced activation of NF-E2-related factor 2 (Nrf2) and expression of heme oxygenase-1 (HO-1) [29]. Nrf2/ARE-dependent detoxification pathway also potentiates the gene expression of several phase II enzyme genes like γ-glutamylcysteine synthetase, glutathione peroxidase, and glutathione *S*-transferase [30], and it is logical to expect that zerumbone potentiates the gene expression of these crucial detoxification enzymes and thereby mediates the chemopreventive effect atleast in part through this mechanism. Cell culture studies have also shown zerumbone to suppress TPA-induced JB6 cell transformation and the intracellular accumulation of reactive oxygen species that mediate the mutagenesis and triggers inflammation and carcinogenesis [29].

Numerous studies in the past two decades have equivocally shown that ginger rhizome and its compounds possess chemopreventive effects against cancers of different histological origins by mediating several mechanisms like free radical scavenging, antioxidant, antimutagenic, anti-inflammatory; increase in the antioxidant enzymes, modulation of phase I and II enzymes, modulation of signal transduction, transcription factors and cell cycle, and induction of selective apoptosis in neoplastic cells [7]. A similar mechanism may also occur in mediating the chemopreventive effects in skin and needs to be validated in detail with both extract and the important phytochemicals.

Conclusions

Preclinical studies in the past two decades have unequivocally shown that ginger rhizome and some of its compounds possess beneficial effects on the skin, prevent UV-induced skin damage and photoageing, inhibit melanogenesis, and prevent chemically induced skin cancer. Although considerable work has been done to exploit the skin care effects of ginger, countless possibilities for investigation still remain. Further in-depth mechanistic in vitro studies, relevant animal model studies, and rationally designed clinical trials at the normally consumed levels are sufficient for the beneficial effects and also to assess for its adverse effects if any at higher concentrations, especially following ginger consumption over longer periods. This will also help establish not only whether ginger is safe and efficacious as a skin care agent. Ginger or its phytochemicals have never been studied for their adverse effects or beneficial effects when applied topically for extended period of time. These studies are also important as this will help in making us realize their importance in skin care. Due to its abundance, low cost, and safety in consumption, ginger remains a species with tremendous potential and countless possibilities for further investigation. Ginger has the potential to develop as a non-toxic skin care agent when gaps existing in the current knowledge are bridged. The outcomes of such studies may be useful for the clinical applications of ginger in skin care and may open up a new therapeutic avenue.

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Chapter 12
Amla (*Emblica officinalis* Gaertn.) the Indian Indigenous Berry in Skin Care

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Key Points

- *Emblica officinalis* Gaertn. or *Phyllanthus emblica* Linn. commonly known as the Indian gooseberry in English or amla in Hindi, is one of the most important Indian medicinal and dietary plant.
- The fruits are of dietary and medicinal use and have wide applications in both traditional and folk systems of medicine to treat various ailments.
- Amla is one of the highly investigated medicinal plant and studies have shown it to possess antibacterial, antifungal, antiviral, antidiabetic, hypolipidemic, anti-ulcerogenic, free radical scavenging, antioxidant, antimutagenic, anti-inflammatory and immunomodulatory, antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, snake venom neutralizing, antiatherosclerotic, gastroprotective, anti-anemic, anti-hypercholesterolemic, wound healing, anti-diarrheal, hepato-protective, nephroprotective, and neuroprotective properties.
- With respect to skin, preclinical experiments with both in vitro and in vivo systems of study have shown amla to possess skin care properties like anti-wrinkling, anti-aging, anti-melanogenic, and cancer preventive effects.
- This review addresses the scientific observations on the skin care effect of amla and emphasizes aspects that need further investigations for it to be of use in clinics in the future.

Keywords *Emblica officinalis* • *Phyllanthus emblica* • Amla • Anti-wrinkling • Anti-aging • Anti-melanogenic • Skin cancer

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Introduction

Ayurveda, the traditional Indian system of medicine is one of the oldest systems of medicine and is actively practiced in the Indian subcontinent [1]. Emphasis in Ayurveda is on disease prevention and promotion of good health by adopting proper life style and following therapeutic measures, which will rejuvenate the body [2]. The Ayurvedic remedies which are both preventive and therapeutic are mostly made of plants and when compared with their synthetic counterparts are either less toxic or non-toxic [1]. In the Ayurvedic system of medicine, references to skin care is seen and are termed under various headings such as Vayasthapana (age defying), Varnya (brighten skin-glow), Sandhaniya (cell regeneration), Vranaropana (healing), Tvachya (nurturing), Shothahara (anti-inflammatory), Tvachagnivardhani (strengthening skin metabolism), and Tvagrasayana (retarding aging) [3].

Some of the Ayurvedic formulations and plants used in these preparations are globally receiving increasing attention. In the recent past, these plants have been investigated for their pharmacological effects in accordance to the modern medicine [1]. One such plant that has been extensively studied is the medium-sized deciduous tree *Emblica officinalis* Gaertn. or *Phyllanthus emblica* Linn. belonging to the family Euphorbiaceae. The plant species which was originally native to India is today found growing in the Pakistan, Uzbekistan, Sri Lanka, South East Asia China, and Malaysia [4–6]. In colloquial terms they are known as Indian gooseberry tree, emblic myrobalans and Malacca tree in English and amla in Hindi. The other vernacular names have been listed in Table 12.1.

All parts of the plant are of use in treating various ailments, but the fruits (Fig. 12.1), which are yellowish-green in color, globular in shape, fleshy and smooth striated with an obovate-obtusely

Table 12.1 Colloquial name of *Phylanthus emblica* in different languages

Language	Names
Sanskrit	Dhatriphala, Amla, Amaliki, Amalakan, Sriphalam, Vayastha, Amalaka, Dhatri
Hindi	Amla
Arabic	Haliilaj or ihliilaj
Chinese	An Mole
English	Emblica myroblan, Indian gooseberry
French	Phyllanthe emblica
German	Amla
Italian	Mirabolano emblico
Lao	Mak kham bom
Malaysian	Popok melaka
Nepalese	Amba, amala
Portuguese	Mirabolano emblico
Thai	Ma kham pom
Tibetan	Skyu-ru-ra
Assamese	Amlakhi
Bengali	Amlaki
Gujarati	Amla
Kannada	Nellikkai
Konkani	Aavalo
Malayalam	Nellikka
Manipuri	Heikru
Marathi	Aavalaa, awla
Odiya	Aanla
Punjabi	Olay
Tamil	Nellikkai
Telugu	Usiri

Fig. 12.1 Photograph of amla

triangular six-celled nut are of immense use in the various folk and traditional systems of medicine (Fig. 12.1) [6]. The fruits are also of culinary use and are used to make pickle, chutneys, and as a vegetable in various dishes. Amla is also used to prepare a sweet delicacy by name murabbah, where the ripe fruits are soaked in concentrated sugar syrup for extended period till the aroma of the fruits exudates in to the sugar syrup. The ripe fruits are also used to prepare fresh juice and are recently marketed as concentrates which on appropriate dilution gives a ready to drink fruit juice even during the off season periods of time [4–6].

Phytochemistry

Amla is one of the extensively studied plants, and reports suggest that they contain tannins, alkaloids, and phenolic compounds. Amla is a rich source of Vitamin C [5]. The fruits also contain gallic acid, ellagic acid, chebulagic acid, emblicanin-A, emblicanin-B, punigluconin, pedunculagin, citric acid, ellagotannin, trigallayl glucose, pectin, 1-*O*-galloyl-beta-D-glucose, 3,6-di-*O*-galloyl-D-glucose, chebulagic acid, corilagin, 1,6-di-*O*-galloyl beta D glucose, 3-ethylgallic acid (3-ethoxy-4,5-dihydroxy benzoic acid), and isostrictinin [5–8]. It also contains flavonoids like quercetin, kaempferol-3-*O*-alpha-L-(6″-methyl)-rhamnopyranoside, and kaempferol-3-*O*-alpha-L-(6″-ethyl)-rhamnopyranoside [5, 6]. The volatile oils are reported to contain β-caryophyllene, β-bourbonene, 1-octen-3-ol, thymol, and methyleugenol [9]. Some of the phytochemical structures are depicted in Fig. 12.2.

Traditional Uses

Amla is ascribed with a number of medicinal properties and is a necessary constituent of many Ayurvedic medicines [2, 4, 10, 11]. It is also of use in Siddha, Unani, Tibetan, Sri Lankan and Chinese systems of medicine [2, 4, 10, 11]. In Ayurveda, amla is considered to be a potent rasayana

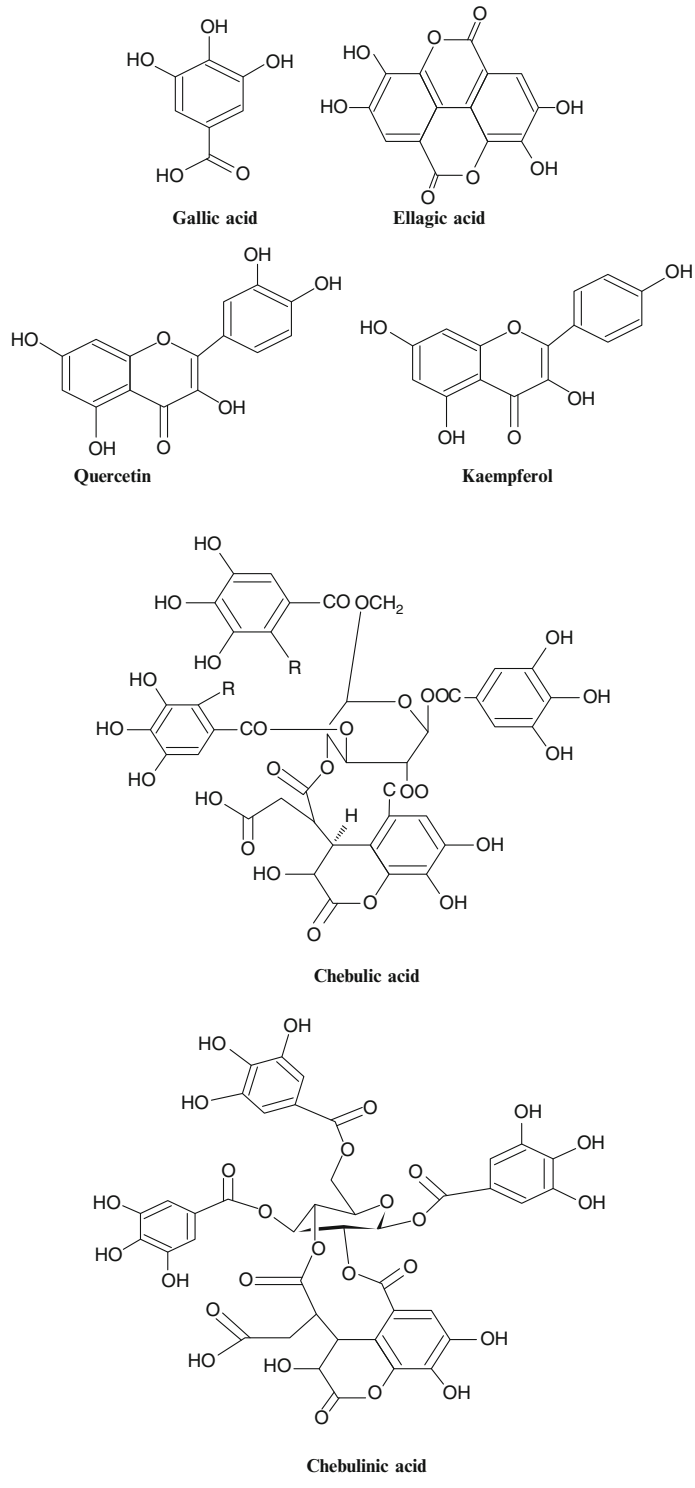

Fig. 12.2 Some important phytochemicals of amla

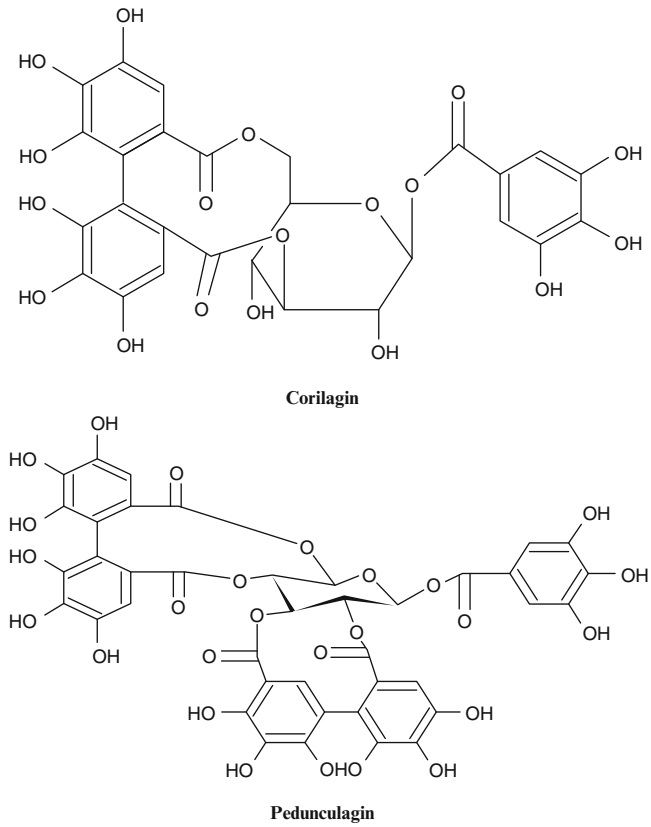

Fig. 12.2 (continued)

(rejuvenator) and to be useful in stalling degenerative and senescence process, to promote longevity, enhance digestion, treat constipation, reduce fever, purify the blood, reduce cough, alleviate asthma, strengthen the heart, benefit the eyes, stimulate hair growth, enliven the body, and to enhance intellect [2, 4, 10, 11].

In the various folk systems of medicine, the amla fruits are useful as astringent, expectorant, ophthalmic, dyspepsia, gastritis, hyperacidity, constipation, colic, colitis, hemorrhoids, hematuria, menorrhagia, purgative, spasmolytic, anemia, diabetes, cough, asthma, osteoporosis, premature graying of hair, weak vision, and fatigue [2, 4, 10, 11]. Amla is also used to treat ailments such as anemia, hyperacidity, diarrhea, eye inflammation, leucorrhea, jaundice, nervine debility, liver complaints, cough, and anomalies of urine [2, 4, 10, 11].

Scientifically Validated Studies

Preclinical studies carried out in the past three decades have validated many of the traditional uses of amla. Experiments have shown that amla possess antibacterial, antifungal, antiviral, antidiabetic, hypolipidemic, anti-ulcerogenic, free radical scavenging, antioxidant, antimutagenic, anti-inflammatory

and immunomodulatory, antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, snake venom neutralizing, gastroprotective, anti-anemic, anti-hypercholesterolemic, wound healing, anti-diarrheal, antiatherosclerotic, hepatoprotective, nephroprotective, and neuroprotective properties [5, 6]. Compelling preclinical studies with both in vitro and in vivo systems have shown that amla possess skin care effects, anti-wrinkling, anti-aging effects, and anti-melanogenic and cancer preventive effects. The following section addresses these aspects in detail.

Antibacterial

Skin and soft-tissue infections are among the most common infections, and may lead to serious local and systemic complications. These infections can be potentially life-threatening and may progress rapidly. Most infections are controlled by the topical application of antibiotic containing creams or by their systemic or intravenous administration. However the indiscriminate use of antibiotics has led to the development of drug resistance in many strains of pathogenic bacteria and studies are ongoing to discover novel agents that are effective and are safe for human consumption [12]. Multiple studies have shown that both polar and nonpolar extracts of amla possess antibacterial effects against important pathogenic bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, and *Enterobacter aerogenes* [13–16]. Fractionated studies have also shown that the chloroform soluble fraction of the methanolic extract [16], the volatile components, and essential oils possess antibacterial effects on both Gram-positive and Gram-negative bacteria [9]. With regard to phytochemicals, studies have shown that both gallic acid and ellagic acid possess antibacterial effects on *S. aureus* [17].

Anti-wrinkling and Anti-aging Effects

Aging of skin is a complex biological process and it is a consequence of both intrinsic and extrinsic factors [18, 19]. While intrinsic factors are genetically programmed and occurs with time, extrinsic factors are, to varying degrees, controllable and include exposure to sunlight, pollution or nicotine, repetitive muscle movements like squinting or frowning, and miscellaneous lifestyle components such as diet, sleeping position, and overall health [19, 20]. The structural changes that occur with the aging of the skin increases the fragility of the skin, decreases the ability of the skin to heal, increases risk for toxicological injuries, promotes development of various cutaneous disorders, and produces aesthetically undesirable effects like wrinkling and uneven pigmentation [19, 21]. Aging of skin has psychosocial impact and has created a demand for effective interventions.

Cell culture studies with human skin fibroblasts have shown that the amla extract stimulated proliferation of fibroblasts and induced production of procollagen in a concentration- and time-dependent manner [22]. Additionally, treatment with amla also decreased the production of MMP-1 and to increase TIMP-1 [22]. Subsequent studies with mouse fibroblast cells have also shown that the treatment with amla increased the type I pro-collagen level and this effect was observed to be better than an herbal collagen enhancer asiaticoside at equivalent concentration [23]. Amla also decreased the levels of collagenase activity in a dose-dependent manner [23].

Recently, Majeed et al. [24] have also observed that the amla extract protected the normal human dermal fibroblasts against the ultraviolet-B (UVB) irradiation-induced reactive oxygen species and collagen damage [24]. The authors observed that at a concentration of 0.5 mg/ml, amla extract was nearly 2.56-fold better than ascorbic acid in protecting against the UVB-induced collagen damage [24]. Additionally, amla was also observed to be 4.26-fold better than ascorbic acid in inhibiting induction of ROS at 0.5 mg/ml [24]. Studies have also shown that when compared to the UVB-treated

cohorts, treatment with ellagic acid stimulated the cell proliferation, protected pro-collagen 1, and inhibited UVB-stimulated MMP-1 induction in skin fibroblasts [25]. Amla also possess inhibitory activity on the activity of hyaluronidase in cell-free assays indicating its usefulness [25]. Recently, Adil et al. [26] have also observed that amla stimulated, the otherwise UVB inhibited cellular proliferation and protected pro-collagen 1 against UVB-induced depletion via inhibition of UVB-induced MMP-1 in skin fibroblasts.

Studies with human dermal fibroblasts and organ cultures of skin biopsies have also shown that treatment with ellagic acid protected dermal elastin from exogenous and endogenous enzymatic degradation and also to deposit significantly more elastic fibers than untreated control cultures without enhancing the transcription of elastin mRNA or cellular proliferation [25]. Detail studies have shown that pretreatment with ellagic acid enhanced biostability of tropoelastin and newly deposited elastin. Additionally, in vitro assays have also shown that ellagic acid bound to the purified elastin and significantly decreased its proteolytic degradation by elastolytic enzymes belonging to the serine proteinase, cysteine proteinase, and metalloproteinase families [25].

Recently, Bae et al. [27] have also observed that ellagic acid attenuated the UV-B-induced toxicity of HaCaT keratinocytes and human dermal fibroblasts and prevented collagen degradation by blocking matrix metalloproteinase production in UVB-exposed fibroblasts [27]. To further substantiate the cell culture observations, animal studies with SKH-1 hairless mice have shown that the topical application of ellagic acid (10 μmol/l) before exposure to UVB (for 8 weeks) decreased the production of pro-inflammatory cytokines IL-1β and IL-6 and blocked infiltration of inflammatory macrophages in the skin. Treatment with ellagic acid mitigated the inflammatory intracellular cell adhesion molecule-1 expression in UV-B-irradiated keratinocytes and photoaged mouse epidermis and together all these observations clearly indicate that ellagic acid to be effective in attenuating UVB-triggered skin wrinkle formation and epidermal thickening and to mediate these effects, at least in part by preventing collagen destruction and inflammatory responses [27].

Skin Lightening

Skin lightening preparations are widely used in dermatology by persons of all Fitzpatrick skin types [28]. Hydroquinone, which has been a standard agent for skin lightening is shown to possess deleterious effects when used on a daily basis for a long time and also to cause cancer when consumed. This has encouraged research into alternative agents that are both safe and efficacious in inhibiting skin pigmentation and as possible alternatives to hydroquinone [28]. Recently, Draelos et al. [28] carried out a double-blind study with 80 multiethnic participants to examine the skin lightening ability of a topical formulation containing kojic acid, emblica extract, and glycolic acid and compared it with the prescription generic hydroquinone cream 4%. The creams were applied twice daily for 12 weeks and the product efficacy, tolerability, and safety using investigator assessment, participant assessment and dermospectrophotometry were noted. The results indicated that both study product and hydroquinone 4% were equally effective in causing skin lightening effects in mild to moderate facial dyschromia [28].

Studies have also shown that both ellagic acid [29] and gallic acid [30] are effective in inhibiting melanogenesis. Cell-free assay with mushroom-derived tyrosinase and cell culture studies with B16 melanoma cells have shown that incubation with ellagic acid and gallic acid decreases the activity of the tyrosinase [29, 30]. Additionally, mechanistic studies have shown that treatment with copper caused a dose-dependent reversal of the inhibitory effects induced by ellagic acid, indicating that the anti-tyrosinase inhibitory effects were mediated by the binding of ellagic acid to the copper in the enzyme [29]. Studies with brownish guinea-pigs having melanocytes in their skin have also shown that the topical application of ellagic acid for 6 weeks was effective in suppressing the UV-induced (2 weeks) skin pigmentation possibly by suppressing melanogenesis by reacting with activated melanocytes and also by not injuring the skin cells [29].

Amla and Some of Its Phytochemicals Prevent Skin Cancer

Globally, skin cancer is the most common type of cancer in the fair-skinned populations and its incidence and mortality rates are dramatically increasing. Individuals with familial genetic syndromes are susceptible to specific types of skin cancers but in most cases exposure to ultraviolet radiation (UV) from sun exposure is the most important cause of skin cancer. Additionally, viral infections by the human papilloma virus, exposure to ionizing radiation, environmental pollutants, chemical carcinogens, and work-related exposures to organic solvents and toxic metals have also been associated with skin cancers [31].

Chemoprevention, a science that has emerged during the past three decades, presents an alternative approach to reducing mortality from cancer. It aims to block, reverse, or delay carcinogenesis before the development of invasive disease by targeting key molecular derangements using pharmacological or nutritional agents at non-toxic concentrations [32].

Scientific studies carried out in the past three decades have shown that natural products possess inhibitory effects on carcinogenesis and amla is shown to prevent liver and skin cancer [33]. Animal studies have shown that amla was effective in preventing two-stage carcinogenesis (DMBA-induced and croton oil promoted) in mice and that there was a considerable delay in tumorigenesis, cumulative number of papillomas, tumor incidence, tumor yield, and burden [34]. The authors observed that feeding amla for 7 consecutive days before and after DMBA application was less effective than when administered during the promotion (starting from the time of croton oil treatment and continued till the end of experiment for 16 weeks) [34]. However the best effect was observed when amla was fed throughout the experimental period, i.e., before and after DMBA application as well as during the promotional stage [34]. Amla is shown to possess free radical scavenging effects, to decrease Phase I enzymes, to increase Phase II enzyme (GST), to decrease ornithine decarboxylase, to increase antioxidant enzymes, to possess immunomodulatory effects, to decrease lipid peroxidation, to possess anti-inflammatory effects and antimutagenic effects [33].

With respect to the phytochemicals present in amla, studies have shown that topical application of ellagic acid resulted in significant protection against MCA-induced skin tumorigenesis in Balb/C mice [35] and DMBA-induced skin tumorigenesis in NMRI Swiss mice [36]. Studies have also shown that topical application of ellagic acid simultaneously with phorbol-12-myristate-13-acetate (PMA) or mezerein protected against DMBA-induced skin tumors in mice [37]. Topical application of ellagic acid before application of the tumor-initiating dose of benzo[a]pyrene and its ultimate carcinogenic metabolite (±)-7 beta, 8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (B[a]P 7,8-diol-9,10-epoxide-2) also inhibited the TPA-induced skin tumorigenesis [38]. Experimental studies with rats have also shown that ellagic acid prevented Tris-ethanol-induced lipid peroxidation and the rate of necrosis [39].

Mechanistic studies have shown that pretreatment with ellagic acid inhibit TPA-induced increase in ornithine decarboxylase [40, 41], production of hydroperoxides in the epidermis [41–43], and DNA synthesis [41]. Gallic acid another phytochemical of amla is also shown to possess inhibitory effects on the TPA-induced induction of epidermal ornithine decarboxylase activity, hydroperoxide production, and DNA synthesis [43]. Ellagic acid is also shown to be a potent inhibitor of epidermal microsomal aryl hydrocarbon hydroxylase activity in vitro [44] and of benzo[a]pyrene (BP)-binding to both calf thymus DNA in vitro and to epidermal DNA in vivo [44], to affect covalent binding of the anti-diol epoxide of benzo[a]pyrene to cellular DNA of mouse skin in organ culture [45], inhibit epidermal microsomal aryl hydrogen hydroxylase activity, and of benzo[a]pyrene (BP) binding to epidermal DNA in vivo [46]. Cell culture studies with keratinocytes have also shown that ellagic acid also caused a dose-dependent inhibition of the cytochrome P-450-dependent monooxygenases, aryl hydrocarbon hydroxylase, and 7-ethoxycoumarin-*O*-deethylase [47].

Cell culture studies have also shown that gallic acid inhibited cell proliferation and induced apoptosis in A375.S2 human melanoma cells. Treatment with gallic acid caused morphological changes, induced apoptosis and cell death in a dose- and time-dependent manner. Gallic acid mediated the apoptosis by down-regulating antiapoptotic proteins (Bcl-2), up-regulating the proapoptotic proteins (Bax), induced reactive oxygen species (ROS), increased intracellular production of Ca^{2+}, and decreased mitochondrial membrane potential. Gallic acid triggered cytosolic release of apoptotic molecules, cytochrome *c*, promoted activation of caspase-9 and caspase-3, and ultimately apoptotic cell death. It also promoted cytosolic release of apoptosis-inducing factor (AIF) and endonuclease G (Endo G) [48]. Additionally, studies have also shown that gallic acid inhibits the migration and invasion of A375.S2 human melanoma cells through the inhibition of matrix metalloproteinase-2 and Ras [49]. Together both these observations clearly indicate the usefulness of gallic acid in the possible treatment of melanoma.

Conclusions

Preclinical studies have unequivocally shown that amla and its phytochemicals gallic acid and ellagic acid possess beneficial effects on the skin thereby validating the ethnomedicinal observations. Although considerable work has been done to exploit the skin care effects of amla, countless possibilities for investigation still remain. Further in-depth mechanistic studies in both in vitro and animal model, and rationally designed clinical trials are required. Amla or its phytochemicals have never been studied for their adverse effects or beneficial effects when applied topically for extended period of time. These studies are also important as this will help in making us realize their importance in skin care. Due to its abundance, low cost, and safety in consumption, amla remains a species with tremendous potential and countless possibilities for further investigation.

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Chapter 13
Review on the Use of *Aloe vera* (Aloe) in Dermatology

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Key Points

- Since antiquity, *Aloe vera* (L.) Burm. f. Syn (*Aloe barbadensis* Miller), commonly known as the aloe, burn plant, lily of the desert, elephant's gall in English, has been one of the most important plant in skin care in different parts of the world.
- The leaf pulp and exudates have wide applications in both traditional and folk systems of medicine to treat various ailments.
- Aloe is reported to possess antidiabetic, antiulcer, anti-septic, antibacterial, anti-inflammatory, antioxidant, anticancer agent and in treating stomach ailments, gastrointestinal problems, constipation, dysentery, and diarrhea.
- With respect to skin, scientific studies have shown aloe to possess skin care properties like preventing aging and senescence, acne, psoriasis, enhancing wound healing, UV-induced skin damage, and chemical carcinogenesis.
- Emodin, an anthraquinone of *A. barbadensis*, is shown to enhance the UV-induced primary cutaneous melanin-containing tumors. However emodin alone was not carcinogenic.
- The present review addresses the scientific observations on the skin care effect of *A. vera* and emphasizes aspects that need further investigations for it to be of use in clinics in the future.

Keywords *Aloe vera* • *Aloe barbadensis* • Aging • Senescence • Acne • Psoriasis • Wound healing • Chemical carcinogenesis

Introduction

Aloe vera (L.) Burm. f. *Aloe barbadensis* Miller (Fig. 13.1), family Asphodelaceae, is a important medicinal plant and history suggest that it was used by the natives of ancient Greece, Rome, Babylonia, Arabian countries, Egypt, India, and China. The plant is supposed to be native of North Africa and

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Fig. 13.1 Photograph of an *Aloe vera* plant

Spain; however, today the plants are found growing also in Mexico, the Pacific Rim countries, India, South America, Central America, the Caribbean, Australia, and Africa [1–3]. *A. vera* plants are shrubby or arborescent, perennial, and pea-green in color. They are extremely sensitive to freezing temperatures but grow profusely in the warm tropical areas as the leaves have water storage tissue and this helps them survive in dry areas where rainfall is minimal or erratic [1]. Historical observations indicate that the name *Aloe vera*, or true Aloe, is probably derived from the Arabic word "*alloeh*," Syrian "*alwai*," or Hebrew "*halal*" meaning a "*shining bitter substance.*" It is commonly called aloe, burn plant, lily of the desert, elephant's gall [1–3]. The terminologies in various other languages are enlisted in Table 13.1.

Phytochemically, *A. vera* is one of the well-studied plants and is reported to constitute 99–99.5% of water, while the remaining solid material (1–0.5%) is reported to contain over 75 different ingredients that include vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, chromones, flavanoids, lignin, saponins, sterols, amino acids, and salicylic acid [4]. The levels and ratio of phytoconstituents are shown to vary with the growing conditions, temperature, harvesting, and processing of the plants. The details on the compounds and the class to which they belong are enlisted in Table 13.2.

Table 13.1 Name of *Aloe vera* in various languages

Indian languages	Names
Sanskrit	Kumari, ghrita-kumari
Hindi	Gheekumari, gheekwar, gvar patta, gvarapatha, musabbar
Bengali	Ghrita kumari, kumari, musabbar
Gujrati	Kunwar
Kannada	Lolisara, kathaligida, kathalae, lavalasaara
Konkani	Lolisara
Malayalam	Chotthu kathalai, kaattu vazha, katar vazha
Marathi	Pivalaboel, korphad, kora-kanda
Punjabi	Elwa
Rajasthani	Gawar patha
Tamil	Kathalai
Telugu	Kathalai
Urdu	Gheekwar, aelwah
English	Indian Aloe
Arabic	Mussavar, alwah haqeeqiyyah, sabr suqutree, sabir haqeeqi, sibr asqootree
Chinese	Nu hui, lu hui, hsiang tan
French	Aloé indiana
German	Aloe, echte aloe, wundkaktus, bitterschopf
Persian	Aelwah
Spanish	Zabila

Table 13.2 Summary of the chemical composition of *Aloe vera* leaf pulp and exudates [1–4]

Class	Compounds
Anthraquinones, Anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B, isobarbaloin, emodin, ester of cinnamic acid
Chromones	8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A, 8-*C*-glucosyl-(*S*)-aloesol, 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, 8-*C*-glucosyl-7-*O*-methylaloediol, 8-*C*-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoaloesin A
Inorganic compounds	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactogalacturan, arabinogalactan, galactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose
Lipids	Arachidonic acid, γ-linolenic acid, steroids (campestrol, cholesterol, β-sitosterol), triglicerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid
Amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, superoxide dismutase
Vitamins	Vitamins B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol

Traditional Uses

Since antiquity, aloe plants have been used in the various folk systems of medicine in health, beauty, and skin care. Historical evidences suggest that aloe was used in almost all ancient civilizations as a wound healing agent, to relieve itching, swelling, reduce inflammation, bacterial infection, and to promote healing of injured tissues. In the various systems of medicine, aloe is also considered to be a blood purifier, anti-inflammatory, diuretic, uterine tonic, spermatogenic, and laxative. In the Indian traditional system of medicine, the Ayurveda, aloe has been used to stimulate appetite, as a purgative, emmenogogue, and antihelmenthic. It is supposed to be effective in treating cough, colds, piles, general debility, asthma, liver ailments, and jaundice. *A. vera* is also believed to be useful in improving immunity, prevent premature graying of hair, protect heart, brain, and other vital organs of the body [1–4].

Validated Studies

Acne is a global problem and scientific studies carried out in the past have shown that *A. vera* possess antidiabetic, antiulcer, anti-septic, antibacterial, anti-inflammatory, antioxidant, anticancer agent and is also useful in treating stomach ailments, gastrointestinal problems, constipation, dysentery, and diarrhea [1–4]. *A. vera* has been studied extensively for its skin care effects and experiments have shown that the aloe gel is useful as a wound healing agent, in heat burns, radiation injury, and skin diseases. Additionally the leaf pulp is also used in the cosmetic and toiletry industry as base material for the production of creams, lotions, soaps, shampoos, facial cleansers, and other products. In the pharmaceutical industries it has been used for the manufacture of topical products such as ointments and gel preparations [1–4]. In the following paragraphs the validated skin care properties of *A. vera* are accordingly discussed.

A. vera in Acne

In vitro studies have shown that the ethanolic extract of both gel and the leaf inhibited the growth of *Staphylococcus aureus*, the gel to be effective only on *Trichophyton mentagrophytes* and only the leaf to be effective on both *Pseudomonas aeruginosa* and *Candida albicans* [5]. Additionally studies have also shown *A. vera* to be effective in inhibiting *Shigella flexneri*, Methicillin-Resistant *S. aureus* (MRSA), *Enterobacter cloacae*, and *Enterococcus bovis* [6] and against the fungal species associated with superficial mycoses [7]. Contradicting these observations, studies have also shown that *A. vera* was ineffective in preventing the *Propionibacterium* acnes-induced ROS and pro-inflammatory cytokine production in polymorphonuclear leukocytes and monocytes [8], indicating aloe to be ineffective against these organisms.

A. vera Prevents Aging and Senescence

Clinical studies with 30 healthy female subjects (>45 years) has shown that the dietary supplementation of *A. vera* gel (1,200 mg/day or 3,600 mg/day) for 90 consecutive days improved wrinkling and

elasticity in the photoaged skin. Mechanistic studies indicated that the *A. vera* increased collagen production and decreased the collagen-degrading MMP-1 gene expression [9] to mediate the observed effects at least in part.

A. vera in Psoriasis

A. vera gel has been used to treat psoriasis in various folk systems of medicine. Recently Dhanabal et al. [10] have observed that when compared with the negative control, the ethanolic extract of the gel was effective and produced a significant increase in relative epidermal thickness in the mouse tail model for psoriasis. Clinical studies with 60 patients with slight to moderate chronic plaque-type psoriasis have shown that the application of *A. vera* extract for 16 weeks with 12 months of follow-up on a monthly basis was effective in curing psoriasis in 83.3% of patients (25/30), while in the placebo control the cure rate was a meager 6.6% (2/30) [11]. To further substantiate these observations, studies by Choonhakarn et al. [12] have also shown that the topical application of *A. vera* for 8 consecutive weeks was effective in decreasing the Psoriasis Area Severity Index score from 11.6 to 3.9 and improving the quality of life of patients with mild to moderate psoriasis. However contradicting these observations Paulsen et al. [13] in their double-blind, placebo-controlled right/left comparison study (2-week wash-out period followed by a 4-week treatment period with two daily applications and follow-up visits after 1 and 2 months) have observed that the commercial *A. vera* gel was not better than the placebo in healing stable plaque psoriasis suggesting that detailed studies with large number of patients are warranted with standardized aloe preparation.

A. vera in Wound Healing

Topical application of aloe both alone and in combination with mafenide acetate, three times a day for 14 consecutive days, was effective in enhancing the process of wound contraction and neutralizing the wound retardant effect seen with the topical mafenide acetate alone. When compared to controls (placebo treated), the application of aloe increased the breaking energy. This effect appears to be due to an increased collagen activity, improving collagen matrix and enhancing the breaking strength [14]. *A. vera* alone and in combination with a microcurrent is also shown to accelerate wound healing in rats, by advancing the proliferative phase [15]. Studies with both rats and rabbits have shown that the application of *A. vera* on the incised open wounds enhanced the wound closure rate thereby substantiating the previous reports [16].

Mechanistic studies have shown that the topical and oral treatments with *A. vera* enhanced the synthesis of glycosaminoglycan components (hyaluronic acid and dermatan sulfate) in the matrix of a healing wound to increase the collagen content of the granulation tissue and the degree of crosslinking [17, 18]. Additionally the type I/type III collagen ratio of the *A. vera* treated groups was lower than that of the untreated controls indicating enhanced levels of type III collagen [18]. *A. vera* increased collagen biosynthesis, degradation of collagen, increased lysyl oxidase and cross linking of the newly synthesized collagen [19]. Studies with diabetic rats have shown that oral and topical application of *A. vera* gel enhanced the wound healing and the wound tensile strength. It reduced the wound closure time by influencing inflammation, fibroplasia, collagen synthesis and maturation, and wound contraction [20].

A. vera in Healing of Burn Wounds

With respect to healing of wounds induced by high temperatures, studies have shown *A. vera* to be effective in promoting wound healing when applied on a burn wound in rats [21, 22]. Additionally, comparative studies with rats have also shown that *A. vera* was better than silver sulfadiazine in healing burn wounds [23]. Clinical studies with a small number of partial thickness burn wound patients (27) have also shown that treatment with *A. vera* gel was more effective than the Vaseline gauze in ensuing healing. Histopathological studies showed early epithelialization in the cohorts treated with *A. vera* gel indicating the effectiveness of *A. vera* gel on a partial thickness burn wound and its benefit in treating burn wounds [24]. Mechanistic studies have clearly shown that *A. vera* inhibited the inflammatory process following burn injury, as characterized by the reduction of leukocyte adhesion, as well as the pro-inflammatory cytokines TNF-α and IL-6 [22], decrease wound tissue nitric oxide release, optimize nitric oxide/endothelin ratio, lighten vascular inflammatory reaction, and lessen permeability and edema [25].

A. vera and Olive Oil Combination Cream Reduces Sulfur Mustard Induced Skin Damage

Exposure to sulfur mustard causes severe lesions that range in severity from mild erythema to blister formation and necrosis in the early stages and xerosis, chronic pruritic skin lesions, hypo or hyper pigmentation, scars, and rarely, skin cancers as long-term effects. Recently, Panahi et al. [26] in their randomized double-blind clinical trial with Iranian chemical warfare-injured veterans have observed that *A. vera*/olive oil combination cream reduced the frequency of pruritus, burning sensation, scaling, and dry skin, reduced the mean pruritus and visual analogue scale scores. The effect of the combination was observed to be at least as effective as betamethasone 0.1% indicating a promising therapeutic option for the alleviation of symptoms in mustard gas-exposed patients [26].

A. vera Against the Ionizing Radiation-Induced Skin Damage

Radiation causes severe skin burns and impairs wound healing thereby aggravating the morbidity of the patient. *A. vera* has been investigated for its radioprotective properties and the results have been mixed and inconclusive. In one of the earliest studies Sato et al. [27] observed that *Aloe arborescens* protected mice against X ray-induced skin injury. Subsequently, it was observed that methanol extracts of the *A. arborescens* was effective in scavenging reactive oxygen and protecting the DNA [28]. Studies have also shown that the *A. vera* leaf extract protects mice against radiation-induced sickness and lethality and mediates the protective effects by increasing the antioxidant (glutathione, catalase, and superoxide dismutase) and decreasing the lipid peroxidation [29]. Studies with C3H mice have also shown that aloe gel when applied daily for at least 2 weeks beginning immediately after irradiation (30–47.5 Gy) was effective in reducing the skin reactions and decreasing the ED50 values for skin reactions by approximately 7 Gy [30].

Atiba et al. [31] studied the effectiveness of oral administration of aloe gel in promoting wound healing in rats previously exposed to radiation (3 days post-irradiation) and observed that it is effective. The authors observed that when compared to the cohorts receiving placebo, administration of *A. vera* gel increased the inflammatory cell infiltration, fibroblast proliferation, collagen deposition, angiogenesis, and the expression levels of TGF-β-1 and bFGF (or FGF-β) [31]. Contrary to the preclinical observations, the human studies have also shown *A. vera* gel does not protect against radiation

therapy-induced dermatitis in patients undergoing radiotherapy for the breast [32–34]. However topical application of the aqueous cream was observed to be significantly better than *A. vera* gel in reducing dry desquamation and pain related to treatment (Heggie et al., 33), indicating a need for detail studies.

A. vera in Preventing the Ill Effects of UV-Induced Damage

In one of the earliest observations Strickland et al. [35] have observed that topical application of the aloe gel extract (0.167–1.67% aloe gel) after exposure to radiation (400 J/m^2 UVB) for 4 consecutive days was effective in preventing the fluorescein isothiocyanate (0.5%) induced contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) in C3H mice. The authors observed that when compared to the skin of mice given only UVR or UVR plus the vehicle (placebo), posttreatment with aloe partially preserved the number and morphology of Langerhans and Thy-1+ dendritic epidermal cells in skin. Pretreatment with the gel and then exposure to a single dose of UVR (2 kJ/m^2) showed that the effect of aloe was not due to screening [35]. Application of aloe after exposure to UVB radiation (5 or 10 kJ/m^2) was effective in suppressing DTH to *C. albicans* or contact hypersensitivity to fluorescein isothiocyanate in C3H mice [35]. Aloe treatment did not prevent the formation of cyclobutyl pyrimidine dimers in the DNA of UV-irradiated skin or accelerate the repair of these lesions thereby indicating that the observed protection was not due to prevention of DNA damage or induction of DNA repair [35].

Subsequent studies have shown that the application of poly/oligosaccharides of *A. barbadensis* to UV-irradiated skin prevented suppression of DTH responses in vivo and reduced the amount of IL-10 in the UV-irradiated murine epidermis. Aloe poly/oligosaccharides were also effective in preventing the suppression of immune responses to alloantigen in mice exposed to UVB irradiation (30 kJ/m^2). When compared to the radiation alone cohorts, treatment of keratinocytes (Pam212 cells) with immunoprotective carbohydrates after exposure to UVB radiation (300 J/m^2) reduced IL-10 production by approximately 50% [36]. Additionally, randomized double-blind, placebo-controlled, phase III studies with 40 volunteers with skin types II and III have shown that the topical application of *A. vera* gel (97.5%) after exposure to 1.5-fold minimal erythema dose of UVB was effective in preventing the inflammation. The anti-inflammatory effects were not as effective as 1% hydrocortisone cream but still indicate Aloe vera's potentiality as a protective agent against the UV-induced immune suppression and inflammation and a useful agent for skin care [37].

Contrary to the observations with the whole *A. barbadensis* poly/oligosaccharides, studies have shown that emodin, an anthraquinone found in *A. barbadensis* and other plants, causes primary cutaneous melanin-containing tumors only in combination with exposure to UV radiation. The authors observed that the combination of emodin and UV caused almost double the number of tumors (20–30%) when compared to the concurrent radiation alone cohorts who received the placebo (ethanol) and then exposed to UV in identical conditions (50–67%). In the absence of UV radiation, neither ethanol nor aloe-emodin induced skin tumors. These observations clearly indicate that increased numbers of melanoma are caused only when emodin and UV are combined [38]. Subsequent studies have shown that the combining emodin with UV caused mutations in the critical tumor-suppressor gene p53 in exons 4, 5, 6, 7, and 8 while UV alone caused mutations predominantly in p53 exons 5 and 8 [39].

A. vera in Preventing Chemical-Induced Skin Cancer

Animal studies have shown that oral administration (1,000 mg/kg body weight/day), application of aloe gel (at a dose 1 ml/9 cm^2/mice/day), and combination of the two were effective in preventing DMBA-induced and croton oil promoted skin carcinogenesis in Swiss albino mice [40, 41]. When compared

to the cohorts receiving placebo and the carcinogen treatment (DMBA and croton oil), treatment with *A. vera* was effective in delaying the appearance, reducing the number and size of the papillomas. To substantiate these observations the ethyl acetate extract of the acetone-soluble *Aloe arborescens* fraction is also shown to inhibit TPA-induced ear edema, putrescine increase, and tumor promotion in mouse skin [42] and to reduce the levels of lipid peroxidation in skin [40]. Together all these observations indicate the usefulness of *A. vera* in the prevention of chemical-induced skin carcinogenesis.

Conclusions

Scientific studies carried out in the recent past have validated the skin care effects of *A. vera* in enhancing open wound healing process, preventing UV-induced skin damage, chemical carcinogenesis preventing aging and senescence. However, contradictory observations have been reported in the case of the psoriasis and this may be due to the variations in the levels of bioactive compounds. Additionally, the observations that emodin when applied before exposure to UV radiation enhances the primary cutaneous melanin-containing tumors, clearly indicate the need for more studies with the phytochemicals considered to be nontoxic. The pharmacological activity of aloe extracts appears to be due to the various constituents like vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, chromones, flavanoids, lignin, saponins, sterols, amino acids, and salicylic acid. The final ratio of these compounds in *A. vera* is determined by a number of factors, including the geographic origin, the maturity of the rhizomes at the time of harvest, and the method by which the extracts are prepared as only then will the observations be more meaningful and of use in human application.

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Part IV
Dietary Components and Skin Health

Chapter 14
Chocolate and Skin Health

Leena Patel and Ronald Ross Watson

Key Points

- Chocolate contains polyphenols, which contain antioxidants that provide numerous health benefits
- Chocolate provides UV photoprotection
- Other nutrients in chocolate provide additional dermal health benefits
- Chocolate's components do not affect acne vulgaris
- Antioxidants in chocolate reduce signs of aging such as wrinkles

Keywords Flavanols • Palatability • Acne vulgaris • Polyphenols • Aging • Cocoa • Photoprotection • Antioxidants • Ephelides

Perceptions About Chocolate

Chocolate is a sweet dessert that brings satisfaction. Nonetheless, chocolate is a complex food that has many benefits for the body. Certain types are known to provide advantages in terms of blood pressure and flow, total and LDL cholesterol, insulin sensitivity, and may have anticancer properties [1]. Recently, the components of chocolate that is most beneficial to the body, flavanols, have received considerable research attention from nutritionists, scientists, and the media [1]. Flavanols are secondary plant constituents found in blueberries, cranberries, grapes, apples, red wine, tea, and cocoa [2]. While chocolate contributes a large amount of kilocalories and sugars to the body, the flavanols provide essential nutrients that should improve the health of the body. In addition, there has been some controversy in the general population about the effect that chocolate provides on the onset or inflammation of acne in adolescents [3]. Nonetheless, research has shown that antioxidants are a necessary part of one's diet, and this information has boosted consumption of chocolate [2]. In fact, the popularity of chocolate and its potential ability to provide health benefits has caused a substantial amount of research because it has a high palatability [4]. Palatability is how acceptable or agreeable

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to the taste a food is. The use of chocolate in order to benefit human health ushers in a new idea that foods enjoyed by much of the population may provide health advantages, and therefore, the role of chocolate in dermal health is reviewed.

Perceptions About Skin Health

Appearance of skin is often one of the most important concerns for many people. More than 17 million Americans suffer from the skin disease acne vulgaris. Many people would also like to boost their skin health in order to prevent signs of aging, sun damage, and the onset of other unpleasant skin problems. Diet is one of the most important modifiers of skin health, and the intake of a variety of micronutrients and macronutrients is crucial [2]. Photoprotection, cutaneous blood flow, skin structure and texture, hydration, and water loss are important factors that affect a person's overall skin health. In order to have healthy skin, one must keep a diet that balances each of these aspects. Wrinkling is also an aspect of skin health that often shows after aging, tanning, or other factors. Freckles on the skin are often seen as displeasing [5], and can be a result of genetics, while age spots and sunspots are a result of direct ultraviolet (UV) radiation [6]. Overall skin health can be greatly affected by diet as the body gains necessary nutrients for dermal health through foods that are consumed.

Chocolate

Chocolate is a solid made of cocoa liquor, cocoa butter, and sugar. The type of chocolate depends on the proportion of the product that is made of cocoa liquor. Milk chocolate contains 10–12% chocolate liquor, while dark or bittersweet chocolate has 35% or more, and white chocolate contains no cocoa liquor [7]. Chocolate is made from cacao beans, which are seeds grown on the *Theobroma cacao* tree, that is found in tropical regions [1]. These seeds are dried and fermented from the fruit on this tree [7]. These beans were traditionally employed in order to reduce pain of fever, cough, and pregnancy [8]. They are known to be filled with polyphenols and therefore have a high antioxidant content [1]. Polyphenols are compounds that promote health and assist in the prevention of certain diseases [9]. Specifically, cacao beans have xanthine derivatives, catechins, and flavonoids [8]. Xanthines are found in most human body tissues and fluids and a number of mild stimulants are derived from xanthines [8].

As a complex food, chocolate has many bioactive compounds. In addition to flavanols, chocolate also contains lipids, vitamins, minerals, fiber, and polyphenols [7]. Unfortunately, not all chocolate products are made with the same methods, and because of this, these products do not have the same concentration of flavanols or antioxidants. The type and origin of the bean, growing conditions, post-harvest handling, and manufacturing process affect component concentrations [1]. Often, these changes, especially those in manufacturing, can affect the antioxidant content negatively, thus making chocolate a less reliable source of these nutrients. Nonetheless, chocolate contains a considerably larger amount of antioxidants than other foods, and thus have been used by scientists to understand the effects of antioxidants to the body [7].

Flavonoids are a subclass of polyphenols from secondary plant metabolites and can alter enzyme activity, influence anti-inflammatory effects, and affect cell division (Fig. 14.1). Some animal studies have found that tea flavanols can decrease reactions (such as burning and damage) based on UV exposure [10]. It is thus expected that cocoa will similarly benefit skin health and decrease sensitivity of skin to UV damage.

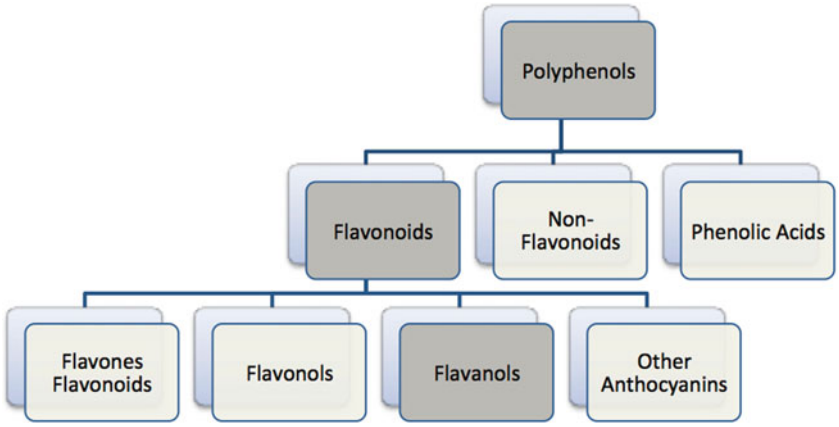

Fig. 14.1 Polyphenol/flavonoid flowchart

Cocoa and its derivatives have been found to contain large amounts of polyphenols (especially flavonoids, and more specifically flavanols). The main types of flavanols found in cocoa are epicatechin, catachin, and procyanidins. The latter provides the majority of the antioxidant content in cocoa [7]. These nutrients are important, as they are a pivotal factor in the activation and deactivation of many internal processes, and act synergistically with vitamin C, selenium, and other nutrients [7]. Because similar polyphenols are found in commonly researched products such as green tea, wine, olive oil, and fish oil, similar benefits are expected to take place by consuming chocolate.

Skin Health

Skin is the largest organ of the body and is most visible to others. There are many things that affect skin because it is exposed to air, sunlight, chemicals, and other environmental hazards. Because skin is the first defense barrier of the body from the environment, it is crucial that it stays healthy and is at optimal condition to perform. Poor dermal health may cause bacteria, viruses, and other pathogens to enter the body and decrease the immunity of the body. If the body's secondary defenses cannot react quickly to breaks in the skin, infections and illness can occur.

Freckles or ephelides are found most often on fair skin, though they can be found on dark skin as well, and are pigmented spots that are obtained genetically and mostly appear at a young age [5]. Solar lentigines, age spots, are darkly pigmented areas of skin; they are produced during aging by photo-damage, and along with ephelides, are risk indicators for skin cancer [6]. Because the only exogenous causal factor for melanoma that has been established is sunlight, it is of special importance that attention be paid to this and how chocolate affects photocarcinogenesis [11]. In addition, chronic UV exposure is an important cause of premature skin aging [1]. Skin ages similarly to other organs with decreased cellular function but can age prematurely by overexposure to UV radiation [12]. Short wavelength UV radiation is especially known to cause skin photo-damage, wrinkling, and skin cancer [8]. This is because the exposure produces radicals in the skin and thus decreases the skin's antioxidant system [1]. Therefore, if antioxidant levels are heightened, this loss is less significant and causes fewer changes to the skin's health. Because of this, skin loses rigidity, elasticity, and resilience, thus making it seem rough, leathery, and wrinkled [12]. Thus, some topical administrations of vitamin E

and C and phytochemicals provide skin protection [1, 12]. With this information, it is expected that similar benefits would occur with topical administration of cocoa.

Acne vulgaris is the result of obstruction and thus the inflammation of hair follicles. It may result in inflammation or non-inflammation and cause the multiplication of bacteria in the follicle and its sebaceous gland. Especially during hormonal changes, the follicle becomes blocked [3]. Acne vulgaris affects 85% of the population between 11 and 30 years of age and is the most prevalent skin condition [13]. There is an interesting association between acne and milk consumption, which can increase risk of acne, potentially because of the hormones it contains. The commonly consumed milk chocolate contains milk products and therefore, it could increase the risk of acne vulgaris. However, chocolate has no effect on acne vulgaris [3]. Specifically, this is because it does not affect sebum production or composition [13]. Thus, the commonly misconceived relationship between chocolate and acne can be disregarded.

Chocolate and Skin Health

Chocolate provides a number of benefits in terms of reducing or even reversing adverse actions on skin. Application of xanthines directly onto the skin reduced wrinkle formation and connective tissue alteration. In addition, antioxidants found in chocolate have also been found to assist in this process of prevention or remodeling [8]. Thus, in the elderly or aging, topical application of chocolate's derivatives can be most beneficial for reducing signs of age and maintaining a youthful appearance.

Regular consumption of cocoa (in beverage form) strengthens photoprotection of the skin and increases microcirculation, which influences nutrient delivery, skin condition and appearance [7, 10]. Photoprotection has been increased by the use of carotenoids (fat-soluble vitamins, such as those found in tomatoes) and vitamin C from supplements and foods, and used to prevent sunburn and skin damage based on antioxidants and interference with UV responses of the skin tissue [10]. Density and hydration of the skin are elevated and loss of water from the skin's surface is lowered by chocolate. Additional benefits include increased quality of the skin with decreased roughness and scaling. This may be for the reason that cocoa's antioxidants in plasma increases, heightening scavenging oxygen [2].

Another interesting use of cocoa derivatives is the commercial usage of cocoa butter in lotions and moisturizing products. Such products have increased moisture and may have antiaging abilities by decreasing the formation of wrinkles as they keep the skin supple and moisturized. This form of cocoa is substantial, as some research has been conducted using topical chocolate. The lipids found in cocoa butter play an important role in keeping dermal moisture at optimal levels, as it creates a barrier that water cannot cross and leave the body through evaporation from the skin's surface.

Conclusions and Perspectives

The use of chocolate has been reviewed in terms of benefiting skin health. There are many forms of chocolate and different levels of cocoa content in many products. While chocolate is highly palatable, it also contains a number of nutrients that aid the body in keeping healthy. Such nutrients include antioxidants, lipids, vitamins, and fibers. The combination of these products provides the body, especially the skin, with important benefits. Skin health is crucial for overall body health and is made up of a number of factors such as microcirculation and nutrient delivery. Skin conditions such as age spots, wrinkles, freckles, and sunburn can be positively affected by chocolate. Both topical and oral chocolate use benefit skin health in terms of improving factors and providing photoprotection from UV rays. However, only association between acne vulgaris and the detrimental effects of chocolate

was unsubstantiated. Rather, this association is due to sugar content rather than chocolate content. Thus, chocolate has been found to be beneficial to dermal health and can be an important food used to increase dermal health. Chocolate is especially desirable as a beneficial food because it is consumed regularly and is well received by the overall population in many forms. Further research should be undertaken in order to understand how chocolate may affect other detrimental skin conditions in order to increase awareness of the benefits of chocolate consumption and to provide an enjoyable avenue for improvement to dermal health.

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Chapter 15
Natural Dietary Factors (Products), Antioxidants, and Skin Health

Howard A. Epstein

Key Points

- Symptoms of vitamin deficiency may be manifested by abnormal skin appearance
- Studies indicate that a diet that is rich in vegetables, legumes, fish and that is low in sugar and dairy intake is correlated with reduced actinic damage.
- Proteins throughout the cell interact with specific receptors to modify the cell's response to the environment.
- Many proteins function by changing their conformation induced by binding of a specific molecule
- The generation of reactive oxygen species (ROS) within certain boundaries is essential to maintain homeostasis.

Keywords Cell function • Metabolism • Vitamins • Flavonoids • Flavanoids • Antioxidants • Skin health

Introduction

The discovery of vitamins and human vitamin deficiency as manifested through the abnormal appearance of skin exemplifies the earliest scientific studies connecting dietary factors with the health of skin. In 1775 scurvy, a deficiency of vitamin C was described as a disease exhibiting a change of facial skin color to a pale bloated complexion, spots appearing on skin that ultimately developed into large ulcerating blotches. Juice from oranges, lemons, and limes were found to cure the disease. It was not until 1932 that ascorbic acid was identified as the curative agent in citrus fruits. Vitamin C in a stabilized form is formulated in cosmetic skin care products to treat fine lines and wrinkles and to promote a more even skin tone. Pellagra, another vitamin deficiency disease, is manifested with typical symptoms that include cutaneous skin lesions. In 1927 Dr. Joseph Goldberger realized that dried yeast was effective in treating the disease. Nicotinic acid and its derivatives were determined to be the active agents in yeast curing the disease. Niacinamide, a derivative of nicotinic acid is currently applied topically to even skin tone. More recently a study involving 177 Greek-born individuals living in Australia and 69 individuals living in Greece, 48 Anglo-Celtic Australians living in Melbourne and 159 Swedes living in Goteborg, Sweden was conducted to evaluate the correlation of diet and skin appearance. A correlation of pooled data suggested that less actinic skin damage may be correlated with a higher intake of vegetables, olive oil, fish and legumes and a low intake of sugar and dairy products.

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The use of molecular biological techniques developed during the human genome program has further improved our understanding of the mechanisms by which natural products either consumed orally or applied topically may impact the health of skin. Example mechanisms are reviewed in this chapter.

Cell Structure and Function

Cells are the most basic units of the body. They differ in size, shape, chemical composition and function yet they have many common properties that include movement, growth, ingest nutrients, excrete waste, and react to their environment [1]. Although cells of the body are specialized they all have basic structure important for their normal function. All human cells have a plasma membrane and a nucleus, most have an endoplasmic reticulum, Golgi apparatus, and mitochondrion. The plasma membrane is the membrane encapsulating the cell. The membrane is a sheet like structure composed primarily of lipids which have hydrophobic and hydrophilic moiety. It permits retention of water and requires transport systems across the membrane permitting passage of compounds. Membrane proteins provide the functionality for the membrane. They serve as pumps, gates, receptors, energy transducers, and enzymes. Cytoplasm inside the cell connects with the membrane and is involved in cell signaling from one part of the cell to another. Chemical differences distinguish one cell membrane from another type of cell membrane. The cellular matrix consists of cytoplasm and contains a variety of proteins that provide structural support and binding surfaces for the cell. Many enzyme-catalyzed reactions occur in the cytoplasm. The enzymes catalyzing the reactions of a metabolic pathway if oriented sequentially near each other will impact activity of the other enzymes. Metabolic pathways of significance include glycolysis, glycogenesis, and glyconolysis, and fatty acid synthesis, including production of nonessential, unsaturated fatty acids [2]. The mitochondria are the primary site of oxygen use in the cell and are responsible for most of the metabolic energy produced in cells. Size and shape will differ in various tissues relative to the function of the specific cell [2]. It is a large organelle that carries out oxidative phosphorylation in this way producing most of the ATP in the cell during cell metabolism. In nonphotosynthetic cells the main fuel for ATP synthesis are fatty acids and glucose. The energy released in mitochondrial oxidation generates heat, transports molecules in and out of the mitochondrion. Molecular oxygen is the oxidizing agent in these reactions [2]. Metabolic enzyme systems functioning in the mitochondrial matrix are those catalyzing reactions of the Krebs cycle and fatty acid oxidation. Other enzymes are involved in the oxidative decarboxylation and carboxylation of pyruvate in certain reactions of amino acid metabolism [2]. The mitochondria contain small quantities of DNA which is inherited from the mother exclusively. The primary function of mitochondrial genes is to code for proteins vital to the production of ATP. All cells in the body other than erythrocytes contain mitochondria. The nucleus of the cell initiates and regulates most cellular activities. Nuclear DNA is encoded with thousands of genes that direct the synthesis of proteins; each gene specifically encodes a single protein [2]. Protein biosynthesis occurs in phases referred to as transcription, translation, and elomgation. Each phase requires DNA and or RNA activity. Other organelles in the cell are lysosomes and peroxisomes, both contain enzymes. The lysosome is believed to function primarily as the cell's digestive system and the peroxisome performs oxidative catabolic reactions [2]. Oxidative enzymes in the peroxisome are involved in pathways that catalyze the release of hydrogen peroxide as an oxidation product. Hydrogen peroxide is very reactive and can cause cellular damage if not properly removed or converted. Catalase is found in the peroxisomes which degrade hydrogen peroxide into water and molecular oxygen.

Many chemical transformations occur in the cells of the body providing energy and molecules necessary to form cell structure and coordinate its activities. The molecules involved include water, inorganic ions, sugars, vitamins, fatty acids, proteins, polysaccharides, and DNA. Hormones and

various growth factors are molecules acting as signals directing cell activity. Proteins are regarded as the workhorses of the cell. Many of these proteins are enzymes which catalyze the chemical reactions. Other proteins facilitate cell function including molecular transport across cell membranes [1]. Proteins may remain within the cell or be secreted. An active area of biomolecular research is understanding how newly synthesized proteins function as they do within the cell and/or are directed outside the cell. This function is related to the amino acid sequence of the specific protein. A signaling sequence on the protein will interact with specific receptors located on the cell membrane. Cellular proteins of specific importance with respect to human health including skin are proteins that modify the cell's response to the environment, transport proteins that regulate the flow of nutrients into and out of the cell, and enzymes that act as catalysts for biochemical reactions taking place in the cell [2].

Many proteins function by changing their conformation induced by binding of a specific molecule, change in the environment, or chemical modification.

Receptor proteins are functionally specialized; they are located in the plasma membrane facing the exterior of the cell. Oligosaccharides are carbohydrates that are bound to the outer surface of these proteins. It is believed that they serve as recognition markers for binding. Membrane receptors act as attachment sites for specific external stimuli including hormones, growth factors, antibodies, lipoproteins, and various nutrients. Other molecules that bind to the receptor are called ligands. Receptors on the membrane and located in other areas of the cell are thought to be glycoproteins necessary for the correct positioning of newly synthesized cellular proteins [2]. Interaction of receptors with relevance to skin include; an internal chemical signal that is frequently produced by a stimulus-receptor is 3′,5′-cyclic adenosine monophosphate (cyclic AMP). It is formed from adenosine triphosphate (ATP) by the enzyme adenylate cyclase; it is frequently referred to as the second messenger in the stimulation of target cells by hormones [2]. Cyclic AMP (cAMP) is an activator of protein kinases. Cellular responses to cAMP vary among different cell types. These are enzymes that add phosphate groups to other enzymes which activate the enzymes from an inactive form. The receptor tyrosine kinase (RTKs) are cell-surface soluble or membrane-bound peptide/protein hormones that include nerve growth factor (NGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin. The RTK signaling pathways have a wide spectrum of functions including regulation of cell proliferation and differentiation, promotion of cell survival and modulation of cellular metabolism [1].

Provitamin, Vitamins, and Skin

Vitamin A refers to retinol and retinal. Retinoic acid is a metabolite of retinal. β-carotene, alpha-carotene and gamma-carotene are referred to as provitamin A. Lycopene, lutein, and zeaxanthin are forms of provitamin A found in nature. Vitamin A is primarily found in foods of animal origin and dairy products such as liver oils of fish, whole milk, cheese, and butter. Carotenoids are found in plants as well as fish. Lycopene is abundant in tomatoes, canthaxanthin is a red-orange carotenoid found in plants, fish, and seafood. Generally yellow, orange, and brightly colored red fruits and vegetables including carrots, papaya, tomato, squash, and pumpkin contain significant amounts of carotenoids [2]. Green vegetables also contain carotenoids. Retinol is not usually found free in foods, it is typically bound to fatty acid esters frequently as retinal palmitate. Retinyl esters and carotenes in food are often complexed with proteins from which they need to be released. Bioavailability may be improved by heating plant foods prior to consumption [2]. Carotenoids not converted to retinol in the digestive system may be absorbed and transported in the blood to tissues. Small quantities of retinal may be irreversibly oxidized into retinoic acid within the intestine [2]. Retinoic acid is essential for normal epithelial homeostasis. This signaling molecule exerts its regulatory actions in target cells by binding to nuclear retinoid receptors. In this way it influences the transcription of retinoid responsive genes [3]. Retinoid acid is available to epithelial tissue from two sources; local metabolism of plasma

that is transported to retinol-binding protein, or in tissue including bone marrow and adipose tissue. Retinoic acid is also present in the circulation bound to albumin where it is taken up by the cell [3]. Differential of epidermal keratinocytes is sensitive to exogenous retinoid, retinoic acid, or retinol. Retinoic acid has been identified in intact epidermis [3]. Vitamin A has been shown to stimulate growth of epithelial cells [2]. Stimulation in part is by growth factors that bind to specific receptors on cell surfaces. Retinoic acid appears to increase the number of specific receptors for growth factors [2]. Retinyl β-glucuronide formed in tissue from retinol has been shown to improve acne lesions [2].

Carotenoids believed to be found in interior cell membranes and lipoproteins. They are soluble in lipids and have the ability to quench singlet molecular oxygen and free radicals including peroxyl radicals. At least 600 different carotenoids have been identified. Lycopene, β-carotene, and the xanthophylls, astaxanthin, canthaxanthin, lutein, and zeaxanthin are of particular interest for human health. Canthaxanthin and astaxanthin are found at significant concentrations in the blood stream at high intake concentrations [4]. In addition to the potential for protecting against atherosclerosis, some forms of cancer, and age-related macular degeneration, studies have shown that they may provide photoprotection against sun damage in skin [4]. With respect to providing health benefits, the reactivity of the carotenoid is dependent upon their structure and incorporation into a membrane environment. β-carotene is less effective at preventing lipid peroxidation when exposed to a water-soluble peroxy radical initiator in liposomal environment. Zeaxanthin effectively protected against both water and lipid-soluble peroxyl radicals. The investigators of this study hypothesized that the antioxidant activity of carotenoids in such environments is related to their biophysical interaction with the lipid membrane [4]. Of the hundreds of known carotenoids found in nature, approximately 20 are found in the human body. Lutein and its isomer, zeaxanthin are found in the portion of the eye where light is focused by the lens, the macula lutea. Studies have indicated that these carotenoids filter high-energy wavelengths of visible light and function as antioxidants to protect against formation of reactive oxygen species [5]. Lutein and zeaxanthin are also found in skin and studies indicate they help protect skin against light-induced skin damage. They are non-provitamin A carotenoids classified as xanthophylls. They differ from other carotenoids because they contain oxygenated substituents. They have free hydroxyl groups at the end of the molecule that provide unique biochemical properties. These hydroxyl groups allow them to orient within cell membranes and lipoproteins in ways other carotenoids are unable to [5]. Typical sources are green leafy vegetables. Consumption of foods containing lutein and zeaxanthin enter the blood serum. One of the first publications reporting the protective effects of lutein was published in 2002. In that study, women ingested an oral antioxidant complex containing 6 mg of lutein and 0.18 mg of zeaxanthin daily for an 8-week period. Lipid peroxidation in skin was reduced after 2 weeks and continued to decrease during the study period. Skin moisturization was also improved [6]. A more recent 12 week placebo controlled study concluded that oral administration of lutein may provide better protection than that afforded by topical application when measured by changes in lipid peroxidation and photoprotective activity in skin following UV light irradiation [7]. The antioxidative function of carotenoids in skin has been investigated by various groups over the years. The development of Raman spectroscopy has enabled investigators to evaluate skin in vivo. The technique has shown that cutaneous carotenoids decreased the influence of stress factors on illness, including fatigue, UV irradiation, and consumption of high quantities of alcohol. Supplementing the diet with antioxidant substances in the absence of stress situations resulted in an increase of β-carotene level in skin. Correlation of skin roughness including wrinkles was also found to be associated with the level of carotenoid in skin [8].

Flavonoids and Flavanoids

A low cardiovascular mortality rate was noted in Mediterranean populations in association with red wine consumption and a high saturated fat intake. This observation is known as the French paradox. The Mediterranean diet is rich in flavonoids which are found in fruit, vegetables, grains and beverages

including tea and wine [9]. Flavonoids represent a family of plant compounds high in phenolic content. Epidemiologic studies suggest that dietary flavonoids provide a protective role against coronary heart disease [9] and protect skin when taken orally. Studies show in addition to oral consumption, topical application can enhance benefits to skin. These benefits are antioxidant, anti-inflammatory, and anti-tumor properties [9–11]. In 1930 a new substance was isolated from oranges believed to be a new class of vitamin, it was designated vitamin P. Vitamin P was later found to be a flavonoid, rutin. Rutin is found in the highest quantities in buckwheat, tomato, apricot, rhubarb, tea, celery, spinach, Brussels sprouts, and lemon. Rutin and quercetin are the most commonly used flavonol compounds in nutritional supplements [12]. Flavonoids are divided into several classes: flavonols, flavonones, flavones, flavanols, flavan-3-ols, and isoflavones. The classifications are made according to the chemical composition of the compounds, specifically the positions of substitute groups present on the parent molecule. Flavonols are the yellow antioxidant pigments found in various plants and flowers. One of the best studied flavonoids is quercetin. Quercetin is found in abundance in apple, onion and numerous medicinal plants. Rutin and quercetin have poor bioavailability and the content varies considerably in fruit, between 0.25 and 700 mg/100 g fresh weight [13]. In vivo biologic activity of flavonoids is dependent upon the bioavailability which is a function of absorption and metabolism. In most plants, flavonoids are bound to a sugar molecule as a glycoside. Flavonoids not bound to a sugar molecule are termed aglycones. Flavonoid glycosides are stable to most normal cooking methods, stomach acid pH, and secreted gastric enzymes. The limited capacity of the stomach to absorb aglycone flavonoids is related to the small absorptive surface of the stomach, 0.05 m^2 compared with 200 m^2 in the small intestine [14]. The data on absorption, metabolism, and excretion of flavonoids in humans is contradictory [9, 10, 12]. Some studies indicate that quercetin is absorbed in significant amounts. The form of flavonoid may influence the rate of absorption and contribute to the variation of data reported in the literature. Some investigators indicate that glycosylated forms of quercetin are absorbed more readily than the aglycone forms. Other investigators note that catechin which is not glycosylated is efficiently absorbed [15]. Many investigators believe that the conjugation pathway for flavonoids such as catechins begins with the conjugation of a glucuronide moiety in intestinal cells. The falvonoid is then bound to albumin and transported to the liver. The liver can extend the conjugation of the flavonoid by adding a sulfite group, a methyl group or both. These additions increase the circulatory elimination time and may decrease toxicity. The type of conjugate and its location on the flavonoid skeleton may determine the enzyme-inhibiting capacity, the antioxidant activity, or both. The regular intake of flavonoids may result in increased formation of several conjugates which would then result in greater activity [17, 18].

Quercetin is a natural antioxidant functioning by inhibiting lipid peroxidation through blockade of the enzyme xanthine oxidase, chelating iron, and directly scavenging hydroxyl, peroxy, and superoxide radicals [9, 12]. Free radicals can attract various inflammatory mediators contributing to a generalized inflammatory response and tissue damage. Flavonoids are oxidized by radicals, resulting in a more stable les-reactive radical [9]. Epicatechin and rutin are other potent radical scavengers [9].

Various flavonoids are able to inhibit the metabolism of arachidonic acid resulting in anti-inflammatory properties [16]. Cyclooxygenase and lipoxygenase are key mediators of inflammation. They are involved in the release of arachidonic acid which is the starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid and also provoke release of cytokines. Various phenolic compounds have demonstrated the ability to inhibit cyclooxygenase and 5-lipoxygenase pathways [19–21]. Inhibition reduces release of arachidonic acid. Quercetin is particularly effective in this regard. Another anti-inflammatory property of flavonoids is the ability to inhibit eicosanoid biosynthesis. Eicosanoids, e.g., prostaglandins, are involved in various immunologic responses and are the end products of cyclooxygenase and lipoxygenase pathways [22–24].

Omega-6 and omega-3 fatty acids are important for human health. Known as polyunsaturated fatty acids (PUFA) they help to stimulate skin and hair growth, maintain bone health, regulate metabolism and maintain the reproductive system. A healthy diet consists of a balance of omega-3 and omega-6

fatty acids. Omega-3 fatty acid helps to reduce inflammation while excessive omega-6 fatty acid will promote inflammation. The typical American diet tends to contain excessive amounts of omega-6 fatty acid. The Mediterranean diet has a better balance between omega-3 and omega-6 fatty acids. The diet referred to in the introduction of this chapter as the Mediterrean diet has been shown to be associated with prevention of wrinkle formation [25]. In addition to wrinkle reduction, there is a connection between PUFAs and atopic dermatitis. Arachidonic acid an omerga-6 fatty acid is converted to proinflammatory *n*–6 prostaglandin and *n*–6 leukotrienes by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX). The pathway continues to form prostanoids including PGE2 or leukotrienes which are pro-inflammatory agents. Omega-3 fatty acids will form eicosapentanoic acid, possibly shutting down the arachidonic metabolic pathway and progress to anti-inflammatory eicosanoids including prostaglandin (PGE3) and leukotriene (LTE5). The proinflammatory pathway can result in the development of dermatitis [26].

Antioxidants

The term antioxidant describes a variety of substances including enzymes that neutralize damaging reactive species. In the body, antioxidant nutrients function together to mitigate destructive radical and non-radical oxygen species [2]. All matter consists of the fundamental unit, an atom. Each atom has two electrons that orbit around the nucleus of the atom. When one of the electrons becomes unpaired, the atom becomes a free radical. When an unpaired electron is found alone in the outer orbital, a superscript dot is indicated next to the element. For example the superoxide radical is denoted as ($O_2^{\bullet}$) or a superscript dash (O_2^{-}). The single electron imbalance is usually highly reactive [2]. Free radicals containing oxygen are referred to as reactive oxygen species (ROS), free radicals containing nitrogen are referred to as reactive nitrogen species (RNS). The reactivity of reactive species varies with different compounds. The term reactive oxygen species or reactive nitrogen species includes free radicals containing nitrogen and oxygen and nonradicals. ROS encompass a variety of diverse chemical species that include superoxide anions, hydroxyl radicals and hydrogen peroxide. Superoxide and hydroxyl radicals are very unstable, hydrogen peroxide is more stable and freely diffusible and relatively long-lived in cells [27]. Symptoms of aging, cancer, atherosclerosis, heart disease have been connected with low density lipoprotein (LDL), cell membranes, and DNA exposed to oxidative stress [28]. Antioxidants are composed of enzymatic and nonenzymatic compounds. Superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and glutathione transferase are enzymatic antioxidants. Nonenzymatic antioxidants are alpha-tocopherol, ascorbic acid, ubiquinol, β-carotene; other examples are in Table 15.1.

Table 15.1 Example antioxidants (adapted from ref. [29])

	Comments
Enzymatic antioxidants	
Superoxide dismutase	Biological
Catalase	Biological
Glutathione peroxidase	Biological
Nonenzymatic antioxidants	
Antioxidant enzyme cofactors: (Selenium, Coenzyme Q10)	Biological, dietary
Oxidative enzyme inhibitors: (aspirin, ibuprofen)	Biological, dietary
Transition metal chelators (EDTA)	Biological, dietary
Radical scavengers (vitamin C and E)	Biological, dietary

The generation of ROS within certain boundaries is essential to maintain homeostasis. ROS generation by phagocytic cells helps to combat infection. An additional function of ROS is cell signaling in response to growth factors regulating proliferation [30]. Mitochondrial-derived oxidants function as signaling molecules under metabolic stress conditions [31, 32]. Numerous reactive species are generated in the body continuously as it is a normal physiological process; excess ROS products lead to aging and can become toxic. It is believed that the majority of intracellular ROS production is derived from the mitochondria. Mitochondrial superoxide production occurs primarily at two points in the electron transport chain. Complex I (NADH dehydrogenase) and at complex III (ubiquinone-cytochrome c reductase) [27]. Complex III is the major source of mitochondrial ROS production. The generation of ROS becomes predominately a function of metabolic rate and the rate of living may be indirectly correlated with a corresponding rate of oxidative stress. In addition to generating oxidants, metabolism can produce numerous other by-products including glyoxal and methylglyoxal which contribute to advanced glycation end-product (AGE) formation that appears to contribute to the appearance of aging skin [27].

Diet, Antioxidant Function and Redox Regulation of Thiols

Redox (reduction-oxidation) reactions refer to all chemical reactions in which atoms have their oxidation state changed. This can be either a simple redox process, such as the oxidation of carbon to yield carbon dioxide or the reduction of carbon by hydrogen to yield methane (CH_4), or a complex process such as the oxidation of glucose ($C_6H_{12}O_6$) in the human body through a series of complex electron transfer processes. The term comes from the two concepts of reduction and oxidation. It can be explained simply in that oxidation is the loss of electrons and reduction is the gain of electrons or a decrease in oxidation state by a molecule or atom

Thiols are a class of organic compounds that contain a sulfahydryl group (−SH) frequently referred to as a thiol group composed of a sulfur and hydrogen atom attached to a carbon atom. Protein thiols in the plasma include a protein sulfhydryl group and proteins mixed with disulphides and homocysteine. Mammalian tissue contains comparatively large amounts of protein thiols. The redox state of the thiol is dependent on the cellular location in the tissue. Thiols have significant redox capability; the balance of oxidized and reduced thiols likely plays a key role in regulating many cellular pathways associated with oxidation. Cellular redox balance can quench excess ROS and signal apoptosis when the degree of damage exceeds the capacity for repair [33]. Diet can influence thiol redox regulation at multiple points. If dietary cysteine is limited, it is possible for the cell to be unable to prevent oxidative damage to tissue. Dietary selenium is also essential for normal cell regulation. Broccoli which contains an aliphatic isothiocyanate is also important for redox cell signaling. Certain polyphenols from fruits and vegetables have also been shown to alter the thiol redox levels in tissue [33].

Hormesis

Many foods and dietary supplements are marketed on the assumption that any addition of antioxidants to the diet is beneficial. Accumulating research is indicating that the redox balance is more important than the quantity of antioxidants consumed. Changing the balance between oxidation or reduction may be deleterious [33]. The theory of hormesis addresses this question. A certain level of ROS may be essential; at low levels ROS may function to trigger antioxidant responses. Vigorous physical exercise increases cellular ROS production. In 2009, it was observed that antioxidant treatment of 1,000 mg vitamin C and 400 IU of vitamin E per day blocked the positive effects of exercise

Table 15.2 Example reactive oxygen species (ROS)

Superoxide anions	Very toxic	
Hydroxyl radicals	Very unstable	
Hydrogen peroxide	Freely diffusible and long-lived in tissue	
NADPH oxidases	Various biological outcomes related to cell type, level of oxidant, duration of oxidant production, species of ROS generated in cell and site of production	Example is nitric oxide synthase (NOS). Generated in normal cellular signal and homeostasis

on the cell signaling of insulin-dependent glucose uptake by muscle tissue. It was hypothesized that exercise-generates ROS may regulate redox potential via the mechanism of hormesis [34]. Redox signaling is activated by specific signaling agents, an example being hydrogen peroxide and not general oxidative stress. For this reason the effects of antioxidant supplementation are not dependent on how it interferes with specific oxidative species [33].

Conclusions

The scientific understanding of how antioxidants function in the body is evolving. With respect to antioxidnats, new findings indicate that compounds previously not considered to be antioxidants do have antioxidant activity in vivo. Available scientific evidence does not necessarily support the opinion that all ROS production is harmful to the body. Questions remaining to be answered include how various genes interact to influence stress and aging of the body. The term antioxidant is a generic term. In food processing antioxidants are added to retard food spoilage. To other individuals it may be considered a material having a high oxygen radical absorbance capacity, which gives a level of antioxidant capacity, but no indication of cell signaling activity. These points suggest that new methods of antioxidant testing and interpretation of results need to be developed. Another challenge is to better understand how natural dietary factors provide benefit to human health.

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Chapter 16
Nutrient-Rich Botanicals in Skin Health: Focus on *Avena sativa*

Khalid Mahmood, Claude Saliou, and Warren Wallo

Key Points

- Oatmeal and oat extracts are important tools in the compendia of dermatology and pediatric dermatology.
- Oatmeal and oat extracts are considered safe for topical use.
- Colloidal oatmeal and other topical oat-based ingredients improve many skin conditions including extra dry, itchy skin, xerosis, and dry skin associated with diabetes, as well as associated pruritus (itching).
- Oatmeal-based moisturizer and gentle cleanser have been proven to improve the symptoms of eczema in both children and adults.

Keywords *Avena sativa* • Oatmeal • Oat constituents • Safety • Clinical efficacy • Dry skin • Atopic dermatitis

Introduction

Botanicals are generally defined as ingredients sourced from a natural feedstock such as a plant, fungus, microorganism or algae. Over time, certain botanicals have been selected for their nutritive or medicinal values. Interestingly some botanicals used as food source exhibit health benefits. Examples of nutrient-rich botanicals include oatmeal, vegetable oils, curcuma, pepper…etc. Until recently however, the mechanism of action of these botanicals was largely unknown. They were also mostly used as crude preparations. In addition, clinical evidence often lacked or was insufficient to support their rational usage. With the advances in preparation and extraction processes, standardized materials are now available. Moreover, research has been conducted in elucidating the chemical composition of such botanicals and determining the contribution of each phytochemical class to its mechanism of action.

While the energy-supplying part of the botanicals may provide a health benefit, like the polysaccharides and proteins in oats, it is often the minor nutrients (e.g., polyphenols or vitamins) that

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Table 16.1 Examples of investigational topical botanicals for which clinical studies are registered at Clinicaltrial.gov

Botanicals	Skin conditions being investigated
Topical indigo naturalis oil extract	Psoriasis
Topical broccoli sprout extract	Radiation dermatitis
Topical green tea extract	Radiation dermatitis and mucositis
Topical *Vitreoscilla filiformis*	Atopic dermatitis
Topical olive oil	Uremic pruritus
Topical sea buckthorn oil	Skin aging

carry their main medicinal properties. For instance, polyphenols in Feverfew (*Tanacetum parthenium*) were found to be potent antioxidants and anti-inflammatories [1, 2]. In milk thistle (*Silybum marianum*), flavonolignans inhibit NF-κB and COX-2 and exhibit anti-carcinogenesis properties etc. [3]. In pine bark extract (*Pinus pinaster*), procyanidins (catechins and polymeric catechins) increased the ultraviolet-induced minimal erythemal dose via inhibition of NF-κB [4]. Many more botanicals [5] have been and still are the subject of clinical studies today as indicated in Table 16.1. This chapter focuses on the dermatological values of *A. sativa* L. (Oats) and materials derived from *A. sativa*, such as colloidal oatmeal. A review of oats constituents, their properties and usage in dermatology and skin care is presented.

Avena sativa (Oats): Cultivation and Economics

The Common Oat (*A. sativa* L.) is an annual grass belonging to the Poaceae family (formerly known as the Gramineae family). Oats are grown in most temperate regions of the world, with the top-4 producers, Russia, Canada, USA, and Poland, constituting 50% of the global production (FAOSTAT—http://faostat.fao.org/site/567/DesktopDefault.aspx). In recent years, other species of oats have been cultivated in subtropical regions, providing highly nutritive fodder to cattle. The global production of oats for grain has however decreased from about 50 million metric tons in the mid 1970s to about 25 million metric tons today. In comparison to wheat (*Triticum* spp.), oats are more flexible cultures, not requiring as much water, fertilizers or pesticides. The lower pesticide need may be explained by the fact that oats are naturally more resistant to certain pathogens than wheat. Oats contain saponins and phytoalexins, which provide a robust defense against common plant pathogens [6].

Only a small proportion (less than 20%) of the oats culture is used for human consumption. Its main usage is as animal feed [7, 8].

Oats Species and Varieties

Many varieties of oats are cultivated across the world. One simple grouping may be based on the hull coverage. Hull is a protective coating of hay on the oat grain. The two common types of oats which are generally cultivated are covered oats and hull-less oats (main variety is *A. sativa* var. *nuda*). Covered oats are a commodity crop whereas hull-less oats are still a specialty crop and mostly grown in China. Hull-less oats do not require the dehulling step but still require high temperature to neutralize lipases and other enzymes responsible for rancidity. Additionally, breeding programs can also help to further increase the potential of oat crops by optimizing a desired composition.

Oats and Oat-Derived Ingredients: Processing, Chemical and Nutrition Perspectives

Colloidal Oatmeal

Among non-food uses the most utilized form of oats is known as colloidal oatmeal or oatmeal powder or oat flour. It is simply the finely ground dehulled kernels of oats with an ability of making a colloidal system in water [9]. A standardized preparation for skin protection is described in the US pharmacopeia. Colloidal oatmeal is included in the US Food and Drug Administration's Skin Protectant Drug Products for Over-The-Counter Human Use Final Monograph [10].

The history of oatmeal is nearly as old as that of oats, with reference in ancient Roman texts. In the recorded scientific literature the term colloidal oatmeal was beginning to appear in early to mid twentieth century. Some of the first reports of use other than food include dermatological uses for aged and compromised skin [11]. It is difficult to define an exact chemical composition of colloidal oatmeal due to seed variations and the chemical changes that occurr during processing of the grains. Generally, colloidal oatmeals are characterized by the mesh size and their ability to create a therapeutic film on the skin. Colloidal oatmeal is made from food-grade oats and undergoes extra steps to produce the extra fine colloidal oatmeal powder.

The preparation of colloidal oatmeal includes a series of physical processes, consequently not requiring the use of any chemical solvents. The grains are cleaned to remove any foreign materials and imperfect grains, weed seeds and other grains. They are then de-hulled to remove the hull around the grains, yielding the groats. The groat size is further reduced with steel cutters to start the particle size reduction process. Oats contain a number of enzyme systems, lipase being one of the most abundant enzymes in the oat. The lipase enzyme is the enzyme which causes hydrolysis or rancidity in the oat and must be inactivated to stabilize the oat. Therefore, the groats are steam-heat-treated. The timing of this treatment is critical as rancidity can be initiated within hours after the raw oat has been de-hulled. This treatment is necessary to maintain low levels of free fatty acids, but also to reduce the bio-burden and prepare the groat for rolling, flake integrity, and proper water holding. The oats are then rolled or flattened and then pulverized to the desired particle size resulting in colloidal oatmeal.

Oat Oil

Fats are a major constituent of oats grains. Paul reported preparation and composition of oil from oats [12]. Amberger and Wheeler-Hill reported that the oat oil composition consists of almost 90% of unsaturated fatty acids and about 10% saturated fatty acids [13]. Novozhilova et al. studied three types of ground oats concluding that exact composition of oat oil is dependent on the type of oat plant and the region [14]. It was in 1977 that Boocock and Oughton described a commercial preparation of oil from oats for food and feed uses. Since then various preparations of oat oil are described in the literature including solvent extractions as well as supercritical liquid carbon dioxide extractions. DPPH radical scavenging ability of oat oil obtained via different extractions were studied by Li and Zhang [15]. Oat oil extracted with methanol exhibited higher radical scavenging ability as compared to oat oil obtained via hexane extraction of liquid carbon dioxide extractions. It is plausible that methanol is not only extracting lipophilic lipids but also polar lipids which are also potentially contributing in the radical scavenging process. Doehlert et al. reported polar lipids from 18 varieties of oat kernels. Their results indicate polar lipids of oats contain glycolipids and phospholipids of great diversity [16].

High contents of oleic acid in oat oil make it a suitable emulsifier and stabilizer [17]. Saastamoinen et al. studied oat oil composition variation across eight locations in Finland over a period of 3 years and concluded that oat grown at low temp produced a fatty acid composition, which is more beneficial to human nutrition compared to the fatty acid composition obtained from oat grown at high temperature [18]. The use of oat oil in the personal care industry is a natural extension of oatmeal use in the food industry. Oat oil can be considered as an enriched fraction with respect to the fatty contents of oatmeal and is expected to deliver correspondingly measurable benefits in clinic. It is no surprise that many claims of the use of oat oil for dermatological conditions have been reported in the last 20 years. Dull published a review in *Cosmetics & Toiletries* with similar emphasis [19].

Other Oat Fractions

Alkaloidal polyphenols from oats, also known as avenanthramides, are another unique class of chemistry with dermatological benefits. Avenanthramides is a group of more than 30 chemicals where scientific learning continues to improve our understanding of their complete role for human health and nutrition. Oats remained their main known natural source which also justifies the term "oat avenanthramides" used in the literature describing these unique oat chemicals. Avenanthramides are susceptible to processing conditions. In one study Dimberg et al. [20] found that one of the three studied avenanthramides are more susceptible to high temp and exposure to UV light as compared to the others. Extraction and a quantitative identification of avenanthramides from oat plant parts and oat based products have been published by Dimberg and Jastrebova [21].

The activity of oat enzymes and conditions for their optimum activities have also been studied and reported by Kazakov et al. [22]. Ground oat seeds are used as a source of peroxygenase enzyme to conduct epoxidation of fats and oils [23]. Oats as a source of enzymes is a new development and has not yet been exploited fully.

Oat beta-glucan concentrates, oat protein concentrates, and oat starch concentrates are relatively better known ingredients and are developed for cosmetic applications and also for food applications. However, oat peptides are a more recent advance in the sequence of novel ingredient development from oats. Dong et al. [24] has claimed efficient extraction of oat peptides with a molecular weight of up to 30,000 Da from oats. These peptides are known to increase collagen content in the skin. Oat peptides with specific amino acid sequence are claimed for antihypertensive and health benefits [25]. (Table 16.2)

Biological/Physical Properties of Oat Constituents [26]

The carbohydrates (starch) are the most abundant constituents of colloidal oatmeal. They are highly hydrophilic substances and can absorb large quantities of water [27, 28]. The proteins contained in colloidal oatmeal contribute further to its water affinity [28, 29]. Finally, the unique lipid (fat) composition of colloidal oatmeal [30] contributes to its emulsifying properties and the formation of a film [11, 31] at the surface of the skin. This hydrolipidic film (emulsion) formed by the combination of water bound to proteins and starch and lipids prevents the loss of water from the skin [11, 32]. The water holding capacity of colloidal oatmeal comes mainly from the carbohydrates and the proteins.

Table 16.2 Constituents of oats [76, 77]

Chemical classes	Subclasses	Main components	Distribution in oats	Comments
Oat starch	Carbohydrates	Amylose and amylopectin	Oat groats, Oat flours, endosperm	Unlike other cereals oat has strong protein–starch matrix which makes it difficult to separate oat starch from other parts of grain
	Lipids	Lysophospholipids and free fatty acids	Seed, bran, hull, endosperm	Higher lipid and phosphorous contents in oat starch vs. other cereal starch
	Proteins	Peptides, amino acids, etc.	Groat, endosperm	Relatively higher protein contents vs. other cereals especially wheat
	Inorganics	Calcium, magnesium, potassium	Hull, ash	Constitute the noncombustible residue
Non starch polysaccharides	Monosaccharides	Glucose, xylose, arabinose, galactose, mannose, uronic acid, fucose, rhamnose	Hull, bran	
	Polysaccharides	Beta-glucan	Groats, endosperm	Soluble fiber is mostly made of beta-glucan
Phenolic compounds	Hydroxy benzoic acids and aldehydes	*p*-Hydroxybenzaldehyde, *p*-hydroxyphenyl acetic acid, *p*-hydroxybenzoic acid, salicylic acid, vanillin, vanillic acid, syringic acid, protocatechuic acid, cinnamic acid, *p*-coumaric acid, *o*-coumaric acid, caffeic acid, ferulic acid, sinapic acid	Whole oats, groats, hulls, flour, trolled oats, wholemeal, kernels	These chemicals are biosynthetic precursors in oats and other cereals. They also contribute towards antioxidant activity of the grain when present in free or as glucosides
	Avenanthramides	Avenanthramide 2, Avenanthramide A Avenanthramide C Avenanthramide B Avenanthramide E Avenanthramide D Z-Avenanthramide E	Leaves, groats, hulls, flour, whole oatmeal	More than 30 avenanthramides are reported. Bisavenanthramides are also reported Avenanthramides are phytoalexins [78]
	Phenolic glucosides	2-Methoxyhydroquinone glucosides, *p*-hydroxybenzoic acid-4-*O*-β-D-glucoside, vanillic acid-4-*O*-β-D-glucoside, *o*-coumaric acid-4-*O*-β-D-glucoside, ferulic acid-4-*O*-β-D-glucoside	Oat seedlings, dehulled oats	
Flavonoid	Aglycones	2′,4,4′,6′-tetrahydroxy-3-methoxychalcone, apigenin, luteolin, tricin, leucodelphinidin, homo-eriodictyol	Oat kernel, whole plant	Flavanoids are a widely distributed class of chemistry in the plant kingdom with relatively few reports from oats

(continued)

Table 16.2 (continued)

Chemical classes	Subclasses	Main components	Distribution in oats	Comments
	Glycosylflavones	Isovetexin, vitexin-rhamnoside, vicenin-2, isoswertisin-rhamnoside, isoorientin, isoorientin-rhamnoside, luteolin glucosides, isoorientin-glucoside, isoscoparin, tricin-arabinoside, tricin-glucoside, tricin-arabinose, salcolin A, salcolin B	Leaves, stem, florets, whole plant, seedlings, kernel	Oat stem and florets exhibit more diversity of these chemicals as compared to leaves
Lignans	Aglycones	Pinoresinol, medioresinol, syringaresinol, lariciresinol, secoisolariciresinol, matairesinol	Oat flour, oat bran, kernel, hull	Lignans from oats are relatively new discoveries and this may be due to the fact that they are minor components in the oat plant. [79]
Saponin	Glucosides	Avenacin A and B	Roots, kernels	Avenacins help protect the oats against pathogens[6]
Phenylpropanoid *n*-alkanol esters	Feruloyl and caffeoyl	Hexocosanols, octacosanol, hexacosadiols, hexacosanoic acid, Octacosanoic acid, and mixed esters	Oat flour, kernel, bran	Sometimes they are also referred as avenacosylates and are part of waxes
Oat protein	Globulins	Globulin, glutelin, and albumin	Groat, kernel, hull, flakes	Conflicting information exist in actual contents of globulin and glutelin in oat proteins and is probably due to differences in extraction and stabilization techniques
	Prolamins	Avenins	Seed, bran, groat	High glutamic cid and proline contents
	Albumins	Limit dextrinase Nuatigenin 3β-glucosyltransferase Sterol 3β-glucosyltransferase More common enzymes include lipase, lipoxygenase, and lipoperoxidase	Oat leaves, seeds, flakes, groat	Water soluble albumin fraction is about 10–20% of the total protein and also contain the enzymes
	Peptides	Avenothionin alpha Avenothionin beta		45 AA residues including 8 cysteines
Oat lipids	Triacylglycerol	oil contents 3–9% Hybrid varieties of oats have triacylglycerol content as high as 18%	Seeds, bran, endosperm	Oil is present as acylglycerols mostly triglycerides. Depending on processing conditions mono- and di-acylglycerols can also be found

	Free fatty acids	Fatty acids	Oat bran, oat oil	Palmitic, oleic, and linoleic acid accounts for 90–95% of oil contents. Other Fatty acids are: stearic, linolenic, myristic, eicosenoic, and decanoic acids As oil contents increased in varieties of oat the proportion of oleic acid also increased
	Phospholipids and glycolipids		Seed, bran	
	Oxylipins		Oat seed, leaves, oat oil	Oxylipins are part of plant defense mechanisms
	Sterol and sterol esters		Leaves, stem, oat lipids	e.g., avenasterol, stigmasterol
	Lipophilic antioxidants	Tocol isomers of all eight kind are main components with alpha-tocopherol and alpha-tocotrienol as the main contributors	Bran, hull	Lipophilic antioxidants
Minerals		Potassium, phosphorus, magnesium, calcium, sodium, iron, zinc, manganese, copper	Ash, hull, bran	Micronutrients Very high phosphorus (mostly complexed with phytic acid)[59, 80, 81]
Vitamins		Vitamin E (tocols), niacin, pantothenic acid, thiamin, vitamin B6, riboflavin, folic acid, biotin, choline	Bran	Micronutrients

Table 16.3 Skin-relevant properties of the main constituents in oatmeal

Oat constituents	Properties	References
Proteins	Water hydrating capacity	[62]
	Oil binding capacity	
	Foaming	
	Superoxide dismutase activity	
Lipids	Emollient	[31]
	Moisturizing	[33]
	Lubricating	
Starch	Moisturizing	
	Water binding	
Beta-glucan	Immuno-stimulatory (increases IL-1, IL-2, IFNg, IL-4 in macrophages)	[82, 83]
Phenolics	Moderate antioxidants	[84, 85]
Avenanthramides	Potent antioxidants	[85–88]
	Anti-inflammatory (inhibits LOX, NF-κB)	
	Anti-itch (anti-histamine)	
Phytic acid	Phosphorus storage	[81, 86]
	Protein–phytate complex	
	Antioxidant	

Colloidal oatmeal provides mild cleansing properties without loss of skin moisture content [11]. As a result, colloidal oatmeal helps to prevent skin dryness and the clinical signs associated with dryness by preserving the moisture content of the skin barrier.

Oats are rich in nutrients and micro-nutrients, each have their own set of properties (Table 16.3).

Clinical Data

Safety

Colloidal oatmeal has been widely used and recommended by dermatologists and pediatricians for patients with dry and itchy skin due to age or skin conditions, like atopic dermatitis, for over 50 years [11, 33–36]. The body of evidence supporting the safe and effective use of colloidal oatmeal resulted in its incorporation in the United States Food and Drug Administration's Skin Protectant Monograph [10, 37]. In fact, oatmeal flour was considered empirically for centuries to be a milder skin cleansing alternative to soap. In 1953, Grais demonstrated that colloidal oatmeal helped restore the skin pH in conditions where is has been raised as a result of the use of alkaline soaps or atopic dermatitis [11].

With an altered and weaker skin barrier, atopic dermatitis patients are sometime more susceptible to develop contact dermatitis (irritant or allergic) upon usage of topical products [38, 39]. These skin reactions could be caused by numerous ingredients found in these products [40]. While colloidal oatmeal was already recommended in the management of dry and itchy skin associated with atopic dermatitis, Pigatto et al. demonstrated that topical colloidal oatmeal can be safely used on children with mild atopic dermatitis [41]. No immediate irritation or allergy was noted among the 65-subject cohort. Similarly, Vie et al. came to the same conclusion that oatmeal extracts did not carry an allergenic potential [42]. In a more recent randomized controlled study, an oat extract-containing emollient helped reduce the amount of corticosteroid usage in infants with atopic dermatitis, while being well tolerated and providing clinical improvements [43]. In addition, in a recent open label study with 12 cereal-sensitized atopic patients, oat-containing products did not increase the risk of allergic reaction [44].

However some rare isolated cases of allergic contact dermatitis or worsening of the atopic symptoms have been reported in relation with oats-containing cosmetic products, all being with atopic patients [45–47]. Using the same oat extract as the one found to exhibit some allergic reactions in the cases reported by Pazzaglia et al. [47] and Vansina et al. [46], Rance et al. estimated at 1.4% the rate of oat-related allergy [48]. Recently, Boussault et al., testing the same oat extract on atopic patients with a history of cereal sensitivity using an atopy patch test and oat pollen in a skin prick test, have found a surprisingly high proportion of subjects with a positive reaction [49]. These results are in contrast with the previous reports on oatmeal and could be explain with the methodology used [50]. Moreover, oat pollen is unlikely to contain the same proteins as in the flour. Consequently an allergic reaction to oat pollen would not be a predictor of an allergy to proteins found in oatmeal.

With celiac disease being more recognized [51], researchers have been looking for nutrient-rich and gluten-free sources of proteins, starches and fibers to replace wheat. Wheat, rye, and barley are the main sources of prolamins (gliadins, secalins, and hordeins, respectively) causing celiac disease and its associated dermatitis herpetiformis. The oats' prolamins (avenins) are less abundant than in wheat. Furthermore, no antiendomysial antibodies were detected in the supernatant of cultured duodenal biopsies obtained from 13 celiac disease patients and exposed to avenin [52]. Altogether, oats have been generally considered safe for celiac patients [53]. Further, long-term (5 years) incorporation of oats (10–70 g/day) in celiac patients' diet provided an equally beneficial improvement (vs. the control gluten-free diet group) and did not result in any complications [54]. The proportion of oats sensitive patients in celiac is considered very low [55]. Contamination of oatmeal flours and oats products with occasional grains from wheat has been cited as the usual explanation to explain occasional harmful effects from oats to certain members of the population at risk [56, 57]. One reason for the relative harmless potential of avenins for celiac patients may be their complexation with phytic acid, thereby limiting their absorption by the gut [58] or even their exposure to the skin' immune cells. Protein–phytate complexes are well documented [59].

With the potential increased risk of allergic reactions due to the presence of proteins in oatmeal, it is important to provide the skin context. Skin penetration of proteins is very limited due to their size, well above the molecular weight threshold usually accepted [60]. This was also the conclusion of the Cosmetic Ingredient Review Expert panel on wheat proteins [61]. There are a couple of additional reasons that prevent oat proteins from penetrating into the skin. First, the oat prolamins are not soluble at skin normal or lesional pH [62, 63]. Solubility of an ingredient is essential for its skin penetration. Second, oat proteins can form a complex with phytic acid, thereby limiting their penetration into a tissue [58, 59].

Efficacy

Since oatmeal has been shown to be an effective skin protectant and anti-irritant, many clinical studies have been done to assess its viability as a treatment option for various common skin ailments. Even half a century ago, researchers were looking toward colloidal oatmeal as a treatment for dermatological disorders. One such study by Melvin L. Grais concluded that Aveeno colloidal oatmeal provided complete or marked relief to almost three fourths of subjects exhibiting common skin conditions. Furthermore, he also noted that colloidal oatmeal exhibited a remarkable lack of skin-sensitizing and irritating properties [11]. More recently, in 2002, a study was performed to assess the anti-inflammatory activity of two oatmeal extracts using the sodium lauryl sulfate (SLS) irritation model and concluded that these extracts displayed a significant effect on irritation and inflammation [64]. These are only two examples of scientific investigations where oatmeal has historically been studied as a potential treatment for a wide variety of dermatological conditions.

Colloidal Oatmeal and Xerosis

The most prevalent condition that has been treated with topical oatmeal is xerosis (dry skin). In a 5-week clinical study, the effectiveness of an oatmeal-based skin protectant lotion in improving the moisture and barrier function of moderate to severe dry skin and to measure the residual skin effects after discontinuing treatment was evaluated. A standard Kligman Regression model was utilized [65]. Following a conditioning period, subjects used an oatmeal skin protectant lotion on their lower leg twice a day for a period of 3 weeks (Days 1–21), and for the following 2 weeks (Days 22–34), subjects did not use the test product or any other lotions on their legs. A statistical analysis of data was performed to determine regimen efficacy. Clinical evaluations of skin dryness showed significant improvements ($p<0.05$) at all time points during the treatment and regression period. Second, skin was significantly more hydrated ($p<0.05$) at all time periods measured during both the treatment and regression period. Finally, transepidermal water loss values showed a significant improvement in skin barrier ($p<0.05$) at all time points during the treatment period and up to day 9 of the regression (no treatment) phase of the study. In addition, many skin benefits continued for up to 13 days after the last application [65]. Furthermore, a separate study following a mini-regression, 9-day, investigator-blinded design was conducted to demonstrate the efficacy of a colloidal oatmeal skin protectant lotion with added avenanthramides in improving skin moisturization after a single application and after repeat applications. This study found that the colloidal oatmeal lotion with avenanthramides was well tolerated and effective in moisturizing and improving the appearance of moderate to severe dry skin and that these benefits persisted at least 2 days (end of study) after the last application. The oatmeal and avenanthramide lotion demonstrated quick efficacy in providing relief in tightness and itching to dry skin patients, and it significantly improved ($p<0.05$) hydration (measured by conductance), visual dryness, and scaliness at all time points, as early as 1 day of use [66].

Another study (a 2-week, double-blinded randomized study) was done to analyze the efficacy of a triple oat skin-protection lotion containing colloidal oatmeal, oat oil and avenanthramides. The lotion was well tolerated in subjects with compromised, itchy extra dry skin. Clinical evaluations showed that the triple oat skin protectant lotion significantly improved ($p<0.05$) dryness, scaling and roughness as early as 1 day after use and maintained over the duration of the study. In addition, adhesive skin sampling demonstrated a significant decrease ($p<0.05$) in both fine and coarse flaking starting after 1 day of application. Transepidermal water loss (TEWL) rates at days 7 and 14 showed a significant improvement ($p<0.05$) in the skin barrier when compared to baseline values [67]. High-resolution digital imaging also showed dramatic visual improvements after 2 weeks (Fig. 16.1).

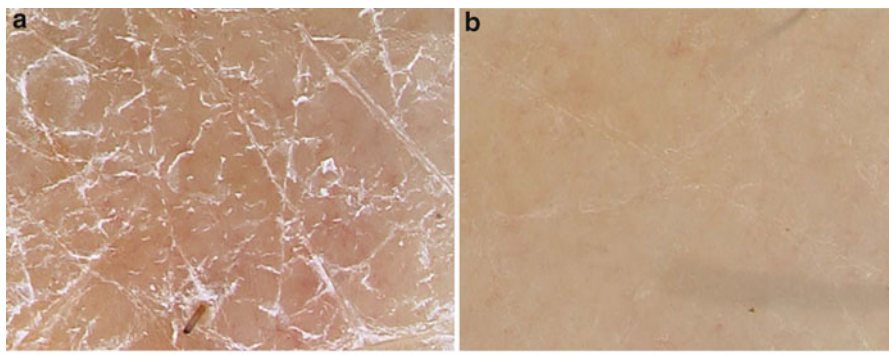

Fig. 16.1 High-Resolution Digital Images Before and After Use of the Triple Oat Skin Protectant Lotion. High-resolution digital imaging shows dramatic visual improvement in skin textural properties including dryness and flaking after 2 weeks of treatment using triple oat skin protectant lotion

It was demonstrated that the triple oat skin protectant lotion did not induce skin sensitization, and provided 24-h moisturization to the skin [67]. The triple oat skin protectant lotion has even been shown to be as effective, if not more so, than a leading prescription medical device barrier cream in improving skin barrier function in a separate double-blinded, randomized, mini-regression study. This study concluded that both creams were well tolerated and efficacious in moisturizing moderate to severe dry skin, and that the triple oat skin protectant cream was as effective as the prescription device cream in delivering rapid and sustained improvements in skin barrier, as evidenced by TEWL measurements during treatment and regression [68]. Additionally, the triple oat skin protectant cream demonstrated superior moisturization efficacy ($p < 0.05$) in skin hydration measurement at day 1 compared to the prescription device cream [68].

Colloidal Oatmeal and Atopic Dermatitis

Another common skin condition for which colloidal oatmeal has shown efficacy is eczema. In an 8-week monadic, blinded, clinical study to evaluate the tolerance and efficacy of an oat based skin care regimen in subjects with mild to moderate atopic dermatitis it was concluded that the oat based skin care regimen was beneficial and well tolerated in subjects with atopic dermatitis, and that the regimen was compatible with the concomitant topical prescription medications used [69]. An Investigators' Global Assessment (IGA) using a 0–5 scale, the Eczema Area and Severity Index (EASI, see Fig. 16.2) scored by a trained investigator and itch severity were all significantly improved as early as week 2 of use, and the Dermatology Life Quality Index was significantly improved at 4 and 8 weeks [69].

Furthermore, a 4 week, investigator blinded, cross-over study to evaluate safety and efficacy a colloidal oatmeal bath (oilated) in patients with history of atopic dermatitis concluded that the colloidal oatmeal bath (oilated) was found to provide relief for dry and sensitive skin. Specifically, there was a 50% reduction in itching ($p = 0.01$) and a 67% reduction in burning ($p = 0.03$) [70].

Of course, one of the most telling signs that oatmeal is safe and effective is that it has been shown to be a safe method of treatment in children. Atopic dermatitis, the most common type of the eczema, affects approximately 17% of children [71]. A 4-week, monadic study to assess the safety and tolerance of a colloidal oatmeal regimen (cream and cleanser) on babies and children with mild to moderate atopic dermatitis showed that this moisturizing regimen was well tolerated and beneficial. Dermatologist evaluations showed significant improvements ($p < 0.05$) in the Investigators Global Assessment (IGA) of eczema severity after 2 and 4 weeks of use. Evaluations also showed significant improvements ($p < 0.05$) in overall dryness and roughness in the same time period [71]. Significant improvements

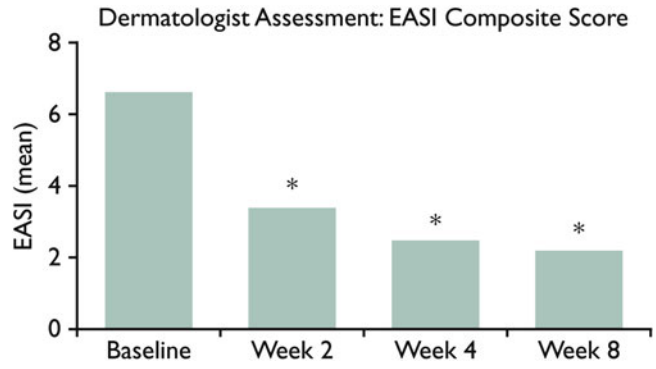

Fig. 16.2 Dermatologist Assessment: EASI Composite Score. Dermatologist evaluations showed a statistically significant improvement ($p < 0.001$) in the EASI composite scores after 2, 4, and 8 weeks of regimen use. *Significant improvement ($p < 0.001$)

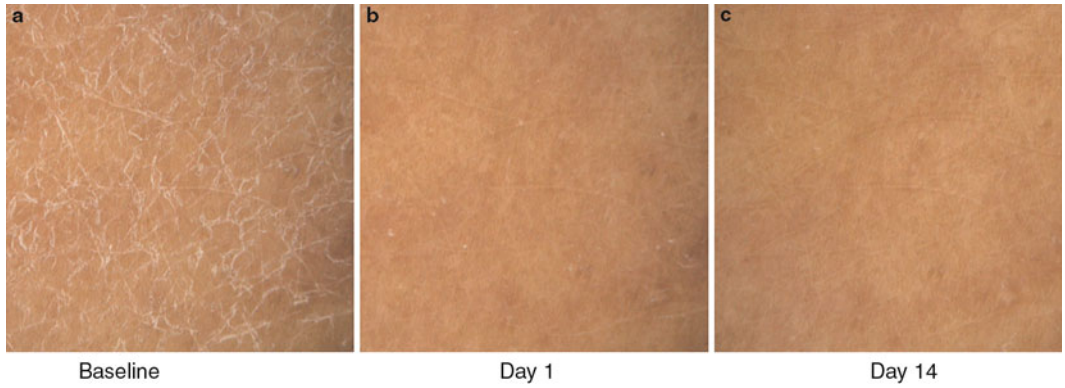

Fig. 16.3 High-Resolution Digital Images Before and After Use of Oatmeal Emollient Lotion. The high-resolution digital images show visible improvements in the appearance of skin ash and scale in skin of color patients, as well as visible improvements in skin textural lines as early as Day 1. There was continued visible improvement in skin ash at Day 14

($p < 0.05$) in itching were perceived after 2 and 4 weeks using the regimen. Improvements in skin condition resulted in overall improvements in the Quality of Life after 4 weeks of using the regimen as determined by standardized Baby/Child Quality of Life Indices for Dermatitis [71].

Interestingly, in 2007, a different research group studied the effect of an oat extract-containing emollient on topical corticosteroid use in infants with atopic dermatitis and concluded that the emollient treatment significantly reduced the need for high-potency corticosteroid consumption [43]

Oatmeal has also shown to be effective in the treatment of ashen skin which can be very apparent, especially in individuals with skin of color. In a 2-week investigator-blinded, randomized clinical trial it was found that an oatmeal containing emollient lotion was shown to significantly reduce the appearance of ashy skin and provide moisturization and textural benefits to skin of color. Clinical evaluations showed significant reductions ($p < 0.05$) in the appearance of ash, skin flaking, dryness, tightness, and itching as early as the Day 1 time point as compared to baseline values. Adhesive skin sampling demonstrated a significant decrease ($p < 0.05$) in fine and coarse flaking starting at the Day 1 time point and continuing throughout the study, and high-resolution digital images further demonstrated and confirmed visible improvements in skin ash, scale, and textural lines following use of the oatmeal emollient lotion (Fig. 16.3) [72].

Colloidal Oatmeal and Diabetic Skin

Oatmeal has been shown to be an effective treatment option for skin conditions in patients with diabetes. Approximately 85% of diabetics in America have dry skin, which can become itchy and cracked, leading to a potential infection [73]. A double-blinded, split body clinical study was conducted to evaluate the tolerance and efficacy of two oatmeal-based skin protecting moisturizing lotions on diabetic skin. This clinical study demonstrated that both the colloidal oatmeal lotion and the triple oat lotion were found to be very well tolerated by patients with diabetes, and both were efficacious, significantly improving ($p \leq 0.05$) skin dryness, roughness and itch on lower legs and feet after only 1 week, with a greater improvement after 4 weeks. In addition, both oatmeal lotions significantly improved skin hydration at weeks 1 and 4 compared to baseline (Fig. 16.4) [73].

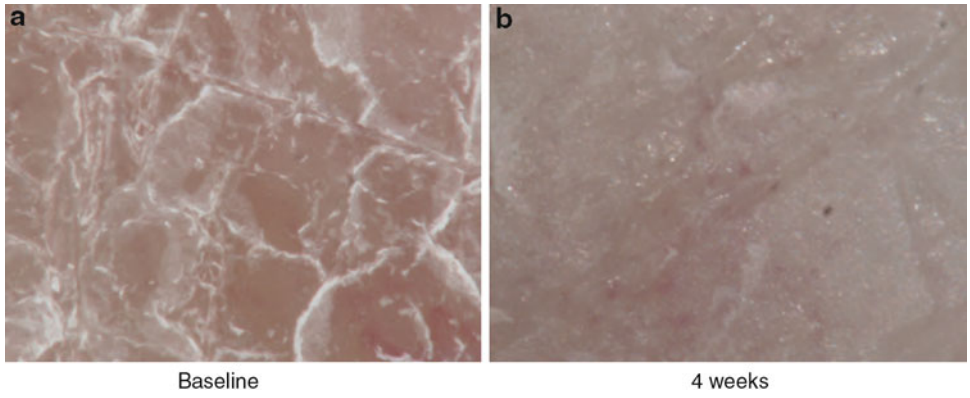

Fig. 16.4 Photos Before and After Use of Skin Protectant Lotions with Oatmeal. High-resolution images clearly show visible improvements in scaling and skin textural lines after 4 weeks of use of the oatmeal lotion with avenanthramides and oat oil

Colloidal Oatmeal and Drug-Induced Dermatitis

Finally, colloidal oatmeal has been shown to be an effective treatment for specific drug-induced therapies. Inhibitors of epidermal growth factor receptors (EGFR) and tyrosine kinase have been used to treat EGFR-positive cancers; unfortunately, this therapy has been known to induce an acneiform rash in patients undergoing treatment [26]. A study of ten patients receiving EGFR-inhibitor therapy and concurrent acneiform rash reported that treatment with an oatmeal-based lotion resulted in a complete or partial response in all patients with no associated toxicity [26, 74]. Furthermore, a separate case study reported that lotion containing colloidal oatmeal successfully controlled pruritus associated with the used of the EGFR-inhibitor erlotinib, completely controlling the outbreak after 10 days of treatment [26, 75].

Conclusion

Oatmeal and oat extracts are very important tools in the dermatologists and pediatricians compendium of adjuvant therapies. Recently, great advances have been made to better characterize its phytochemical constituents and understand their modes of action. Oatmeal and oat extracts are generally considered safe for topical usage. Clinical studies presented here demonstrate the dermatological benefits of colloidal oatmeal and other topical oat-based ingredients. Improvements have been documented for many conditions including extra dry, itchy skin, xerosis, and dry skin associated with diabetes, as well as associated pruritus (itching). Use of an oatmeal-based moisturizer and gentle cleanser has been proven to improve the symptoms of eczema in both children and adults, with added improvements in the Quality of Life indices. Newer learning indicates benefits for chemotherapy patients undergoing EGFR-inhibitor therapy who suffer from acneiform eruptions. With continued investigations, additional topical benefits are expected to be discovered for oats ingredient in other dermatological conditions.

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Chapter 17
The Role of Polyphenols in Skin Health

Grace Emily McClain and Ronald Ross Watson

Key Points

- Polyphenols are a class of phytochemicals found in fruits, vegetables, and other plants that have powerful antioxidant properties.
- Damage to the skin from ultraviolet radiation and other factors can lead to oxidative stress, a known contributor to the wrinkles, loss of elasticity, and abnormal pigmentation of aging.
- Plant polyphenols found in the diet have been shown to dramatically reduce free radical damage caused by oxidative stress, thereby improving skin condition.

Keywords Antioxidant • Cancer • Dietary polyphenols • Flavonoids • Oxidative stress • Photoaging • Resveratrol • Ultraviolet radiation

Introduction

Polyphenols are a unique group of phytochemicals that are present in a large number of fruits, vegetables, and other plant products. They are classified according to their chemical structure, which contains multiple linked phenol groups [1]. These compounds have powerful antioxidant properties that counteract harmful free radicals in the body. Nutrition has long been identified as a critical factor of health and well being, and the investigation into the costs and benefits of ingesting certain compounds has just begun; polyphenols in particular have been a topic of interest since the discovery that they may prevent or even reverse the effects of photoaging due to ultraviolet radiation [2]. Here, we explore the various types and properties of polyphenols, the deleterious effects of free radicals on skin health, and the possible protective and regenerative ability of dietary polyphenols.

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Polyphenols

This class of organic molecule is found in a large number of edible plants as a secondary metabolite [3, 4]. Polyphenols have a wide range of chemical structures that can be simple or extremely complex, and therefore also have a variety of functions [5]. The molecular weight of these molecules ranges from 3,000 to 20,000 g/mol, which indicates a huge variation in size. All polyphenols, however, have certain traits that differentiate them from other bioactive molecules. As was mentioned, many polyphenols are excellent antioxidants. This arises from the chemical structure of these compounds, which contain functional groups capable of accepting a free radical's negative charge. Polyphenols also have the ability to bind to proteins, are astringents, and are responsible for many of the distinctive flavors of tea, cocoa, and wine [4]. Flavonoids and resveratrol are two types of polyphenols that have potential to protect against damage that causes aging of the skin [6].

Flavonoids

Flavonoids are some of the most abundant and varied polyphenols; almost all plants produce this kind of molecule through their metabolic processes, and there are more than 2,000 known naturally occurring flavonoids [7]. Current research suggests that flavonoids are powerful bioactive agents that can have a significant effect on risk factors for cancer and other degenerative diseases [8]. The ability of flavonoids to scavenge free radicals very effectively and therefore prevent oxidation damage has led to their classification as antioxidants [1]. These compounds are structurally favored for receiving free radicals in the skin that are created by exposure to UV light, effectively capturing them and preventing further damage. Other polyphenols act as antioxidants, but flavonoids have the ability to scavenge superoxide anions (O_2^-) where others may not [9]. Studies have shown that flavonoids have the ability to inhibit kinase activity, a biological process that results in the formation of free radicals [7]. In addition, the high prevalence of flavonoids present in citrus fruits and other foods such as chocolate and wine make it a protective agent that can be easily and willingly accessed; the average American's daily intake of flavonoids is estimated at around a gram [7, 10].

Resveratrol

Resveratrol is a phenolic compound that grape vines produce in order to combat fungal infection or other forms of stress, such as UV radiation [11]. It is a sirtuion activating compound, or STAC, which have antiaging and anti-neurodegenerative properties [6, 12]. Intake of resveratrol has been shown to imitate caloric restriction in its antiaging properties by decreasing oxidative stress and inflammation. Studies also indicate that this compound may increase the life span of yeast (*Saccharomyces cerevisiae*) and short-lived vertebrates [6].

Skin

Function

Skin is the largest organ of the human body, and it serves as the first line of defense against harmful external agents. The two main layers are known as the dermis and epidermis. The epidermis is the outer layer that is directly exposed to air, sunlight, and other environmental factors. Keratinocytes are

the cells that make up about 90% of the epidermis [2]. Their secretion of the chemical keratin is what gives skin its strength and waterproof nature. The dermis is larger than the epidermis, and contains cells that are responsible for strength as well as flexibility. It is this layer of the skin that is the most affected by aging, and possibly by the positive contribution of phytochemicals [13].

The protective functions of skin span four main areas of exposure: biological, mechanical, chemical, and physical [14]. Biological agents are pathogens such as microorganisms and viruses, while mechanical traumas are defined as lacerations, bruises, abrasions, and other injuries [14]. Most research about the benefits of polyphenols focuses on the areas of chemical and physical exposures, as these are areas where ingestion of a chemical may have more of an impact on skin's effectiveness as a barrier.

Oxidative Stress

Oxidative stress has been identified as the key causal element of skin aging and damage [15, 16]. It is defined as the buildup of free radicals and partially reduced oxygen molecules, which make up the reactive oxygen species (ROS) [16]. Free radicals are charged particles that have broken free from a molecule and can cause severe damage to intracellular structures by oxidizing nucleic acids, proteins, and lipids [16]. The skin comes into contact with free radicals through both extrinsic and intrinsic means; for example, nitric oxide radicals are produced naturally in the skin by the enzyme nitric oxide synthetase [15]. The breakdown and synthesis of adenosine triphosphate (ATP), an essential biological process, also introduces free radicals into the body. It should be noted, therefore, that the benefits of antioxidants to skin health do not come from completely eradicating the presence of free radicals, but rather preventing an excess that could result in oxidative stress [16]. External sources such as air pollutants, pathogenic bacteria and viruses, and irradiation due to UV rays are some of the detrimental exposures that can lead to oxidative stress [15]. Primary among these carcinogens and contributors to skin aging is sunlight, which is discussed in depth.

Research on plant polyphenols' antioxidant properties can be measured the activity of processes caused by free radicals and oxidative stress. One such process is lipid peroxidation, which occurs to a greater extent with an increased presence of free radicals. Some of the harmful effects of lipid peroxidation on cell membranes are loss of fluidity, inactivation of membrane enzymes, increased cell permeability, and a greater likelihood of ruptured cells [17].

The human body already possesses an extensive defense system against free radicals that comes in the form of either enzymes or low molecular weight antioxidants. Many polyphenols fall under the low molecular weight antioxidant category, but they have not been studied to the extent of antioxidant enzymes. Research thus far suggests that increasing the amount of low molecular weight antioxidants in the diet can supplement the body's antioxidant defense system, although the long term implications of this information in terms of aging is not yet fully understood [15, 18].

Types of UV Damage

Long-wave UVA radiation (320–400 nm) makes up most of the UV radiation that reaches the earth's surface, but UVB (290–320 nm) has also proven to be very harmful to the skin. Almost all of the much more dangerous UVC rays (200–290 nm) are deflected by Earth's atmosphere [2]. When UV rays invade the skin, they do so by means of transcellular permeation [14]. This means that the light passes directly through the cells as opposed to chemicals, which can enter the body between the skin cells through intercellular lipid pathways [14]. UV radiation's ability to directly penetrate the cells contributes to its mutagenic and carcinogenic nature. UVA and UVB radiation combined can lead to oxidative

stress, immunosupression, DNA damage, premature aging, and the formation of free radicals [2]. UVA radiation induces the formation of free radicals by exciting primarily oxygen atoms and hydroxyl groups to the point that they gain enough kinetic energy to separate from the molecule. This results in a small, charged particle that acts as a reducing agent to fragile biological systems [16].

Premature Aging and Cancer

A primary component of these external aspects of aging is prolonged exposure to the sun and its UV rays, or photoaging due to oxidative stress. Aspects of photoaging include deep wrinkles, uneven pigmentation, brown spots, and a leathery appearance. These changes are due to differences in the extracellular matrix; collagen, one of the key components of the dermis that gives it resilience and elasticity, can be structurally altered by cumulative UV radiation. This leads to the characteristic wrinkles as well as the loss of rigidity of photoaged skin. In addition to impeding the function of collagen, sunlight has been shown to alter the activity of matrix metalloproteinasses, which play an important role in tissue destruction and possibly wrinkle formation [17]. In addition to weakening elastin and collagen in the skin, oxidative stress can slow the growth of new skin cells to replace old ones [16].

Cancer also occurs more frequently in skin that is photoaged, which implies that UV rays cause substantial damage to the DNA of skin cells, especially those of the dermis [19]. As one of the most common types of cancer, skin cancers deserve a great deal of attention from the health community. Melanoma, the most deadly form of skin cancer, has been indisputably linked to sun exposure due to UV rays' ability to cause mutations of DNA [19]. More specifically, radiation from the sun induces free radicals in the skin that can cause the loss of a base pair, a missense mutation, as well as single and double breaks in DNA [15] When this mutation occurs in the p53 tumor suppressor gene, the result is cancerous growth of skin cells [20].

Dietary Polyphenols

The skin health benefits of eating a greater number of polyphenol-rich foods have been suggested by a number of studies [21]. The use of green tea polyphenols as a topical treatment has also shown a great deal of potential for reducing free radical damage to the DNA in studies on hairless mice [2]. Certain dietary antioxidants and vitamins have been shown to reduce the severity of skin lesions, which are precursors to nonmelanoma skin cancer [22]. There is no shortage of polyphenol-containing foods, and a diet rich in fruits, vegetables, and certain beverages may result in healthier skin.

Citrus Flavonoids

The polymethoxyflavonoids that are found only in citrus have been attributed with a great deal of positive bioactive processes, including the differentiation of leukemia cells, antimutagenic activity, and antiproliferative effects on squamous cell carcinoma, a cancer that often affects the skin [10, 23]. Out of four flavonoids examined in a study, only the polymethoxylated flavones nobiletin and tangeretin showed a marked ability to inhibit growth of cancerous cells [23]. More extensive research has shown that luteolin, natsudaidain, quercetin, eriodictyol, and 3,3′,4′,5,6,7,8-heptamethoxyflavone

also exhibit antiproliferative effects in multiple cancer lines, including melanoma [24]. Considering the high incidence of squamous cell carcinoma and the deadliness of melanoma, the properties of these molecules have significant implications for the preventative role of citrus fruit in skin health.

Tea Polyphenols

While green and black tea both contain certain polyphenols, green tea contains the powerful flavonoid (−)-epigallocatechin-3-gallate, or EGCG [2, 10]. EGCG acts as an inhibitor of free radical formation and lipid peroxidation, along with other anticarcinogenic fuctions [10]. The polyphenols present in green tea are incredibly powerful antioxidants that are even stronger than vitamins C and E [16]. Animal studies on hairless mice exposed to UV rays have revealed the benefits of green tea polyphenols (GTPs). Compared to mice who were not given any tea, those that ingested a mixture of GTPs had a decreased incidence of non-melanoma skin tumors, as well as tumors that were smaller in number and size. Topical application of ECGC proved to be an even more effective administration of the polyphenols, as the mice with ECGC had an even smaller incidence, size, and number of tumors. These results were indicative of green tea polyphenols as valuable protection against UV induced immunosuppression and photocarcinogenesis [25]. The mice that were given GTPs also showed a regression of growths that were already present, which demonstrates the remarkable ability of these chemicals to not only prevent but also reverse damage caused by the sun. For GTPs to have effect, however, the presence of interleukin (IL-12) is essential; without this cell-signaling protein, mice did not experience the benefits of the polyphenols [2].

In addition to their anti-carcinogenic properties, green tea also exhibits anti-inflammatory, and as was mentioned, antioxidant properties. Both oral and topical absorption of GTPs resulted in protection against sunburn and photoaging, two main forms of skin damage [16].

Black tea also contains a number of bioactive ingredients, with the antioxidant theaflavin as the primary component [10]. While studies have shown that green and black tea are equally effective as antioxidants, green tea components have proven to be more active in the proliferation of cancer cells [10, 26]. In fact, there has been no real association, positive or negative, between black tea consumption and the incidence of the four main cancers: stomach, colorectal, lung, and breast [27]. Because of this, it is highly unlikely that black tea polyphenols can protect against skin cancer, in spite of its antioxidant properties.

Red Wine

One of the many phenolic compounds in red wine is the polyphenol resveratrol, which comes from the antioxidant-rich grape skin [6]. Wines may have as many as 1,000–5,000 mg of total polyphenols per liter [4]. However, the concentration of resveratrol in red wine is not very high: about 1–10 mg/L. In white wine, the concentration is even lower. It has been suggested, therefore, that wine must be ingested fairly regularly in order to obtain any of the benefits from resveratrol. These benefits are similar to those provided by other antioxidants; elderly people in particular may benefit from drinking wine. Resveratrol's ability to act as a superoxide anion scavenger helps to prevent against lipid peroxidation, neurodegenerative diseases, and perhaps the natural degeneration of skin with time [6]. Since wine helps to prevent against oxidative stress, it could play a key role in slowing the process of photoaging, like other polyphenols. Topical administration of resveratrol, like tea polyphenols, has shown to be especially effective in reducing and reversing skin damage [28].

Pomegranates

Yet another dietary source of bioactive polyphenols is the pomegranate fruit, which has been used in traditional medicine around the world. Recently, it has been valued as a substance rich in polyphenols, including flavonoids and hydrolyzable tannins. Regular consumption of pomegranate products has been thought to prevent UV-induced deterioration of the skin. Its strong antioxidant effect has been noted due to the compound's reduction in lipid hydroperoxides (LPOs), a harmful indicator of peroxidation. LPOs are especially harmful to biological membranes and enzymes, but pomegranate consumption was shown to inhibit an increase in LPOs due to UV rays. Therefore, it is highly likely that a diet rich with pomegranate products would result in skin less affected by photoaging and photocarcinogenesis due to ROS scavenging [17].

Other Polyphenols

A truly vast number of natural plants contain polyphenolic compounds that could prove beneficial to human skin health. Another beverage that contains a number of polyphenols is coffee. Extract from the *Coffea arabica* plant show potential to improve dyspigmentation, photoaging, and erythema, or redness of the skin. The antioxidants contained in coffee could possibly be even more powerful than green tea extract, vitamin C, pomegranate, and vitamin E [16].

Finally, oliomeric proanthocyanids (OPCs) have many of the antioxidant properties that have been discussed. These compounds can be found in grape seed extract, teas, berries, and apple skins, but most notably in pycnogenol. Pycnogenol is a French pine bark extract that is known for its benefits to skin health. Topical application has the ability to stabilize collagen and elastin, thereby improving the functioning and aesthetics of the skin. It has also been shown to decrease UV-induced pigmentation, as well as to reduce scarring [16].

Conclusion

Research has indicated that polyphenols, a bioactive component of fruits, vegetables, and other foods and beverages, have the ability to significantly improve the condition of skin. Much of their protective and regenerative qualities come from their interactions with free radicals (especially ROS) that are produced during vital biological processes and exposure to UV radiation. Because oxidative damage caused by the sun is one of the key components of skin aging, polyphenol-rich foods may act as preservative and regenerative agents to skin health. Topical administration of various polyphenolic extracts, such as ECGC, has been shown to protect skin much more effectively than sunscreen alone [16]. The numerous benefits provided by antioxidant polyphenols suggest that a diet of a sufficient amount of the compounds would result in significant positive changes in the condition of the skin.

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Chapter 18
Resveratrol in Dermal Health

Dipak K. Das and Hannah Vasanthi

Key Points

- Resveratrol, a polyphenol phytoalexin, possesses diverse health benefits by virtue of its numerous biochemical and physiological actions including anti-inflammatory, anti-angiogenic, and antiplatelet properties.
- Skin is the outermost layer of the body, and is always exposed to environmental toxins including pro-oxidants and harmful radiations that could lead to skin aging, inflammatory disorders, and even skin cancers.
- In this review, we discuss all these skin problems including skin cancers, UV radiation, aging, wound healing, inflammation, and acne vulgaris and how resveratrol can protect the skin from such problems. Discussion also includes the mechanisms of dermal protection by resveratrol.

Keywords Skin cancer • Skin aging • Wound healing • UV radiation • Psoriasis

Introduction

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic phytoalexin present in the skins of a variety of foods and vegetable including grapes, berries, and peanuts. The richest source of resveratrol is the roots of *Polygonum cuspidatum* (Ko-jo-kon) mainly cultivated in China and Japan. The skins of grapes contain about 50–100 mg of resveratrol, and believed to be responsible for the cardioprotective properties of red wine, which contains 0.2–7 mg/l of wine. In addition to grape, a large variety of fruits including mulberry, bilberry, lingo berry, sparkleberry, deer berry, partridge berry, cranberry, blueberry, and jackfruit, peanut, and a wide variety of flowers and leaves including gnetum, white hellebore, corn lily, butterfly orchid tree, eucalyptus, spruce, Poaceae, Scots pine, and rheum also contain

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resveratrol. Resveratrol is synthesized in response to environmental stressors that include water deprivation, UV radiation, and especially, fungal infection. Thus, the production of resveratrol in plants can be considered part of the defense mechanism.

Although resveratrol was initially found as a chemoprotective compound and depressed tumor growth [1], subsequent studies determined diverse health benefits of resveratrol including its ability for cardioprotection [2–5]. Cardioprotective properties of red wine (stemmed from popular French Paradox [6]) are believed to be due to the presence of resveratrol in red wine [7]. Among the numerous cardioprotective effects of resveratrol, anti-thrombin activity inhibiting platelet adhesion [8], inhibition of LDL peroxidation [9], induction of vasorelaxation [10], stimulation of angiogenesis [11], depression of ventricular arrhythmias [12]. Reduction of ischemia/reperfusion injury [13], inhibition of endothelin [14], and inhibition of lipid peroxidation [15] are worth mentioning. Many of such cardioprotective activities are mediated by the ability of resveratrol to depress inflammatory response [16, 17]. In addition, resveratrol possesses numerous other health benefits including, protection from UV radiation injury [18], cerebral ischemia [19], growth of H. pylori [20], neurological damage [21], and antiaging activity [22].

Beneficial Effects of Resveratrol on Skin

Compared to the studies on the abilities of resveratrol to protect heart and different kinds of cancer, very little information is available on dermal health. One of the earliest reports include a study demonstrating that pretreatment the mouse skin with resveratrol significantly lowered the oxidative stress induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and restore glutathione levels an superoxide dismutase (SOD) activity suggesting that resveratrol protects skin via antioxidant action [23]. Subsequent studies, however, revealed that protection of dermal health is associated with mechanisms beyond the antioxidant effects of resveratrol. This review discusses diverse benefits of resveratrol in dermal health

Skin Cancer

Skin cancer is very common malignancy, which is rapidly increasing throughout the world with approximately 1.3 million new cases per year. The use of pat-derived products is increasingly recognized in the management of skin cancer. Resveratrol dose-dependently lowered TPA-mediated carcinogenesis by abrogating the pathways for reactive oxygen species (ROS) formations [24]. TPA-mediated increases in the induction of the expression of COX-1 and COX-2 as well as c-myc, c-fos, c-Jun, and TGFα were depressed after resveratrol treatment [24, 25]. In another study, resveratrol induced apoptosis in DMBA (7,12-dimethylbenz[a] anthracene)-initiated and TPA (12-*O*-tetradecanoylphorbol-13-acetate)-promoted mouse skin tumors [26]. Resveratrol resulted in regression of tumors after withdrawal of the TPA. Western blot analysis combined with multivariable flow cytometry showed that resveratrol induced the expression of the p53 and pro-apoptotic Bax, with concomitant decrease in anti-apoptotic protein Bcl-2. Alteration in Bax/Bcl-2 ratio by resveratrol resulted in apoptosis associated with the release of cytochrome c and induction of apoptotic protease-activating factor-1 (APAF-1) leading to the formation of caspase 9 and 3 and poly (ADP-ribose) polymerase (PARP). The same group subsequently reported that chemopreventive potential of resveratrol in moue skin tumors were mediated through the PI3K/AKT signaling pathways [27]. Resveratrol treatment also increased the DMBA suppressed p53 and Bax while decreased the expression of Bcl-2 and survivin. Another study revealed that resveratrol inhibited proliferation of cancer

cells by manipulating JAK/STAT pathway, where resveratrol prevents phosphorylation of JAK, thereby inhibiting STAT1 phosphorylation [28]. This was followed by the stimulation of ROS leading to the mitochondrial membrane depolarization

Interestingly, when several polyphenols were compared, resveratrol was found to be absorbed much more efficiently than other polyphenols in humans, and appeared to be the most effective anticancer polyphenol present in red wine when consumed by human subjects [29]. Another related study also compared several flavonoids to inhibit melanoma growth, and determined that EGCG or apigenin was the best while resveratrol was the worst [30]. The order of potency to inhibit melanoma growth was EGCG [(−)epigallocatechin-3-gallate] > apigenin = qercetin = tamoxifen > resveratrol. In contrast, a recent study showed that resveratrol induced cell-cycle disruption and apoptosis in chemoresistant B16 melanoma [31]. This study clearly showed that resveratrol inhibited the growth of a doxorubicin-resistant B16 melanoma cell subline (B16/DOX) (IC50 = 25 μM after 72 h). Yet another study demonstrated synergistic effects of resveratrol with other polyphenols in the prevention of skin cancer in mice [32]. In this study, ellagic acid and calcium D-glucarate was used as components of diets, while resveratrol was applied topically using DMBA-induced skin carcinogenesis model. All combinations of resveratrol with other compounds showed a synergistic effect on hyperplasia and Ha-ras mutation Skin tissue receiving the combinations showed decreased cell proliferation and Bcl-2 expression, decreased p21 and decreased marker of inflammation cyclooxygenase-2. Inhibition of skin cancer by resveratrol was found to be related to its bioavailability [33]. This study found the half-life of resveratrol in plasma to be very small (half-life after i.v. administration of 20 mg/kg was 14.4 min in rabbits). Highest concentration of resveratrol was reached within the first 5 min of resveratrol administration.

In a recent study, SirT1-null mice were used to develop tumors. Although tumors were developed at normal rates, they were poorly protected by resveratrol [34]. The authors found that the presence or absence of SirT1 had no effect on incidence and tumor load of skin papillomas induced by the classical two-stage carcinogenesis protocol. Resveratrol topically applied to the skin profoundly reduced tumorigenesis. The chemoprotective effect was significantly reduced but not ablated in SirT1 null mice, suggesting that part of the protection afforded by resveratrol might require SirT1-encoded protein. In a related study, toll like receptors (TLR4) was required for the chemopreventive action of resveratrol in DMBA (7,12-dimethylbenz(a)anthracene) skin carcinogenesis [35]. In this study, mice with normal and deficient TLR4 function were compared when pretreated with resveratrol and then subjected to a DMBA-induced skin carcinogenesis protocol. There were fewer tumors/group in resveratrol treated TLR4 competent C3H/HeN mice than in TLR4 deficient C3H/HeJ mice. In addition, the size of tumors in C3H/HeN mice was reduced in vivo and their survival in vitro was inhibited by resveratrol to a significantly greater extent than in C3H/HeJ mice. Resveratrol also inhibited angiogenesis to a much greater extent in the TLR4 competent mice than in TLR4 deficient mice suggesting that TLR4 is an important mediator of resveratrol chemoprevention in DMBA skin tumorigenesis.

Resveratrol treatment resulted in an induction of the cyclin kinase inhibitor WAF1/CIPI/p21 which, by inhibiting cycline, D1 and D2 as well as cyclin-dependent kinases cdk2, cdk4 and cdk6 results in a Go/G1-phase arrest followed by apoptosis of A431 human epidermoid carcinoma cells [36]. The results of this study suggest that resveratrol treatment of the cells causes an induction of WAF1/p21 that inhibits cyclin D1/D2-cdk6, cyclin D1/D-cdk4 and cyclin E-cdk2 complexes, thereby imposing an artificial checkpoint at the G_1-⟶ transition of the cell cycle. This series of events results in G1-phase arrest of the cell cycle, which is an irreversible process that ultimately results in the apoptotic death of the cancer cells. The same authors subsequently provided evidence for the involvement of the pRb-E2F/DP pathway as an important contributor of resveratrol-mediated cell cycle arrest and apoptosis [37]. This study demonstrated that resveratrol treatment of A431 cells results in a dose- and time-dependent decrease in the hyperphosphorylated form of pRb with a relative increase in hyperphosphorylated pRb. This response was accompanied by downregulation of protein expression of all five E2F (1–5) family members of transcription factors and their heterodimeric partners DP1 and DP2. Possible mechanisms of action of resveratrol on skin cancer are summarized in Table 18.1.

Table 18.1 Possible mechanism of action of resveratrol on skin cancers

Effects	Mechanisms	Dose/duration	References
Reduces the number of skin tumors initiated with DMBA and promoted by TPA in female CD-1 mice	↓COX-1; ↓COX-2; ↓c-myc; ↓c-fos; ↓c-Jun; ↓TGF-β1; ↓TNF-α	1, 5, 10, 25 μmol Twice/week for 18 weeks	Jang et al. [38]; Jang and Pezzuto [24]
Suppresses the development of DMBA-initiated and TPA-promoted papillomas in female ICR mice	Free radical scavenging	85 nmol/l 21 weeks	Kapadia et al. [39]
Reduces the onset of skin tumors with DMBA-TPA model in CD-1 mice	Antioxidant effect	1, 5, 10, 25 μmol Twice/week for 18 weeks	Soleas et al. [29]
Inhibits the development of DMBA-TPA-induced skin tumors in male Swiss albino mice	↑Apoptosis; ↑Bax; ↑p53; ↓Bcl-2; ↑cytochrome c release; ↑APAF-1	50 μmol/mouse 3–24 weeks	Kalra et al. [26]
Prevents UVB-mediated photocarcinogenesis in female SKH-1 mice	↓COX; ↓ODC; ↓lipid peroxidation	25 μmol/mouse	Afaq et al. [40]
Decreases UVB-induced skin hyperplasia in female SKH-1 mice	↑CDK-2, CDK-4, and CDK-6; ↑cyclin D1 and cyclin D2; ↑MAPK; ↑p21; ↑p53; ↓COX-2; ↓ODC; ↓survivin mRNA and protein	10 μmol/mouse 7 times on alternate days	Reagan-Shaw et al. [41]; Aziz et al. [42]
Prevents UV radiation-mediated skin tumorigenesis in female SKH-1 mice	↑Survivin mRNA and protein; ↑ phospho-survivin; ↓Smac/DIABLO	25, 50 μmol/mouse Twice/week, 28 weeks	Aziz et al. [42]
Delays tumor growth in female C57Bl/6N mice transplanted with B16-BL6 melanoma cells		50 mg/kg 19 days	Caltagirone et al. [30]
Does not slow down the growth of B16M melanoma cells inoculated into the footpad of male C57Bl/6J mice		20 mg/kg, 23 mg/ml 10 days	Asensi et al. [33]
Does not inhibit the growth of A375 human melanoma cells xenografted in male nu/nu mice		10, 25, 50, 100 mg	Niles et al. [43]

Skin Aging

Skin aging can occur in both intrinsic and extrinsic processes. In addition, aging may occur through metabolic processes, free radicals and cosmic irradiation. The outcome of the intrinsic aging include smooth, dry, and thinner skin with accentuated expression lines while extrinsically aged skin shows signs of photo-damage that include appearance of wrinkles, pigmented lesions, actinic kerasotes, and patchy hypo-pigmentations. Resveratrol has the ability to protect photo-damaged skins [44], UV radiation leading to photo-damage elicit premature skin aging (photoaging) resulting in extensive damage to dermal connective tissue. This causes the wrinkle formation by disrupting the normal dermal structure of skin connective tissue, which mainly contains collagen. A recent study demonstrated that

SirT1 reduced MMP-9 transcriptional expression in skin [45]. Resveratrol and metformin significantly inhibited MMP-9 expression and protected collagen from degradation after UV radiation. This study suggest that resveratrol through the activation of SirT1 could be used as a therapeutic agent to delay or block photoaging.

In another study, resveratrol exhibited anti-inflammatory action on the mouse skin via the inhibition of cyclooxygenase (COX) [46]. UVB exposure to the skin significantly increased epidermal COX activity at 24 h following UVB exposure and the pre-application of resveratrol prior to UVB irradiation resulted in a significant inhibition of UVB-mediated increase in epidermal COX activity, Anti-inflammatory activity and DNA repairing ability of resveratrol was also discussed in a recent paper [47].

Acne Vulgaris

A recent study investigated the therapeutic effects of resveratrol for the treatment of acne vulgaris [48]. In this study, resveratrol incorporated in a carboxymethylcellulose-based gel was applied to the right side of the face of the patients affected by acne vulgaris for 60 days, while the left side of the face was treated with vehicle only. Clinical evaluation showed a 53.75% reduction in the global can grading system score on the resveratrol-treated sides of the faces compared with 6.10% on the vehicle-treated sides of the face. These data were supported by histological analysis, which showed a 66.7% reduction in the average area of microcomedones on the resveratrol-treated sides of the face, compared to 9.7% reduction in the vehicle-treated sides.

Wound Healing

In another study, resveratrol (5,000 ppm) from grape seed proanthocyanidin (GSPE) extract induced VEGF expression in cultured keratinocytes, with pretreatment of HaCaT keratinocytes upregulating hydrogen peroxide and TNFα followed by VEGF expression. The authors concluded that resveratrol has the potential to heal dermal wounds [49]. Subsequently, the same investigators used topical GSPE to treat the dermal wounds in vivo [50]. GSPE increased the rate of wound contraction and closure in concert with the expression of VEGF and tenascin in wound edge tissue. Another related study showed that resveratrol ad GSPE together promoted VEGF expression and induced wound angiogenesis [51]. In a related study, resveratrol showed accelerated healing of left colonic anastomosis. [52]. In this study, 32 male Wistar albino rats were randomized into two groups and subjected to colonic anastomosis. The study group was treated with RSV and the control group received tap water instead. The rats were sacrificed 3 and 7 days postoperatively. Wound complications, intra-abdominal abscesses, and anastomotic leaks and stenosis were recorded. Four types of assessment were performed: bursting pressure, hydroxyproline (OHP) content, histopathology, and biochemical analysis. Compared to the control group, the resveratrol-treated rats displayed a higher bursting pressure ($p<0.001$) and anastomotic OHP content ($p<0.05$). RSV treatment leads to significant increase in PON activity at both time points and decrease in malondialdehyde levels on postoperative day 3 ($p<0.001$). Histopathological analysis revealed that RSV administration leads to a better anastomotic healing in terms of mucosal ischemia, neovascularization, reepithelialization, fibroblast, and lymphocyte infiltration. The study results suggest that exogenous RSV administration exerts a positive effect on experimental colonic wound healing in the rat. Although the precise cellular mechanisms by which RSV enhances anastomotic wound healing is not clear, stimulation of neovascularization, generation of collagen synthesis, inhibition of over-inflammation, and restriction of oxidative injury seems to be of paramount importance.

In another related study, however, resveratrol was found to delay wound healing process due to the inhibition of angiogenesis [53]. In this study, resveratrol directly inhibited capillary endothelial cell growth. It blocked both VEGF and FGF receptor-mediated angiogenic responses. In addition, resveratrol inhibited the phosphorylation of mitogen-activated kinase isoforms (MAPKp44/MAPKp42) induced by FGF-2 in proliferating endothelial cells in a dose-dependent manner.

UV Radiation

Ultraviolet radiation A (UVA) is an important factor in the development of skin carcinogenesis. A recent study using human keratinocytes demonstrated that resveratrol could protect the skin cells from UVA-induced damage [54]. In this study, human keratinocytes were UVA irradiated and the effects of resveratrol on cell viability; reactive oxygen species (ROS) generation and membrane lipid peroxidation were measured. The proteins and mRNA of Nrf2 and Kelch-like ECH-associated protein 1 (Keap 1) were determined by immunofluorescence staining. Resveratrol could effectively increase the viability of keratinocytes after UVA exposure presumably by increasing the level of Nrf2 protein and facilitating its accumulation in the nucleus. Resveratrol reduced the Keap1 protein, a repressor of Nrf2 in the cytoplasm by degrading Keap1 protein.

In related studies, resveratrol protected against UVB-mediated injury in SKH-1 hairless mice through the inhibition of survivin [42, 55]. In this study, the mice were subjected to chronic UVB exposure (180 mJ/cm^2, twice weekly, for 28 weeks). The experimental animals received either a pre-treatment (30 min before each UVB) or post-treatment (5 min after UVB) of topical resveratrol. The topical application of skin with resveratrol (both pre- and post-treatment) resulted in a highly significant inhibition in tumorigenesis and also a delay in the onset of tumorigenesis. This study found survivin was the primary factor for resveratrol-mediated protection of the skin. The same authors reported that modulations of cki-cyclin-cdk network and MAPK pathway were also involved in skin protection afforded by resveratrol [41, 55]. Topical application of resveratrol resulted in significant decrease in UVB-induced bi-fold skin thickness, hyperplasia and infiltration of leukocytes. The data from immunoblot and/or immunohistochemical analyses revealed that multiple exposure to UVB radiations caused significant upregulation in proliferating cell nuclear antigen (PCNA), a marker of cellular proliferation, and cyclin-dependent kinase (CDK)-2, -4 and -6, cyclin D1 and cyclin D2. Resveratrol treatment resulted in significant downregulation in UV-mediated increases in these critical cell cycle regulatory proteins. Resveratrol also resulted in further stimulation of UVB-mediated increases in cyclin kinase inhibitor WAF1/p21 and tumor suppressor p53. Overall, the results of this study suggested that he modulations of cki-cyclin-cdk network by resveratrol as associated with the inhibition of MAPK pathway. Resveratrol also resulted in significant inhibition of UVB-mediated induction of cyclooxygenase and ornithine decarboxylase (ODC) enzyme activities and protein expression of ODC, which are well-established markers of tumor promotion as well, increased oxidative stress-induced lipid peroxidation [40]. In another recent study, resveratrol modulated transforming growth factor β2 (TGFβ2) signaling to block UV-induced tumor progression. [56]. This study showed that oral administration of resveratrol to highly tumor-susceptible p53+/–/SKH-1 mice markedly delayed UV-induced skin tumorigenesis and reduced the malignant conversion of benign papillomas to SCCs. TGFβ2 was predominantly over-expressed in UV-induced SCCs and its expression was diminished in resveratrol-treated SCCs/ skin. In addition, resveratrol increased the level of epithelial cadherin. Resveratrol treatment decreased phosphorylation of Akt and CREB. The authors concluded that resveratrol suppresses UV-induced malignant tumor progression in p53+/–/SKH-1 mice through the Akt-mediated down regulation of TGFβ. In another related study, resveratrol enhanced UVB-induced cell death through nuclear factor κB pathway in human epidermoid carcinoma A431 cells [57, 58]. The authors demonstrated that resveratrol and UVB treatment of A431

cells disrupted the NFkB pathway by blocking phosphorylation of serine 536 and inactivating NFkB and subsequent degradation of 1kBα, which regulates the expression of survivin. Their study showed that combination of resveratrol and UVB acted synergistically against skin cancer cells.

Resveratrol Binding Sites in Human Skin Tissue

Human skin tissue has been found to contain specific ^{3}H-resveratrol binding sites [$K_D = 10$ nM] that are localized in the epidermis [59]. Exposure of human keratinocyte cells to nitric oxide free radical donor sodium nitroprusside resulted in cell death, which was reduced by resveratrol [$EC_{50} = 200$ μM] and also to a lesser extent by a resveratrol analog piceatannol [$EC_{50} = 95$ μM] The authors concluded that the protective action of resveratrol was likely to be due to the anti-apoptotic effect since at the same concentration resveratrol reduced the number of apoptotic cells as well as apoptotic events triggered by SNP. In clinical studies, resveratrol performed amazingly well. Caudalie products that use resveratrol as ingredient claim that it created a denser and firmer skin texture while boosting the life expectancy of the skin cells by a whopping 160%. It stimulated cellular renewal, enhanced the skin's support system (collagen and elastin) and improved the multiplication of fibroblasts. After only 6 days, patients using Caudalie products experienced skin with double the thickness. After 28 days, deep wrinkles were reduced by 24%. These incredible results work together to achieve a truly younger-looking complexion that exhibits a healthy tone and attractive texture.

Psoriasis, Eczema and Other Exfoliative Skin Disorders

Recent research has hinted that resveratrol can be an effective treatment for psoriasis lesions, acne eczema and other exfoliative skin disorders. Psoriasis is a chronic inflammatory disease of the skin that typically follows a relapsing/remitting disease course. Psoriasis is primarily a T-cell-mediated disease, but intrinsic alterations in epidermal keratinocytes also play an important role. Tumor necrosis factor alpha (TNFalpha) has been identified as an important cytokine in the inflammatory cascade of psoriasis [60]. Resveratrol, a polyphenol interferes in the TNFalpha function via the NFkB thereby modulating the inflammatory condition [61]. The therapeutic efficacy of compounds such as resveratrol that interfere with TNFalpha function thus acts as a potential agent in the management of psoriasis. Clinical tests on human patients indicated that all the subjects treated with ointment containing 1% resveratrol compared to those in the control group without showed rapid decrease in their symptoms to 50% during the first 2 weeks of treatment. Eighty percent of the patents showed remarkable improvements while only 20% showed acceptable improvements while none in the control group exhibited any improvement at all. In all the subjects, no unwanted side effects were noted. In contrast to current treatment of exfoliative skin disorders, resveratrol has no systemic or topical side effects during and after treatment [62].

A patent was filed in the year 2001 by Pelliccia and her coworkers on the use of resveratrol (3,4′,5-trihydroxy-trans-stilbene) and derivatives thereof, for the preparation of medicaments for the treatment of exfoliative eczema, acne and psoriasis, topical pharmaceutical formulations containing resveratrol or derivatives thereof in combination with other active principles. Treatment consists in topical administrations of resveratrol at concentrations of 0.01–20%, in the form of lotions, creams, or ointments, optionally in combination with other active principles such as melatonin, vitamins D, E, and A and derivatives thereof, hormones, vegetable and/or animal extracts [63]. Another recent investigation by Clement and his team have found that compositions for topical treatment comprising of solubilized resveratrol in a stable emulsion form with dimethyl isosorbide solvent system does not

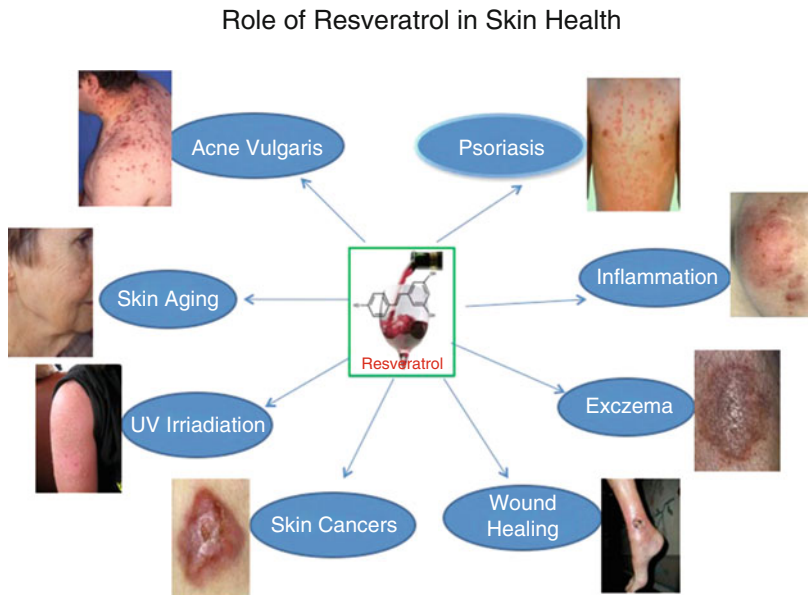

Fig. 18.1 Role of resveratrol in skin health

cause irritation to the skin [64]. The compositions can preferably be used for transdermal applications for the treatment of various skin diseases such as psoriasis, acne, and herpes simplex virus. However, the exact mechanism of action of the phytoalexin on the dermal cells is to be confirmed.

Summary and Conclusion

Skin is always exposed to environmental toxins including pro-oxidants and harmful radiations that could lead to skin aging, inflammatory disorders, and even skin cancers. In this review, we have discussed all these skin problems and how resveratrol can protect the skin (Fig. 18.1). Skin has its own antioxidant system. Resveratrol can certainly protect the skin from harmful UVB radiation and cancer by causing cell cycle arrest in the G1 phase or in the S-G2 phase transition or trigger apoptosis leading to the destruction of the tumors. The possible signal transduction pathways include up regulation of oncosupressor p53 and cyclin kinase inhibitor p21/Waf1/Cip1. In skin several molecular targets have been identified that include p27Kip, NFkB, Akt, ERK, p53, p51, FoxO, and SirT. The existing results indicate that resveratrol possesses a great deal of potential as a skin protecting agent ranging from cancer to aging. Antiaging potential of resveratrol appears to be of great potential. Resveratrol does not combine with other ingredients in creams very well; genetically created micronized resveratrol particles could be our future hope.

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Chapter 19
Skin Care Properties of Grape Seed Polyphenols, a By-Product of the Winery Industry

Manjeshwar Poonam Baliga Rao, Raghavendra Haniadka, Karadka Ramdas Thilakchand, Princy Louis Palatty, and Manjeshwar Shrinath Baliga

Key Points

- In recent years grape (*Vitis vinifera*) seeds, which are an abundantly available waste product of the winery and grape juice industry, has been the subject of intense study as numerous experiments have shown them to possess a range of unique biological and pharmacological properties that include free radical scavenging, antioxidant, anti-mutagenic, anti-inflammatory, and antimicrobial activities, as well as cardioprotective, hepatoprotective, neuroprotective, and anticarcinogenic properties and prevention of chemical- and UV-induced skin cancer in mice.
- The myriad pharmacological properties are accredited to the presence of high concentration of polyphenols like catechins, epicatechins, and procyanidins (also known as proanthocyanidins) in them.
- This review attempts at addressing the beneficial effects of grape seed polyphenols in skin care.

Keywords *Vitis vinifera* • Grape seed polyphenols • Skin care

Introduction

Globally, grapes (*Vitis vinifera*) are one of the most important fruits and are of dietary, medicinal, and industrial use. Grapes have been used right from the ancient Greek and Roman civilizations to prepare wines and today it is a major industry. Grape seeds generated during the production of wine were initially used as soil conditioner, as adsorbent for heavy metals, as fertilizer, and a minor component of the feed for domesticated animals [1, 2].

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Innumerable scientific studies carried out in the past two decades have shown that the seeds of grapes (*V. vinifera*), which are a waste product of the winery and grape juice industry, possess myriad biological and pharmacological effects. Grape seeds are rich in polyphenols and are primarily composed of dimers, trimers, and oligomers of monomeric catechins or epicatechins commonly known as procyanidins or proanthocyanidins [1, 2]. Commercial preparations of grape seed polyphenols are marketed in the United States as GSE3, with 95% standardized procyanidins as dietary supplement due to their health benefits [3].

Grape seed proanthocyanidins are shown to possess free radical scavenging, antioxidant, antimutagenic, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, and anticarcinogenic properties [3]. With regard to skin care, preclinical studies have shown grape seed polyphenols to possess antibacterial effects on the methicillin-resistant *Staphylococcus aureus* (MRSA), to promote hair growth in mice, to prevent melasma in humans, and to prevent the chemical- and UV-induced skin cancer in mice. In the following sections each of the validated properties is addressed.

Grape Seed Extract Possesses Potent Antibacterial Effects on MRSA

Historically, *S. aureus* has been a significant human pathogen known to cause broad spectrum of illnesses, ranging from minor skin infections to life-threatening deep tissue infections. The development of MRSA that are resistant to the commonly used antibiotics is a serious threat to patients. It is associated with higher morbidity and mortality, prolongs hospitalization, and makes treatment difficult and costly [4]. Therefore, new agents are needed to treat infections from MRSA. Al-Habib et al. [5] investigated the antibacterial activity of the grape seed extract against 43 strains of MRSA by gel diffusion, growth, and respirometric studies. The authors observed that all strains were sensitive to the grape seed extract and a complete inhibition of all bacterial strains tested was observed at a concentration of 3 mg/ml. Scanning and transmission electron microscopy confirmed that the observed antibacterial activity was mediated by the disruption of the bacterial cell wall [5].

Grape Seed Prevents Melasma

Chloasma or melasma, an acquired hypermelanosis disorder, typically is an undesired change and has negative psychological and emotional effects on the patients. Melasma is often recalcitrant to various treatments and an amenable as well as safe pigment-reducing modality is exigently needed. Yamakoshi et al. [6] investigated the reducing effect of proanthocyanidin on woman with chloasma for 6 months in an open trial. The investigators observed that the oral administration of the proanthocyanidin-rich grape seed extract was effective and decreased the melanin-index (hyperpigmentation) significantly. The beneficial effects of grape seed extract were maximally achieved after 6 months in 83% of the women, with no further improvement after this period. Continuation of the grape seed extract intake for 5 more months was observed to be preventing chloasma from becoming worse during the summer season. Cumulatively, these observations indicate the usefulness of grape seed extract in improving chloasma and at nontoxic concentrations.

Grape Seed Promotes Hair Growth

Globally, hair loss (alopecia) is a common and distressing symptom and affects a considerable population of the world. The most common forms of alopecia include androgenic alopecia or common baldness, telogen effluvium, chemotherapy-induced alopecia, and alopecia areata. The conventionally used medications like finasteride and minoxidil although beneficial possess undesired side effects. This has necessitated the need for nontoxic effective agents that can prevent hair loss and also promote the growth of hair. Preclinical studies with mice have shown that grape seed proanthocyanidins promoted proliferation of hair follicle cells by about 230% when compared to the controls [7].

The proanthocyanidins were also observed to possess remarkable hair-cycle-converting activity from the telogen phase to the anagen phase in C3H mice [7]. In vitro studies showed that the procyanidin dimer and trimer exhibited higher growth-promoting activity than the monomer [8]. Additionally, when compared to placebo-treated cohorts, the topical application of 1% procyanidin oligomers on shaven C3H mice in the telogen phase caused significant hair regeneration demonstrating the hair-growing activity of procyanidin oligomers both in vitro and in vivo [8].

Grape Seed Polyphenols Are Effective in Preventing Chemical Carcinogenesis

Preclinical studies have shown that grape seed extract was effective in inhibiting the 7,12-dimethylbenz [α]anthracene (DMBA)-induced TPA-promoted two-stage skin carcinogenesis in SENCAR mouse [9] and CD-1 mice [10]. The investigators in both these studies observed that the topical application of the grape seed extract following tumor initiation with DMBA but before 12-O-tetradecanoylphorbol-13-acetate (TPA) application caused a dose-dependent-manner reduction in tumor incidence, tumor multiplicity, and tumor volume [9, 10]. Mechanistic studies have shown that the topical application of grape seed extract (5, 10, 20, and 30 mg) inhibited TPA-induced tumor promotion by decreasing the activity of myeloperoxidase (MPO) activities in a concentration-dependent manner [10]. However the application of grape polyphenolics (20 mg) at 60, 120, and 240 min after treatment with TPA resulted in no significant changes in ornithine decarboxylase (ODC) activity [11]. In vitro studies have shown grape seed extract to be a competitive inhibitor of ODC activity [11]. Application of grape seed extract prior to TPA decreased protein kinase C (PKC) activity at 10 and 30 min following TPA treatment [11]. Grape seed extract is also shown to be effective in decreasing the DMBA-induced inflammatory hyperplasia in and to reduce percentages of mice with mutation in codon 61 of Ha-ras oncogene [12].

Recent studies have also shown that the dietary feeding of grape seed extract (0.2 and 0.5%, w/w) was also effective in preventing DMBA-induced TPA-promoted two-stage skin carcinogenesis in C3H/HeN mice. The mice treated with grape seed extracts developed a significantly lower tumor burden in terms of the percentage of mice with tumors, total number of tumors per group, and total tumor volume per tumor-bearing mouse as compared with the mice that received the control diet. Grape seed extract also delayed the malignant progression of papillomas into carcinomas [13]. Mechanistic studies showed that grape seed extract inhibited the expression of cyclooxygenase-2 (COX-2), prostaglandin E(2) (PGE(2)), and markers of proliferation (proliferating cell nuclear antigen and cyclin D1) in DMBA-initiated/TPA-promoted mouse skin and skin tumors [13].

Grape seed extract inhibited the TPA-induced edema, hyperplasia, leukocyte infiltration, myeloperoxidase (MPO), COX-2 expression, and PGE(2) production in the mouse skin, clearly indicating that the grape seed extract mediates the chemopreventive effects by inhibiting the inflammatory responses caused by tumor promoters [13]. Grape seed extract scavenges peroxyl and superoxide

radicals and protects cells from hydrogen peroxide-induced DNA damage [12]. In vitro studies with three murine keratinocyte cell lines, i.e., non-tumorigenic (3PC), papilloma-derived (MT1/2), and squamous cell carcinoma-derived (Ca3/7) cell lines, have also shown that the IC50 values ranged from 20 to 35 mM for all the three cell lines [12].

Cell culture studies with human epidermoid carcinoma cells (A431) have shown that grape seed extracts inhibited cellular proliferation and induced cell death in a concentration- and time-dependent manner [14]. Mechanistic studies showed that the inhibition of cell proliferation was caused by increasing the G1-phase arrest, and was mediated by decreasing cyclin-dependent kinases (Cdk) Cdk2, Cdk4, and Cdk6 and cyclins D1, D2, and E. A concomitant increase in the protein expression of cyclin-dependent kinase inhibitors (Cdki), Cip1/p21 and Kip1/p27, and enhanced binding of Cdki–Cdk was also observed [14]. Grape seed extract also caused dose- and time-dependent apoptosis by increasing the expression of proapoptotic Bax, decreased expression of antiapoptotic Bcl-2 and Bcl-xl, loss of mitochondrial membrane potential, and cleavage of caspase-9, caspase-3, and PARP [14].

Grape seed extract inhibited both constitutive as well as EGF-induced higher levels of phosphorylated proteins of mitogen-activated protein kinase (MAPK) family with concomitant reactivation of MAP kinase phosphatases. It decreased the levels of phosphatidylinositol 3-kinase (PI3K) and phosphorylation of Akt at ser473. It also decreased the constitutive activation of NF-kappaB/p65 and inhibited expression of COX-2, inducible NO synthase (iNOS), proliferating cell nuclear antigen (PCNA), cyclin D1, and matrix metalloproteinase-9 (MMP-9) [15]. Oral treatment of athymic nude mice with grape seed extracts (50 or 100 mg/kg body weight/mouse) reduced the growth of A431-xenografts in mice by inhibiting transcription of PCNA and cyclin D1, and reduced the activity of nuclear factor-κB (NF-κB) [15].

Grape Seed Polyphenols Are Effective in Preventing UV-Induced Skin Carcinogenesis

Multiple exposures to ultraviolet radiation (UVR) cause severe damage to skin and may lead to the development of several cutaneous disorders including skin cancer. Animal studies by Mittal et al. [16] have shown that the dietary feeding of proanthocyanidins extracted from grape seeds (0.2 and 0.5%, w/w) was effective in preventing UVB-induced complete (both initiation + promotion), initiation, and promotion stages of photocarcinogenesis in the SKH-1 hairless mice. The investigators observed a concentration-dependent decrease in the tumor incidence, tumor multiplicity, and tumor size. When compared with non-grape seed extract-treated mice, feeding grape seed extract (0.5%, w/w) prevented the malignant transformation of UVB-induced papillomas to carcinomas in terms of carcinoma incidence, carcinoma multiplicity, and carcinoma size [16]. Biochemical studies indicated that the grape seed extract mediates the protective effects at least in part by the reduction in UVB-induced oxidative damage and tissue fat content in the skin [16].

Studies with cultured human epidermal keratinocytes (NHEK) have also shown that pretreatment with grape seed extract inhibited UVB-induced hydrogen peroxide (H_2O_2), lipid peroxidation, protein oxidation, and DNA damage and also inhibited UVB-induced depletion of antioxidant defense components like glutathione peroxidase, catalase, superoxide dismutase, and glutathione [17]. To further substantiate these observations recent studies by Filip et al. [18] have also shown that the topical application of the extract of red grape seed (Burgund mare variety) was also effective in preventing UVB-induced oxidative stress. The topical application of the extract (4 mg/mouse/cm^2) 30 min before a single dose of UVB 240 mJ/cm^2 decreased glutathione (GSH) formation and glutathione peroxidase activity and inhibited UVB-induced lipid peroxidation and nitric oxide production [18].

UV-induced oxidative stress is mediated by the activation of mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways and its inhibition contributes to prevention of the ensuing damage. Treatment of NHEK with grape seed extract inhibited UVB-induced phosphorylation of ERK1/2, JNK, and p38 proteins of the MAPK family at the various time points studied [17]. Grape seed extract also inhibited UVB-induced activation of NF-κB/p65 and this effect was mediated through the inhibition of degradation and activation of IkappaBalpha and IKKalpha, respectively [17]. Additionally recent studies have also shown that the topical application of the extract of red grape seed reduces caspase 3 activity, indicating that the cells were protected against apoptosis [18].

Grape seed extract is also shown to inhibit UVB-induced infiltration of proinflammatory leukocytes and the levels of MPO, cyclooxygenase-2 (COX-2), prostaglandin (PG) E(2), cyclin D1, and proliferating cell nuclear antigen (PCNA) in the skin and skin tumors [19]. Grape seed extract modulates UVB-induced inflammatory reaction and immunosuppression by reducing IL-10 (immunosuppressive cytokine) in skin and draining lymph nodes and by concomitantly increasing the IL-12 (immunostimulatory cytokine) in the draining lymph nodes [20] and decreasing proinflammatory cytokines (TNF-a, IL-1b, and IL-6) in UVB-exposed skin and skin tumors of mice [19].

Exposure to UVB causes cyclobutane pyrimidine dimers (CPD) and studies have shown that grape seed extract reduced the levels of CPD-positive cells in UVB-exposed skin of normal wild-type mice but not in the skin of interleukin-12p40 (IL-12) knockout mice [21]. Mechanistic studies have shown that grape seed extract (0.5%, w/w) was effective in reducing the UVB-induced skin tumor development in wild-type mice by increasing the mRNA levels of nucleotide excision repair genes, such as XPA, XPC, DDB2, and RPA1. However a similar effect was not seen in the IL-12 KO mice, suggesting that IL-12 is required for the repair of CPD by grape seed extract [21]. Additionally cell culture studies have also shown that grape seed extract repaired UV-induced CPD-positive cells in xeroderma pigmentosum complementation group A (XPA)-proficient fibroblasts but failed in the XPA-deficient fibroblasts from XPA patients. Furthermore, grape seed extract also enhanced nuclear translocation of XPA and enhanced its interactions with other DNA repair protein ERCC1 to ensue the repair [21].

Conclusions

Grape seed extract is a promising antioxidant that is currently being investigated for a variety of disease conditions including that of the skin disorders and cancer. Skin is particularly well suited for the use of this promising agent because the antioxidant properties of grape seed extract work well against the high oxidative stress that skin cells come under frequently. Grape seed extract has shown promise against skin diseases and even more prospects are yet to be explored, especially on the delivery systems for topical application. Studies are also required to ascertain the transdermal efficacy, cytotoxicity on the skin, topical skin penetration, and stability and safety of grape seed extract is required. Grape seed extract contains many phytochemicals and their pharmacological properties may be affected by their ratio (Fig. 19.1). Therefore studies should be performed with well-characterized standardized extracts and with the important bioactive compounds present in it.

Toxicity studies have shown that subchronic (0, 0.63, 1.25, or 2.5% for a period of 1 month) and chronic administration of grape seed extract (0.5, 1.0, or 2.0% for a period of 90 days) was well tolerated by male and female Sprague-Dawley rats [22, 23]. The results from this study showed that it was devoid of any evidence of toxicity. In milieu of these observations it is safe to suggest that requirement for interventional studies in humans to investigate whether skin functions and conditions can be modulated by supplementing the diet with grape seed extract. The outcomes of such studies may be useful for the clinical applications of grape seed extract and may open up a new therapeutic avenue.

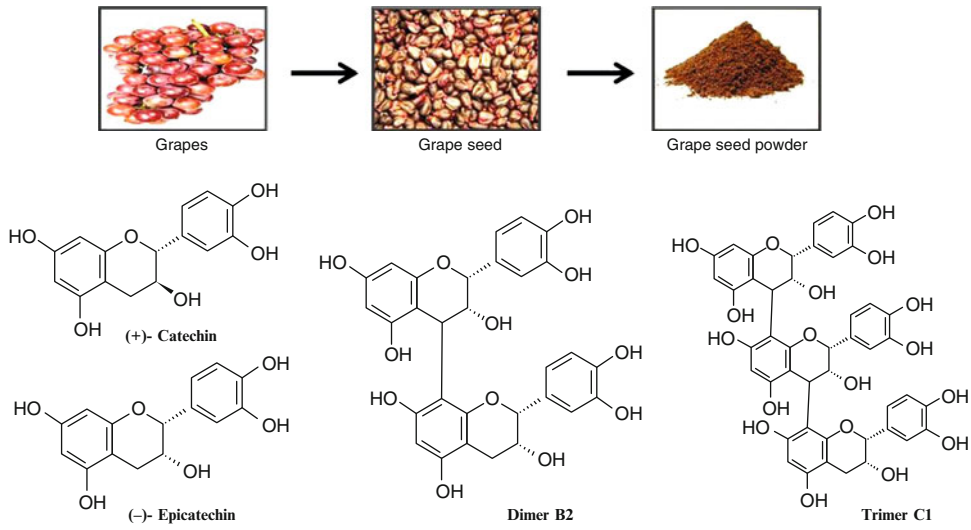

Fig. 19.1 Phytochemicals present in grape seed

In addition to the skin care effects, grape seed extract is also shown to possess free radical scavenging, antioxidant, anti-mutagenic, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, and anticarcinogenic properties, and regular consumption of nontoxic concentration of grape seed extract as a dietary supplement may be beneficial for these diseases too. Due to its abundance, low cost, and safety in consumption, grape seed extract has tremendous potential to develop as a nontoxic skin care agent, but only when the lacunae in the existing knowledge are bridged.

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Chapter 20
Skin Health Benefits of Coenzyme Q_{10}

Jarmila Hojerova

Key Points

- Two most remarkable effects of coenzyme Q_{10} (CoQ_{10}) in relation to human skin lie in its indispensability in the cellular energy production and protection of cell against oxidative stress.
- The body's ability to produce CoQ_{10} declines with age or due to certain diseases, resulting in, inter alia, various adverse skin manifestations.
- Oral and dermal supplementation with CoQ_{10} (1) may suppress UV light-induced oxidative stress in skin cells, (2) may inhibit the activity of some degradative enzymes, (3) may accelerate the proliferation of dermal fibroblasts and production of types IV and VII collagens, and (4) may stimulate biosynthesis of hyaluronic acid.
- Skin benefits of CoQ_{10} supplementation for patients with psoriasis and melanoma are in the research stage only.
- Recommended daily allowance (RDA) for supplemental CoQ_{10} cannot be uniformly determined because it depends not only on skin health, age, and other factors but also on the specific bioavailability of applied CoQ_{10} form. The supplement with better bioavailability requires a lower dose.
- New nanostructure CoQ_{10} carriers have better skin permeability and bioavailability in comparison to traditional CoQ_{10} crystalline suspensions.
- New virtually water-soluble CoQ_{10} formulations as well as new ubiquinol products may have greater bioavailability for skin than traditional ubiquinone products, but there is a need for more scientific evidence.

Keywords Coenzyme Q_{10} • Ubiquinone • Ubiquinol • Dietary supplement • Cosmetics • Energy • Antioxidant • Skin ageing • Wrinkles • Skin-care • Permeability • Bioavailability

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Abbreviations

ATP	Adenosine triphosphate
CoQ	Coenzyme Q
CoQ_{10}	Coenzyme Q_{10}
CoQ_{10}-NLC	Coenzyme Q_{10}-nano-structured lipid carrier
CoQ_{10}-PMLs	Coenzyme Q_{10}-plurilamellar multivesicular liposomes
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
IPM	Isopropylmyristate
J	Joule
M	Mol
Mcg, mcM	Microgram, micromol
mg	Milligram
nM	Nanomol
PMLs	Plurilamellar multivesicular liposomes
RDA	Recommended daily allowance
SQ	Squalene
TPA	Tocopherolacetate
TPA-PMLs	Tocopherolacetate-plurilamellar multivesicular liposomes
UPE	Ultra-weak photon emission
UQ	Ubiquinone, ubiquinone-10, ubidecarenone
$UQH^{\cdot}$	Ubisemiquinone-10 (radical)
UQH_2	Ubiquinol, ubiquinol-10, dihydroquinone-10
UV	Ultraviolet (radiation, rays)
VE	Vitamin E
WA	Wrinkle area
WV	Wrinkle volume

Introduction

Coenzyme Q_{10}, also known as CoQ_{10}, ubiquinone-10, ubiquinone, or ubidecarenone, is only one of the substances that occur naturally in the body including skin. But what is so special about CoQ_{10} that over the last two decades it has become the subject of more than 4,000 scientific studies, 7 conferences of the International Coenzyme Q_{10} Association, and many international symposia, and, moreover, is linked with the Nobel Prize in chemistry? And why since the nineties, CoQ_{10} dietary supplements and skin-care cosmetics have become one of the best-selling products? However, what are the real benefits of these products for human skin? The purpose of this chapter is to answer these questions.

Coenzyme Q_{10} Properties and General Health Benefits

Discovery and History of Coenzyme Q_{10}

Although members of coenzyme Q family have developed together with biological evolution over millions of years [1], they were uncovered only a half century ago. As with other amazing breakthroughs

in medicine, CoQ_{10} was discovered by chance. It was in 1957 during experiments conducted on beef heart, when biochemist Crane at the University of Wisconsin, USA, noticed unusual yellow crystals in the lipid from mitochondria [2]. Because of its quinone structure Crane's group named this substance coenzyme Q. The same year, researchers from Morton's laboratory in Liverpool, England, isolated an unknown compound from rat liver. As its widespread occurrence within organic materials was later confirmed, Morton's group introduced the name ubiquinone (UQ), meaning the "ubiquitous quinone" (lat. ubique—everywhere) [3]. The exact chemical structure of this substance was determined in the next year by two groups of researchers independently. The Morton's group [4] established the structure of UQ. Subsequently the group of Folkers [5] at the Merck laboratories in Rahway, USA, decoded the structure of CoQ, demonstrating that CoQ and UQ are the same. In 1978 British biochemist Mitchell [6] earned a Nobel Prize in chemistry for his work on cellular power production involving CoQ_{10}. This award has become very important for further recognition of CoQ_{10}. The first organized clinical trial in humans was performed by Yamamura of Japan in 1965, where CoQ_7 (similar to CoQ_{10}) was given to patients with heart failure [7, 8]. Initially tests were small scale as CoQ_{10} had to be extracted from the animal heart. It often took hours to obtain only 1 g of CoQ_{10} and the cost of 1 g was over 1,000 USD [8]. In 1974, the Japanese managed to develop production of CoQ_{10} by the yeast fermentation. Thanks to reduced prize, hundreds of scientists and physicians have begun to study CoQ_{10} in relation to various diseases as well as health promotion. Since 1998, every 2–3 years researchers from around the world meet at the conference of the International Coenzyme Q_{10} Association to discuss the latest knowledge on CoQ_{10}.

Chemical and Physical Properties of Coenzyme Q_{10}

Coenzymes are generally nonprotein organic molecules that serve as cofactors for the activation of protein apoenzymes. Coenzymes Q perform this function for at least three mitochondrial enzymes as well as enzymes in other parts of the cells. CoQ family is a series of homologues with the same benzoquinone ring, but with different length (1–12) of *trans*-monounsaturated isoprenoid units depending on the species. Each organism has one dominant homologue of CoQ and minor amounts of other homologues. The most common homologue both in humans and many mammals consists of ten isoprenoid units, so the name coenzyme Q_{10}; a minor homologue is CoQ_9. There are some other polyisoprenoid compounds structurally similar, such as squalene, beta-carotene, and vitamins A, E, and K_1 in nature.

CoQ_{10} or 2,3-dimethoxy-5 methyl-6 decaprenyl-1,4 benzoquinone ($C_{59}H_{90}O_4$) is a yellow-orange (ubiquinone), tasteless, lipid-soluble powder having a melting point 48–52 °C and molecular weight 863.34 g/M. Its functional group is the quinone ring, the reduction of which to quinol produces a carrier of electrons and protons. So, CoQ_{10} is present in three alternative redox states in the body that are responsible for its metabolic functions: (1) the fully oxidized *ubiquinone*; (2) the univalently reduced ($1e^- + 1H^+$) *ubisemiquinone* ($UQH^{\cdot}$), a free radical; and (3) the fully reduced ($2e^- + 2H^+$) *ubiquinol* (UQH_2; see Fig. 20.1). The predominant state should be UQH_2. But its value in various cell membranes of the body ranges 95–30% depending on metabolic state of the cell. In blood plasma of healthy young individuals about 95% of CoQ_{10} should circulate in this reduced form. The plasma ratio of UQH_2/UQ is therefore a good marker of oxidative stress [9, 10].

Biosynthesis and Distribution of Coenzyme Q_{10} in the Human Body

CoQ_{10} is endogenously synthesized in nearly all of living body cells; hence it cannot be considered a vitamin. Its biosynthesis is a multistep process, with the following three main steps: (1) synthesis of the quinone moiety from amino acids tyrosine or phenylalanine; (2) synthesis of the isoprene chain

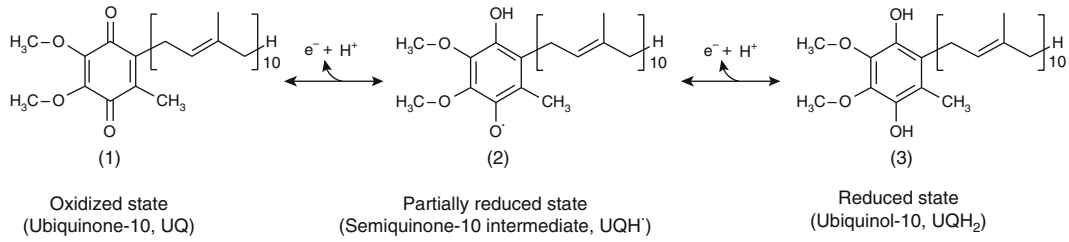

Fig. 20.1 Three alternate redox states of coenzyme Q_{10}: (1) oxidized, (2) intermediate, and (3) reduced states of coenzyme Q_{10}. In mitochondria, like in other subcellular organelles, coenzyme Q_{10} is present in its all states

from acetyl-coenzyme A via the mevalonate pathway; and (3) the joining of these two structures. Reasonable amount of at least seven vitamins (B_6, B_2, B_3, B_{12}, C, folic and pantothenic acids) and some trace elements are necessary for this biosynthesis which must be supplied by diet [11]. CoQ_{10} level in cells of different body parts varies considerably in dependence of their metabolic demands. The highest values of it were found in organs and tissues with high energy turnover such as the heart, kidney, liver, brain, and muscle (see Sect. "Coenzyme Q_{10} Content in the Human Skin").

Biosynthesis in the young healthy body together with normal diet provides the necessary CoQ_{10} level. In adulthood production of CoQ_{10} significantly decreases with age. Also any lack of required nutrients, defects in different biosynthetic enzymes, or gene mutation restrict the biosynthesis. Since human body produces CoQ_{10} in the same way as cholesterol, statins [11] that inhibit production of cholesterol also inhibit formation of CoQ_{10}. Thus, the reduced biosynthesis of CoQ_{10} may cause deficiency not only in mature age but even at a young age with all health consequences.

Coenzyme Q_{10} Intake Through Normal Diet

A small part of CoQ_{10} is acquired through diet. Unfortunately, most foods contain negligible amounts of it. Only organ meats (heart, liver, and kidney from animals), beans, nuts, whole grains, oily fish, soybean, and canola oils contain higher CoQ_{10} level. The usual value of CoQ_{10} in beef heart is 113.3 mcg/g, in beef liver 39.2 mcg/g, in the rapeseed oil 63.5 mcg/g, and in herring and canned tuna 15.9 mcg/g of fresh weight [12]. Boiling has little impact, but frying can deplete it by as much as 30% [13]. In addition, intestinal absorption of CoQ_{10} is limited. So, the average dietary intake of CoQ_{10} in men and in women is only about 5 and 3 mg/day, respectively [12] (see Table 20.1).

To complement the deficiency of CoQ_{10} higher values of this substance are required than are available in food. Here is an example. Plasma values of CoQ_{10} in adults of both sexes are usually 0.43–1.65 mcg/mL [10, 14]. The therapeutic value of CoQ_{10} in plasma was established in 1980 to be at least 1.0 mcg/mL CoQ_{10}, in 1990 was recalibrated to 2.5 mcg/mL, and at present—to 3.5 mcg/mL [7]. In any case, to increase plasma value of CoQ_{10} at least of 1 mg/mL, an external addition of 100 mg/day of CoQ_{10} is necessary [15]. Even when receiving a huge amount of beef heart or herring in the diet, it would be difficult to supply over 100 mg/day of CoQ_{10}.

The Main Functions of Coenzyme Q_{10} in the Human Body

CoQ_{10} has a number of cellular functions in the organism, three of which have been well documented: (1) the cellular energy formation; (2) the protection of cell against oxidative stress; and (3) the expression of genes.

Table 20.1 Average dietary intake of coenzyme Q_{10} (CoQ_{10}) and coenzyme Q_9 (CoQ_9) from different food groups

	Consumption of food (g/day)		CoQ_{10} (mg/day)		CoQ_9 (mg/day)	
Food group	Men	Women	Men	Women	Men	Women
Meat	130	79	3.02	1.9	0.09	0.05
Eggs	22	16	0.03	0.02	0	0
Fish	32	25	0.44	0.35	0.01	0.01
Dairy products	537	405	0.37	0.28	0	0
Cereals	209	150	0	0	0.47	0.33
Fruits	173	214	0.18	0.23	0.04	0.05
Vegetables	243	206	0.31	0.26	0	0
Vegetable oils	29	19	0.99	0.66	0	0

Adapted from Mattila and Kumpulainen [12]

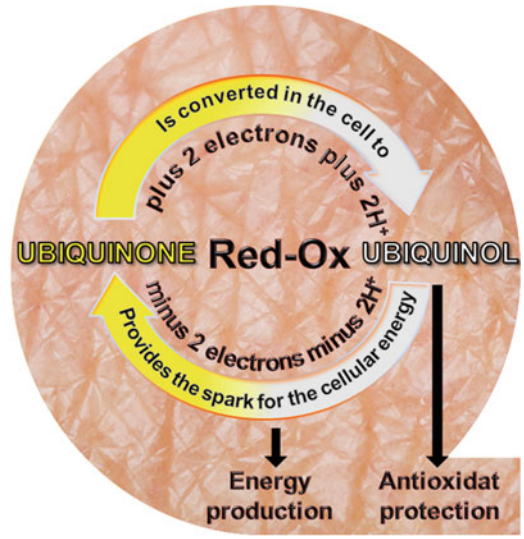

Fig. 20.2 The "Coenzyme Q cycle." The redox functions of coenzyme Q_{10} in skin cellular energy production and antioxidant protection are based on the ability to exchange two electrons and two protons in a redox cycle between ubiquinol (reduced) and ubiquinone (oxidized) state. Image created by Hojerova, inspired by Kaneka [57]

Coenzyme Q_{10} Contributes to the Energy Formation

The primary role of CoQ_{10} is to act as a catalyst for a complex of chemical reactions during which food is broken down into packets of energy that the body can use. Explained in more details, food energy is converted to chemical energy in the form of adenosine triphosphate (ATP) by oxidative phosphorylation in mitochondria, the energy-producing center of every cell. UQ, localized in mitochondrial membrane, accepts electrons from the complexes I and II. In doing so, the basic quinone structure absorbs them and becomes first, ubisemiquinone, and then ubiquinol (UQH_2) delivers electrons to complex III. UQH_2 reverts back to ubisemiquinone and then to UQ. At the same time as electrons, UQ transfers protons to the outside of the mitochondrial membrane. This transfer results in a proton gradient across the membrane. As the protons return to the interior through the enzymatic machinery for making ATP, they drive the formation of ATP. This CoQ_{10} cycle repeats itself over and over and each component is as important as the others [15, 16] (see Fig. 20.1 in the Sect. "Chemical and Physical Properties of Coenzyme Q_{10}" and Fig. 20.2 in the Sect. "Antioxidant Effects of Coenzyme Q_{10} on the Human Skin").

ATP, the molecule probably busiest in the body, serves as a source of cellular energy that cells need for their growth and development, wound healing, maintenance of all muscles including heart, digestion of food, and for performance of numerous other bodily functions, including skin—the subject of

this chapter. Figuratively expressed, CoQ_{10} is an irreplaceable spark that ignites the creation of energy for cells. Energy is life and CoQ_{10} is a crucial component of the energy cycle and therefore of life itself. Without CoQ_{10} we would die.

Coenzyme Q_{10} Protects Against Oxidative Stress

Reduced form of CoQ_{10} (ubiquinol) is one of the endogenous body antioxidants. UQH_2 is able to donate its electrons to free radicals such as hydroxyl radical, thereby breaking the free radical cascade. So, in sufficient quantity, it provides a strong first-stage defense against a cellular oxidative stress in all types of cellular membranes. UQH_2 can also rescue alpha-tocopheroxyl radicals by a direct reduction back to alpha-tocopherol. However, a decline in UQH_2 concentration implies a reduction of antioxidant protection and damage of cells, including lipids, proteins, and DNA, and also regeneration of tocopherol is very slow. For this reason, antioxidative activity of CoQ_{10} depends not only on its value but also on its redox status [15, 17].

Coenzyme Q_{10} Influences in Gene Expression

Recent studies also reveal function of CoQ_{10} in the expression of genes involved in human cell signaling, metabolism, and transport [18].

Coenzyme Q_{10} Supplementation

Hundreds of laboratory and clinical trials over the last 30 years have shown prophylactic and therapeutic effects of supplementation with CoQ_{10} on a variety of disorders such as cardiovascular, Parkinson's and gum diseases, mitochondrial encephalomyopathies, migraine, diabetes, cancer-breast, and others. More information is available in papers [19–23] and in our work [24].

Sources of Coenzyme Q_{10}

There are two main ways to obtain CoQ_{10}. First is a chemical synthesis, the product of which contains a mixture of both *trans*- and *cis*-isomers, but shows only low bioavailability [15]. Second way is an extraction from certain fermented yeast strains that synthesize a substance identical to human CoQ_{10}. The majority of CoQ_{10} in the world market is produced by this way in Japan. Until recently, the most popular CoQ_{10} supplements were hard-shell capsules containing typically 30–60 mg of oil-crystalline suspensions of ubiquinone. These forms should be taken with fatty meals to help intestinal CoQ_{10} absorption [24]. But new types of CoQ_{10} products with improved bioavailability are currently placed on the market (see Sect. "Enhancement of Coenzyme Q_{10} Bioavailability and Efficacy"). Some of CoQ_{10} supplements are fortified with secondary bioactive agents, such as tocopherol, resveratrol, folic acid, alpha-lipoic acid, L-carnitine, omega-3-fatty acids, magnesium, selenium, etc.

Legislative Classification of Products with CoQ_{10}

In most countries, such as the United States, those of the European Union (EU), and New Zealand, CoQ_{10} has not been approved to treat any disease, and it should not be substituted for prescription

medications. In these countries CoQ_{10} is sold for more than 20 years as a dietary supplement, so is regulated as foods not drugs. However in Japan, in the period 1974–2001, products with CoQ_{10} were prescribed as "drug," and then were reclassified also as dietary supplements [24–26].

Safety Profile of Coenzyme Q_{10}

Conclusive evidence on the health benefits of CoQ_{10} as well as the marketing claims of manufacturing companies are causing enormous interest in CoQ_{10}. Recently, there has been an increase in the number of people taking CoQ_{10} as a dietary supplement, and they are able to take it without any limitation imposed on the daily dose. Therefore, safety concerns have received attention [24].

However, there is no official recommended daily allowance (RDA) for CoQ_{10}. In part, this fact stems from the difficulty in estimating CoQ_{10} intake when bodies already produce it internally. Also, RDA depends on circumstances, particularly a health status and age of individuals. Lastly, there may be variability in the absorption of different CoQ_{10} supplements (see Sect. "Enhancement of Coenzyme Q_{10} Bioavailability and Efficacy"). The supplement with better bioavailability may require a lower dose. The European Food Safety Authority (EFSA) [27], on the basis of the relevant published data, stated that the most frequent value of CoQ_{10} (not RDA) used as a prophylactic supplement is 30–200 mg/day.

On the one hand, it is not clear how the distribution of CoQ_{10} in body tissues is controlled. On the other hand, it is certain that the supplementation with CoQ_{10} does not increase tissue levels above normal, except in liver and spleen [15]. The published reports concerning safety studies indicate that CoQ_{10} has low toxicity and does not induce serious adverse effects in humans [14, 26]. Risk assessment for CoQ_{10} based on various clinical trial data indicates that the observed safety level (OSL) for CoQ_{10} is 1,200 mg/day/person [25, 26]. Much higher levels have been tested without adverse effects and may be safe, but the data for intakes above 1,200 mg/day are not sufficient for a confident conclusion of safety [26]. Therapeutic doses of CoQ_{10} for adults generally range 100–300 mg/day [24], although doses as high as 3,600 mg/day have been used to treat some disorders under medical supervision [19].

Due to some exaggerated marketing slogans about the effects, EFSA [27] in 2010 issued a regulation on health claims permitted on CoQ_{10}, which is valid in the EU.

Skin Benefits of Coenzyme Q_{10}

Since the half of the nineties, there are also evidences of CoQ_{10} importance for healthy functioning of the human skin. Just as the American scientist Karl Folkers is considered "the father of CoQ_{10}," the German scientist Udo Hoppe is considered "the pioneer of CoQ_{10} research related to the skin." A large contribution to research of the CoQ_{10} effects on skin brought also several Japanese scientists.

Coenzyme Q_{10} Content in the Human Skin

While the usual content of CoQ_{10} in the internal organs has been relatively well uncovered, data on its level in the human skin are only limited.

In 1994 Shindo et al. [28] determined values of CoQ_{10} in epidermis and dermis from six healthy volunteers (aged 45–70) undergoing surgical procedures. Average values of total CoQ_{10} ($UQ + UQH_2$) were found 7.76 nM/g in the epidermis and 3.15 nM/g in the dermis. There were twofold higher values of UQ than UQH_2 in the epidermis and tenfold higher values of UQH_2 (3.53 nM/g) than in the

Table 20.2 Age and sex variations of coenzyme Q_{10} in sebum from healthy volunteers

Age range (years) and sex	Coenzyme Q_{10} (μg/10 cm^2 of skin surface)
6–10	
Males	0.120 ± 0.024
Females	0.116 ± 0.027
15–20	
Males	0.161 ± 0.022
Females	0.160 ± 0.017
21–40	
Males	0.176 ± 0.023
Females	0.173 ± 0.026
45–60	
Males	0.156 ± 0.024
Females	0.153 ± 0.023
65–80	
Males	0.128 ± 0.024
Females	0.124 ± 0.032

Adapted from Passi et al. [30]
Each result represents the mean ± SEM of 20 experiments

dermis (0.35 nM/g). According to Aberg [9], the tissues of heart, kidney, liver, muscle, brain, and intestine contain 114.0, 66.5, 54.9, 40.0, 15.5, and 11.5 mcg/g of CoQ_{10}, respectively. Unlike CoQ_{10} levels in the internal organs, the total value of CoQ_{10} in the skin is low: 9.4 mcg/g (recalculated by the author of this chapter), from which 6.7 mcg/g were in the epidermis. Other scientists [29] found CoQ_{10} value of 4.9 mcg/g in the stratum corneum from the back of 50 females aged 21–40.

In 2002 Passi et al. [30] intended to determine the values of UQ and UQH_2 in sebum extracted from the upper chest of 100 healthy volunteers (aged 6–80). They found that it is not possible to establish separately UQ and UQH_2, because UQH_2 is unstable outside the human body and is easily oxidized to UQ (see Sect. "Ubiquinol-Based CoQ_{10} Supplements"). Within the same group of volunteers there were constantly, but not significantly, higher CoQ_{10} levels in sebum of skin surface in males than females [30]. Authors also demonstrated that CoQ_{10} has increased value from childhood (0.116 mcg/10 cm^2 in female) to adulthood, with the highest levels in age 22–42 (0.173 mcg/10 cm^2 in female) and then has decreased value with age (see Table 20.2) [30]. It is obvious that the decline of CoQ_{10} values in mature skin is caused by two circumstances: a reduced biosynthesis of it and an increased consumption of it due to extreme amounts of free radicals.

Bioenergetical Needs of the Human Skin

Although the actual number of skin cells cannot be determined precisely, their amount in adults is estimated at a few trillion (10^{12}). Each of these cells must produce its own energy to carry out its vital function. Moreover, skin lose tens of thousands of dead cells per minute. So, it must have enough energy to create new cells to replace those lost. Being directly exposed to ultraviolet (UV) radiation and harmful environmental factors, skin cells need also a lot of energy to regenerate and repair them. As described in the Sect. "Coenzyme Q_{10} Contributes to the Energy Formation," this energy in ATP form is created through cellular respiration in the mitochondria, in which CoQ_{10} is a critical component. Therefore, the most remarkable characteristic of CoQ_{10} in terms of the human skin lies in its indispensability in the cellular energy production [24, 31–33].

While we are young, repair processes of disturbed skin cells usually work well. But, as we age the number of damaged mitochondria in skin is increased and production of ATP energy is reduced. If skin cells lack sufficient energy, the repair mechanism cannot keep place. Later the detrimental changes in skin structure can occur, leading to visible signs of ageing, especially the formation of wrinkles in sun-exposed skin areas [32, 33]. The functional loss of mitochondria represents an inherent part in modern theories trying to explain the cutaneous ageing process [32].

Skin Cell Benefits from Bioenergetical Ability of Coenzyme Q_{10}

Hoppe et al. [1] investigated effects of CoQ_{10} on dermal matrix synthesis. As is known, chronologically aged skin has lower content of hyaluronic acid and disorganized collagen fibers in the dermal matrix. Using artificially aged human fibroblasts they demonstrated that 50 mcM of CoQ_{10} increased the level of hyaluronic acid. This supplemental amount of CoQ_{10} also enhanced (by about 20%) the proliferation of fibroblasts that are necessary for the production of collagen.

Significant knowledge of the skin health benefits of CoQ_{10} later brought the Japanese researchers. Terada et al. [34] showed that CoQ_{10} increases the production of laminin 5 (laminin 332) and type IV and type VII collagens, which are significant constituents of basement membrane anchoring the epidermis and dermis. Given that damage to the basement membrane is one of the causes of wrinkle formation, supplementation with CoQ_{10} can lead to the alleviation of these. They also found that the stimulation of the fibroblasts' proliferation using CoQ_{10} is dose dependent [34]. The same team of scientists [35] later expanded their study also on keratinocytes. They confirmed that CoQ_{10} increases the number of fibroblasts but not keratinocytes. It also accelerated the production of basement membrane components, i.e., laminin 332 and type IV and VII collagens, in keratinocytes and fibroblasts, respectively; however, it had no effect on type I collagen production in fibroblasts [35].

Antioxidant Effects of Coenzyme Q_{10} on the Human Skin

Oxidative stress plays a significant role in skin ageing process, which is a combination of chronological ageing and photoageing. Reactive oxygen species formed inside and around skin cells cause their damage or death. Although the antioxidant properties of ubiquinol have been known for 50 years (see Sect. "Coenzyme Q_{10} Protects Against Oxidative Stress" and Fig. 20.2), scientists began to examine this potential for skin cells little more than a decade ago.

Skin Cell Benefits from Antioxidant Ability of CoQ_{10}

Several works have shown antioxidative effects of CoQ_{10} on the skin using in vitro methods. Great contribution in this field was again the work by Hoppe et al. [1]. Firstly they showed that pretreatment of keratinocytes with CoQ_{10} is able to maintain activity of phosphotyrosine kinase and levels of glutathione, two indicators of oxidative stress in human keratinocytes, at about the values found in non-stressed cells. Secondly, they demonstrated that 24-h pretreatment with 23 mcM of CoQ_{10} can protect human keratinocytes from UVA-induced (5 J/cm^2) oxidative DNA damage. Thirdly, since another result of oxidation stress is the degradation of collagen fibers by the enzyme collagenase, they found that the expression of collagenase messenger ribonucleic acid (mRNA) can be significantly (50%) reduced by pretreatment with 10 mcg/mL of CoQ_{10}. CoQ_{10} can also suppress collagenase expression over 6 weeks with weekly UVA-radiation. Finally they showed that 0.3% of CoQ_{10} has the beneficial effect of suppressing the reduction of the mitochondrial membrane potential following UVA-radiation

(20 J/cm^2) in fibroblasts from both young and old donors [1]. Another scientists [36, 37] later confirmed the protective effect of CoQ_{10} on culture of human skin fibroblasts injured also by UVB-radiation.

Improving protection against oxidative stress in culture keratinocytes from old donors with administration of CoQ_{10} was published by Terada et al. [34]. Prahl et al. [32] demonstrated that detrimental effects after both age-induced and UV-induced damages on mitochondrial function of keratinocytes isolated from skin biopsies of young and old donors can be reversed by exogenously added CoQ_{10}. Inui et al. [38] found that CoQ_{10} suppressed excessive interleukin-6 production in human keratinocytes exposed to UVB-radiation. In addition, they showed that cytokine production in keratinocytes is inhibited by CoQ_{10}, resulting in a decrease of matrix metalloproteinase-1 leading to wrinkle reduction. Muta-Takada et al. [35] showed protective effects against cell death induced by several reactive oxygen species in keratinocytes, but only when its cellular absorption was enhanced by pretreatment of the cells with highly CoQ_{10}-loaded serum. These results suggest that protection of epidermis against oxidative stress and enhancement of production of epidermal basement membrane components may be involved in the anti-ageing properties of CoQ_{10} in skin.

Human Skin Benefits from Exogenous Administration of Coenzyme Q_{10}

Examining of CoQ_{10} importance in human skin was carried out only in the last decade.

Skin Benefits from Oral CoQ_{10} Administration

Ashida et al. [39] investigated the effects of CoQ_{10} (60 mg/day by oral route) on wrinkles of eight female volunteers (mean age 43 ± 3 years). For quantitative evaluation of wrinkle reduction, silicone rubber was applied to the skin at the corner of the eye to make a replica before and after test. Three-dimensional analysis of the replica was performed by the laser cutting method to calculate the wrinkle area (WA, in %) and the wrinkle volume (WV, in mm^3) in the target area. After 2 weeks of intake, the WA decreased on average by 33% relative to its value before intake, and so did the WV (on average by 38%). As a concrete example the authors stated the effect on wrinkles in 43-year-old women. The WA and WV, which were 24.2% and 1.53 mm^3, respectively, before starting intake with CoQ_{10}, have been significantly mitigated to 16.1% and 0.89 mm^3, and remained at this level for 3 months [39, 40].

Skin Benefits from Topical CoQ_{10} Administration

To demonstrate that also in vivo application of CoQ_{10} can act as an effective antioxidant, Hoppe et al. [1] evaluated skin levels of ultra-weak photon emission (UPE). In the basal state, human skin cells emit low levels of photons. When UVA-radiation is applied there is an excited state with a large increase in the level of photons which decays with time. Therefore the level of photons emitted is an indication of the antioxidant status of the skin [1]. Thirteen middle-aged volunteers were treated on the volar forearm, twice daily for 7 days with 0.3% CoQ_{10} in vehicle or vehicle alone. The skin areas were then exposed to 50 mJ/cm^2 UVA-radiation. The evaluation confirmed that the skin sites treated with CoQ_{10} had significantly lower level of UPE. In a next long-time study, 0.3% CoQ_{10} in vehicle was applied around one eye and a vehicle alone was applied around other eye of 20 elderly volunteers once daily for 6 months. Using microtopography a 27% reduction in the depth of wrinkles treated with CoQ_{10} compared to controls has been shown [1].

In a randomized, double-blinded, placebo-controlled trial held by Ichihashi et al. [41], subjects with aged skin used skin-care preparation containing 1% CoQ10 twice daily for 5 months.

Dermatologist assessed the wrinkle grade of subjects at initiation and termination of trial based on the wrinkle grade standard of the guideline for evaluation of anti-wrinkle products by Japanese Cosmetic Science Society and also by the replication method. After 5 months, significant reduction of wrinkle grade and improvement of skin condition were observed. The same team of scientists [38] later held a clinical trial under the same conditions as previously but on sun-damaged face skin. There was confirmed significant reduction of the wrinkle grade score in subjects after 5 months use of 1% CoQ_{10} cream, but no reduction in the placebo group [38].

Vinson and Anamandla [42] investigated skin effects of two CoQ_{10} forms (a chemically synthesized and a yeast-fermented) after topical application to young and older humans. The aim of this study was to determine which of them has better skin absorption and antioxidant properties. Two groups of volunteers (aged 21–29 and 51–70) were tested not only after a single dose (1 h, on the inner wrist) but also after a long-term study (1 month, twice daily on the same ventral forearm) with 0.1% CoQ_{10} in lotion. After a 1-h application significantly more yeast-fermented CoQ_{10} was absorbed into the stratum corneum than the chemically synthesized CoQ_{10}. The skin of older subjects absorbed about twice as much CoQ_{10} as did the younger subjects. The antioxidant activity determined after the long-term supplementation significantly increased by yeast-fermented CoQ_{10} but not by chemically synthesized CoQ_{10}. The skin of older subjects had significantly higher baseline levels of lipid peroxides than did the younger group, indicating an increase in skin oxidative damage with age. Peroxides declined in the stratum corneum with both forms but the decrease was greater with the yeast-fermented form. According to the authors, the yeast-fermented CoQ_{10} is the superior form of CoQ_{10} for human skin application [42].

Skin Benefits from Combined Use of Oral and Topical CoQ_{10}

Passi et al. [29] published the results of extensive study whose aim was to assess the skin effect of the oral and topical supplementation with CoQ_{10} and some other lipophilic antioxidants. The face and back of 50 female volunteers aged 21–40 were treated once daily for 2 months with a cream containing 0.05% of CoQ_{10}, 0.1% of vitamin E (VE), and 1% of squalene (SQ). In addition 50 mg of CoQ_{10} + 50 mg of alpha-tocopheryl acetate + 50 mcg of selenium were administered orally to half of the volunteers (group A). Group B was represented by volunteers who were treated only topically. Every 15 days during treatment the levels of CoQ_{10} and other antioxidants were verified in sebum, stratum corneum, and plasma. The topical application of the cream led to a significant increase of the volumes of CoQ_{10}, alpha-tocopherol, and SQ in the sebum (group B). The volumes peaked after 60 days, without significantly affecting the stratum corneum or plasma volumes of CoQ_{10} and VE. The concomitant oral administration of antioxidants in group A resulted in a significant increase of CoQ_{10} volumes and VE volumes both in plasma and stratum corneum after 15 and 30 days of treatment, compared to group B (see Table 20.3) [29]. However the sebum volumes of CoQ_{10}, VE, and SQ did not show a significant increase. After the treatments, the volumes of CoQ_{10}, VE, and SQ went back to basal levels within 12–16 days in the stratum corneum, 6–8 days in sebum, and 3–6 days in plasma. This means that topical application was able to increase the level of these antioxidants including CoQ_{10} in sebum only, while the concomitant oral route affected the levels of CoQ_{10} and VE both in the stratum corneum and sebum [29].

Skin Benefits of Coenzyme Q_{10} in the Research Stage

Rusciani et al. [43] have shown that recombinant interferon alpha-2b and coenzyme Q_{10} are a postsurgical adjuvant therapy for melanoma. Results of some preclinical study [44] suggest that oral supplementation with CoQ_{10} may be beneficial for the management of patients with severe forms of psoriasis.

Table 20.3 Level of coenzyme Q_{10} (CoQ_{10}) and vitamin E (VE) in the skin sebum or stratum corneum in the group A (oral + topical administration) and group B (topical administration only) of female volunteers

	t = 0		*t* = 15 days		*t* = 30 days		*t* = 60 days	
	CoQ10	VE	*CoQ10*	VE	*CoQ10*	VE	*CoQ10*	VE
Skin sebum (μg/10 cm2 of skin surface)								
Group A	*0.147* ± 0.028	0.151 ± 0.025	*0.254* ± 0.040	0.268 ± 0.036	*0.314* ± 0.065	0.337 ± 0.036	*0.409* ± 0.072	0.425 ± 0.053
Group B	*0.143* ± 0.027	0.163 ± 0.031	*0.234* ± 0.045	0.250 ± 0.043	*0.294* ± 0.053	0.305 ± 0.039	*0.377* ± 0.060	0.397 ± 0.053
Stratum corneum (μg/g of proteins)								
Group A	*4.9* ± 1.5	8.6 ± 1.9	*5.8* ± 1.3	9.0 ± 2.0	*7.4* ± 1.4	9.9 ± 2.0	*9.9* ± 1.3	13.7 ± 2.7
Group B	*4.7* ± 1.6	8.3 ± 1.7	*4.9* ± 1.0	8.5 ± 0.9	*5.4* ± 0.6	5.7 ± 1.2	*5.7* ± 1.2	9.1 ± 1.6

Adapted from Passi et al. [29]

Each result represents the mean ± S.E.M. of 25 determinations

Jung et al. [45] showed the efficacy of 0.5% CoQ_{10} in liposomes against UV-induced free radicals on human hair cutoff. Very remarkable is also the finding published by Giesen et al. [46] that CoQ_{10} stimulates gene expression of different cultivated hair keratinocytes, especially those which are reduced in hair follicles with age and could be effective against hair loss. There are some indications of possible skin whitening effect of ubiquinol administered orally [40] and topically [47]. Ashida [40] implies that CoQ_{10} does not inhibit tyrosinase activity, but it significantly inhibited the autooxidation of 3,4-dihydroxy-L-phenylalanine (L-DOPA), a precursor of melanin, and a consequent pigmentation [40]. However, further scientific studies are needed to confirm all the above effects.

Enhancement of Coenzyme Q_{10} Bioavailability and Efficacy

Recent years have confirmed that intestinal bioavailability and skin permeability of products based on traditional oil-crystalline suspensions are poor due to large and hydrophobic molecule of CoQ_{10}. Therefore, extensive effort has been made regarding the modification physicochemical properties of this substance. There are three main strategies: (1) enhancement of water-solubility CoQ_{10} formulations; (2) size reduction of CoQ_{10} carriers using new oral and transdermal vehicles; and (3) supplementation with ubiquinol instead of ubiquinone.

The Virtually Water-Soluble CoQ_{10} Products

In order to improve CoQ_{10} dissolution profile and thereby its absorption, some new technologies were aimed at alleviating a huge lipophilicity of CoQ_{10}. The "Bio-Solv" technology [48] became the revolutionary process, developed by division of American Corporation Tishcon. The so-called dry emulsions are characterized as microsphere systems, encapsulating CoQ_{10} into its matrix, and they formed emulsions when dispersed into water [49]. The increase in hydrosolubility of CoQ_{10} allowed to produce various products, such as hydrogel capsules, chewable wafers and tablets, intra-oral sprays, syrups, fortified beverages and liquid/gel foods, etc.

However, very few studies have been carried out about the pharmacokinetics of these new CoQ_{10} forms. The relative bioavailability of four traditional CoQ_{10} supplements available on the market were compared with a novel CoQ_{10}-solubilizate [50]. Study assessed 54 healthy volunteers after single and multiple intakes of 60 mg CoQ_{10} over a time period of 14 days. In summary, a solubilizate shows superior intestinal bioavailability in humans compared to traditional oil-crystalline suspensions of CoQ_{10}. Better intestinal absorption of water-soluble forms compared to oil-based CoQ_{10} was also confirmed by other authors [49, 51]. Unfortunately, there have not yet been published relevant scientific studies on the effects of CoQ_{10} water-soluble formulations on the skin.

The Nanostructure Carriers of CoQ_{10}

The relative bioavailability of CoQ_{10} is markedly influenced by its delivery systems.

Swarnakar et al. [52] found that Q_{10}-loaded polymeric nanoparticles (size <100 nm) improved 4.3 times the oral bioavailability of the traditional lipophilic crystalline substance. Viability of three groups of UVA-irradiated fibroblasts, (1) non-treated, (2) treated with traditional CoQ_{10}-emulsion, and (3) water-soluble CoQ_{10} nano-structured lipid carrier (CoQ_{10}-NLC), was compared by Yue et al. [53]. They found that the fibroblasts treated with CoQ_{10}-NLC had the highest viability (41.7%), fibroblasts treated with traditional CoQ_{10}-emulsion showed 25% viability, and non-treated fibroblast had no viability.

In our recent study [54] concerning skin permeability we evaluated one traditional and one new delivery system of CoQ_{10}. Suspension of 0.2% of CoQ_{10} (traditional yeast-fermented crystalline powder of CoQ_{10}) in isopropylmyristate (IPM) and 0.2% of CoQ_{10} encapsulated in the plurilamellar multivesicular liposomes (PMLs), just as 2.5% of tocopherolacetate (TPA) in IPM and 2.5% of TPA encapsulated in the PMLs (TPA-PMLs), was studied. The experiments were carried out using dermatomed 200 μm pig-ear skin (the epidermis + part of the dermis) in Franz diffusion cells (ten for each vehicle) with 10 mg/cm^2 of tested vehicle. The values of substances absorbed into skin from IPM vehicles after 24 h were 19% of applied CoQ_{10} dose and 10% of applied TPA dose, but from the liposome vehicles were 30% of the applied CoQ_{10}-PMLs dose and 28% of TPA-PMLs dose. Even more pronounced differences were detected in permeability of these substances through skin into the receptor fluids. No value of CoQ_{10} and only 0.5% of applied TPA dose from IPM vehicle were detected, despite the fact that IPM is a well-known transdermal enhancer. However, there were 10% of applied CoQ_{10}-PMLs dose and 4.5% of the applied TPA-PMLs dose, so about ten times more than from IPM vehicle. As is known, each occurrence of a substance into receptor fluid (under the experimental conditions) is confirming its permeability through/into live skin layers. So, results of this study indicated that the liposomal vehicle permeated through/into live layers of skin better than the oil-crystalline suspensions of CoQ_{10} [54]. Increased bioavailability and improved benefits for the skin of various liposomal systems were documented also by several other scientists [55, 56].

Ubiquinol-Based CoQ_{10} Supplements

Although ubiquinol (UQH_2, the reduced form) is the dominant CoQ_{10} state in the body (see Sect. "Chemical and Physical Properties of Coenzyme Q_{10}"), ubiquinone (UQ, the fully oxidized form) has only been available as a commercial supplement for the last years. But there is experimental evidence that UQ must be reduced to UQH_2 within the body and this process may be a rate-limiting step in CoQ_{10} assimilation. However supplementation with UQH_2 could not be done because it is unstable outside the human body and is easily oxidized to UQ. In 2000, the Kaneka Corporation in Japan managed to stabilize UQH_2 and since 2006 the next generation of ubiquinol-based CoQ_{10} products is available [57].

Evaluation of whether supplementation with UQH_2 is more useful than with UQ is only the beginning. But results of several studies [58, 59] are very promising. Hosoe et al. [58] observed a significant absorption of UQH_2 from the gastrointestinal tract to the plasma after 4-week multiple oral administration of 90–300 mg UQH_2 to healthy volunteers. However, future clinical studies are required to more accurately quantify enhanced absorption of UQH_2, and what effect patient age or medical condition may have on this value. And it should also be scientifically validated whether stabilized UQH_2 products tend to be oxidized back to the UQ or not after opening the container, and during the period of their life. Consumers can check for themselves by opening a capsule and examining color of the supplement. Ubiquinol is white/gray compound whereas ubiquinone is yellow/orange [16].

Conclusion

Skin has a high energy requirement not only to stay metabolically active but also for protection against oxidative stress and regeneration. Lack of mitochondrial activity with age and due to various reasons causes the amount of endogenously produced energy to be insufficient for skin cells. Equilibrium is disturbed, repair mechanisms cannot keep place, and detrimental changes in skin structure can occur. This may be in part through a decline in the levels of the endogenous CoQ_{10} in plasma and skin, primarily in people over 30/40. Summarizing the information referred in this chapter, it can be stated that

CoQ_{10} administered by oral, by topical, and mainly by simultaneous oral and topical route may be beneficial for mature skin.

There are following scientifically proven skin benefits of supplemental CoQ_{10}: (1) reduces oxidative stress in cells; (2) inhibits the activity of some degradative enzymes such as collagenase and matrix metalloproteinase-1; (3) accelerates the proliferation of dermal fibroblasts and subsequent production of lamina 332, just as types IV and VII collagens, significant constituents of basement membrane; and (4) stimulates biosynthesis of the dermal glycosaminoglycan (hyaluronic acid).

Taken together, CoQ_{10} administrated in adequate oral and topical formulations and applied at least some weeks or some month (depending on the bioavailability) may have prophylactic just as therapeutic effects on skin health and recovery. It may reduce also skin ageing, especially wrinkling of sun-exposed areas. Nevertheless, it should be noted that the mechanism of anti-ageing activity of CoQ_{10} is not fully understood.

There have been several major advances in bioavailability of CoQ_{10} over the past years and there will be even more in the future with an aim to improve CoQ_{10} benefits for human health, including skin. The global market for CoQ_{10}'s products aimed at skin care will grow at a rapid pace.

Summary (5) Points

- CoQ_{10} is an essential substance synthesized in all living body cells, including skin, necessary for the cellular energy formation, the protection of cells against oxidative stressors, and the expression of genes.
- For most people over 30/40, level of CoQ_{10} in the skin lies below optimum, resulting in the various adverse skin manifestations associated with UV-induced photoageing especially.
- CoQ_{10} supplementation through oral and topical route may be beneficial for appearance as well as for health and convalescence of mature skin, mainly due to suppression of oxidative stress in the epidermis and dermis, inhibition of the activity of some degradative enzymes, and/or stimulation of skin fibroblasts' metabolism.
- How much CoQ_{10} should one take? RDA for CoQ_{10} cannot be uniformly determined because it depends not only on skin health, age, and other factors but also on the specific bioavailability of supplemental CoQ_{10}. The most frequent value of CoQ_{10} (not RDA) used as a prophylactic oral supplement is 30–200 mg/day.
- New virtually CoQ_{10} hydro-soluble forms, new types of oral and transdermal nano-carriers of CoQ_{10}, as well as ubiquinol-based supplements appear to be beneficial for skin than traditional oil-crystalline suspensions of ubiquinone, but there is a need for more scientific evidence.

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Chapter 21
Protection Against Free Radicals (UVB Irradiation) of a Water-Soluble Enzymatic Extract from Rice Bran. Study Using Human Keratinocyte Monolayer and Reconstructed Human Epidermis

C. Santa-María, E. Revilla, E. Miramontes, J. Bautista, A. García-Martínez, E. Romero, M. Carballo, and J. Parrado

Key Points

1. EERB in form of a sterilized sirup is a product absolutely soluble in water, the reason why it can be used in all type of cosmetic formulations.
2. The EERB has very good skin compatibility and it is not cytotoxic, for concentrations inferior or equal to 100 μg/mL.
3. This product presents antioxidant effect in cells, which would reduce the cellular damage. In reconstructed human epidermis, 100 μg/mL of EERB showed an antioxidant effect similar to vitamin E at 666 μg/mL.
4. The extract has a capacity of solar filter of 4.8 ± 0.3. This fact indicates a good photoprotection and dispersibility in order to be a totally natural product. This value is very elevated for a totally natural product, with absence of physical and chemical filters.
5. The enzymatic extract of rice bran, with high content in tocopherols, tocotrienols, phytosterols, polyunsaturated fatty acids, and ferulic acid, may have an important place among cosmeceuticals.

Keywords Rice bran enzymatic extract • Antioxidant • Cytotoxicity • Keratinocyte culture

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Abbreviations

AOAC	Association of official analytical chemists
AP-1	Activator protein-1
AU	Arbitrary units
COX-2	Cyclooxygenase-2
DOPA	3,4-dihydroxy-phenylalanina
EERB	Enzymatic extract rice bran
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
GSH	Glutathione
IL-1 beta	Interleukin 1 beta
IL-6	Interleukin 6
iNOS	Inhibit inducible nitric oxide synthase
IUPAC	International Union of Pure and Applied Chemistry
MAP kinase	Mitogen-activated protein kinase
MDA	Malondialdehyde
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NFKB	Nuclear factor kappa B
NPC1L1	Niemann-Pick C1-like 1 protein
NS	Nonsignificant
PBS	Phosphate buffer solution
PEP	Phosphoenolpyruvate
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SPF	Sun factor protection
TNF-beta	Tumor necrosis factor-beta
UI	Unities internationals
UV	Ultraviolet
UVR	Ultraviolet radiation

Introduction

The skin is constantly exposed to oxidative stress induced by reactive oxygen species (ROS) that are generated both from endogenous sources, such as by-products of aerobic oxidation or cellular response to inflammation, and external pro-oxidant stimuli, such as ultraviolet radiation [1]. Oxidative stress can provoke chemical changes and degradation of cellular components (lipids, proteins, and DNA) and extracellular elements (collagen and elastic fibers). Besides, it is now known that ROS influence the expression of a number of genes and signal transduction pathways. The most significant effects are observed in the MAP kinase/AP-1 and NF-κB signaling pathways [2]. In this way, ROS reduce collagen synthesis, induce expression of proinflammatory cytokines (IL-1beta, IL-6, and TNF-beta), and inhibit apoptosis [3]. All these changes contribute to adverse effects on the skin, expressed as erythema, edema, wrinkling, photoaging, inflammation, autoimmune manifestation, hypersensitivity, keratinization abnormalities, preneoplastic lesions, and skin cancer [4].

The endogenous antioxidant capacity of the skin contains many antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) as well as nonenzymatic antioxidant molecules (tocopherols, coenzyme Q_{10}, ascorbate, and carotenoids). Dietary protection is provided by carotenoids,

tocopherols, ascorbate, flavonoids, or *n*-3 fatty acids. These micronutrients can act as UV absorbers, as antioxidants, or can modulate signaling pathways elicited upon UV exposure [5]. However, the cutaneous antioxidant network is less than completely effective and tends to deteriorate with age [3]. Topical administration of antioxidants has proven to be effective in protecting the skin against oxidative damage [6], and provides the most straightforward way to strengthen the endogenous protection system. In the past, antioxidant agents were used in pharmaceutical and cosmetic formulations mostly to prevent autooxidative deterioration of lipidic raw materials [7], but, at present, antioxidants are introduced as primary ingredients in cosmetics [3].

Many antioxidants with vitamin (vitamin C, vitamin A, and vitamin E) and no vitamin (coenzyme Q, lipoic acid, and glycolic acid) have been included frequently in cosmetics [8]. A new tendency in cosmetic formulations is the use of biotechnological raw materials for antioxidant, immunomodulatory, and photoprotective purposes. As a result, formulators are being offered a host of new plant sources such as green tea, soybean, rosemary, propolis, oatmeal, olive oil, grape seed, lavender, mushrooms, and coffee berry [9].

A natural product of great interest in the last years is the rice bran. Rice bran is discharged in the process of the polished rice production and contains many unique bioactive compounds. Rice bran has been used as a starting material to extract single products with a biological significance, protein, oil, or phytochemical as oryzanol, sterols, tocopherols, ferulic acid, etc. Besides, another process has been used to achieve a mixture of the above products in form of extracts with functional properties [10]. Rice bran is especially enriched in antioxidants such as gamma-oryzanol (a mixture of ferulic acid esters of triterpene alcohols and sterols) and vitamin E (tocopherols and tocotrienols) [11]. However, although rice bran is a good source of phytochemicals, it is currently underutilized because some of its fat components are quite unstable due to the presence of lipase, which decomposes triglycerides, promoting rancidity.

We have developed a new product from rice bran, a water-soluble enzymatic extract (EERB) that includes all rice bran components [12]. EERB is a source of natural active principles such as gamma-oregano, inositol, fatty acids (35% PUFAs), peptides, etc., recognized in cosmetic applications. EERB shows a high content in sulfured amino acids (cysteine more methyonine 6%), and of arginine 12.7%, as well as of amino acids implied in tissue regeneration (synthesis of glutation). We have recently reported an antioxidant activity and hypocholesterolemic effect of a water-soluble enzymatic extract from rice bran [13]. The specific aim of the present study was to evaluate the skin compatibility of EERB, its potential cytotoxicity, and possible protection against free radicals produced by ultraviolet radiation. We conclude that this soluble enzymatic extract from rice bran, which presents a texture of easy application, has antioxidant activity and can be useful as a new cosmeceutical.

Main Text

Materials and Methods

Preparation of Rice Bran Enzymatic Extract

EERB was prepared according to an enzymatic process that we have recently described [12]. Briefly, rice bran was modified by enzymatic hydrolysis, using an endoproteases mixture (trypsin- and chymotrypsin-like) as hydrolytic agent, in a bioreactor with controlled temperature (60°C) and pH (pH 8), using the pH-stat method. The processing of this product follows different steps, including centrifugation, filtration, and concentration. The final product is a brown syrup completely soluble in water. EERB was chemically characterized using the AOAC standard protocols [14].

Skin Compatibility

This study was performed according to the general conditions of EVIC International and those particulars of EVIC Hispania, established for the performance of human test project [15]. A total of 11 healthy subjects of sexes, phototype (Fitzpatrick) I–V, and ages between 18 and 70 years participated in this study after having given their consent. Twenty microliters of EERB, diluted at 10% with water, was applied to the back skin under the patch for 48 h. Skin irritation responses were graded 15 min after patch removal and also after 48 h, if a positive reaction was observed. The primary skin irritation was evaluated by visual scoring according to International Contact Dermatitis. The subject was used as own control.

Keratinocyte Monolayer Preparation

Human skin keratinocytes (foreskin, 4th passage), checked free from mycoplasma, were used. Cells were seeded at the starting density of 50×10^3 cells/cm² in multiwell culture plates. Cells were incubated, at 37°C in a humidified atmosphere containing 5% (v/v) CO_2, for 24 h. Culture medium was MCDB 153 (Gibco) supplemented with EGF (5 ng/mL), insulin (5 μg/mL), hydrocortisone (5 ng/mL), and PEP (70 μg/mL) supplemented with 0.5% (v/v) antibiotics. Twenty-four hours later, subconfluent morphology of the cell layer was checked using a microscope. Culture medium was removed and replaced with culture medium supplemented with 0.5% (v/v) antibiotics (10,000 U penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin) [16].

For cytotoxicity experiments, seven series were set up: five containing various concentrations of EERB (1, 10, 50, 100, and 500 μg/mL), one positive control, phenol (640 μg/mL), and one negative control with complete culture medium. Cultures were incubated for 24 h at 37°C in a humidified atmosphere containing 5% (v/v) CO_2. Culture medium was withdrawn and cell layer used to cytotoxicity evaluation (MTT assay).

For antioxidant experiments, six series were carried out: three series of EERB (10, 50, 100 μg/mL), a positive control (vitamin E, 200 μg/mL), and two negative controls of culture medium. All series were incubated for 24 h in a humidified atmosphere containing 5% (v/v) CO_2 (preventive treatment). Thereafter the series were irradiated with UVB (0.1 J/cm²) (Spectroline model X-15N/F, $\lambda = 312$ nm) except for the series corresponding to the second negative control (absolute negative control). Then a curative treatment is performed as described for the preventive treatment over a 24-h incubation period. At the end of the experiments, the cell population of the epidermis was evaluated using MTT test and the MDA levels were measured in the culture medium.

Preparation of the Reconstructed Human Epidermis

Keratinocytes (foreskin, 4th passage), checked free from mycoplasma, were used. Cells were seeding at the starting density of 1×10^5 cells/cm² on the polycarbonate membrane of culture inserts. Cultures were incubated at 37°C in a humidified atmosphere containing 5% (v/v) CO_2, for 24 h, submerged in culture medium CM1:MCDB 153 (Gibco) supplemented with EGF (5 ng/mL), insulin (5 μg/mL), hydrocortisone (5 ng/mL), and PPE (70 μg/mL). Then culture medium CM1 was supplemented with 1 mM of Ca^{2+} (culture medium CM2) in order to induce stratification over a 6-day period. Culture medium CM2 was changed every 3 days. Thereafter the epidermal sheet was cultured for 6 days at the air–liquid interface. On day 14, culture medium was changed. 150 μL of the EERB (three nontoxic concentrations), positive control (vitamin E), and negative controls (culture medium) were deposited on a disc of filter paper. Dilutions were prepared in culture medium CM3 (culture medium CM2 supplemented with 0.5% (v/v) antibiotics) [17].

Six series were carried out using culture medium CM3: three series of *EERB* (1, 10, and 100 μg/mL), a positive control with vitamin E (666 μg/mL), and two negative controls. All series were incubated for 24 h in a humidified atmosphere containing 5% (v/v) CO_2 (preventive treatment). Thereafter the discs were carefully withdrawn and the series irradiated with UVB (0.6 J/cm²) (Spectroline model X-15N/F, $\lambda = 312$ nm) except for the series corresponding to the second negative control, or absolute negative control. Then, curative treatment was performed incubating over 24 h. At the end of the treatment, filter paper disc was carefully withdrawn, the "stratum corneum" was rinsed with culture medium, and the epidermis was detached from the insert together with its substrate (insert membrane) using a scalpel. Culture media were taken for MDA assessment. The epidermis sheet was rinsed with PBS for cytotoxicity evaluation.

Cytotoxicity Evaluation (MTT Assay)

At the end of the incubation period (24 h), culture medium was withdrawn. The cell layer or epidermis sheet was rinsed with PBS and incubated for 3 h, at 37°C, in the dark, with 0.2 mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), at 0.5 mg/mL of culture medium. Formozan violet crystals, induced by MTT cleavage by mitochondrial enzymes, were dissolved in 0.2 mL dimethyl sulfoxide for 1 h at room temperature. Samples of 200 μL were distributed in a 96-well plate for spectrophotometric analysis at $\lambda = 540$ nm [18].

MDA Assessment

A standard series of MDA dilutions (0.1–10 μM) were prepared in culture medium from a stock solution of malondialdehyde at 10 μM in 1% (v/v) sulfuric acid. Aliquots of 500 μL of culture medium (or of standard dilution) were vortexed with 1 mL of a solution of trichloroacetic acid (0.15% w/v), thiobarbituric acid (0.375% w/v), and HCl 0.25 M. The mixture was incubated at 100°C for 15 min, cooled, and absorbance measured at $\lambda = 535$ nm [19].

Determination of Sun Protection Factor

The factor of solar protection was determined by means of a spectrophotometric method initially described by Diffey BL and Robson J [20], modified and improved, in view of the evaluation of the protection of the product against effects of UVB on the skin.

Statistical Analysis

The data comparison between control and treated groups was done by two-tailed *t*-test. The significance was set at 95% of confidence. Significant differences are referenced as $p < 0.05$ in the text.

Results

Skin Compatibility by Evaluation of Primary Skin Irritation

During the development of new topic formulations, skin irritation potential is investigated prior to human exposure, in order to identify chemicals which might induce adverse skin reactions.

Table 21.1 Checking in human of the skin compatibility of EERB after single application under patch

Control time after patch removal	Number of reactive volunteers	Type of reaction	Mean daily irritation score	Percentage of reactive volunteers
15 min	0	/	0	0
24 h	0	/	0	0
Maximal mean irritation score			0	

The study has been realized on 11 healthy subjects of both sexes, phototype (Fitzpatrick) I to V and ages between 18 and 70 years. Twenty microliters of enzymatic extract rice bran (EERB), diluted at 10% with water, was applied to the back skin under the patch for 48 h. Skin irritation responses were graded 15 min after patch removal and also after 48 h, if a positive reaction was observed. Any erythema in all the volunteers 15 min after the patch test removal or 48 h after it was observed

Table 21.2 Cytotoxicity evaluation (MTT assay) using human keratinocyte monolayer

Test substance		MTT (AU) mean ± standard error	Percentage
EEBR (μg/mL)	1	0.744 ± 0.054	100 (NS)
	10	0.742 ± 0.03	99 (NS)
	50	0.746 ± 0.060	100 (NS)
	100	0.751 ± 0.058	101 (NS)
	500	0.632 ± 0.024	85 ($p < 0.05$)
Positive control phenol (640 μg/mL)		0.108 ± 0.008	15 ($p < 0.001$)
Negative control culture medium		0.746 ± 0.024	100

Cytotoxicity was evaluated using MTT test in human dermal keratinocyte monolayers exposed to various concentrations of EERB (1, 10, 50, 100, and 500 μg/mL) for 24 h. The EERB did not induce cytotoxic effect for concentrations inferior or equal to 100 μg/mL. Phenol was used as positive control

Percentage: Calculated versus negative control

Abbreviations: *AU* arbitrary units, *EERB* enzymatic extract bran rice, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *NS* statistically nonsignificant ($p < 0.05$).

Results of patch testing demonstrated that the formulation containing all active substances under study provoked any erythema in all the volunteers 15 min after the patch test removal; 48 h after its removal no reaction was observed (Table 21.1).

EERB Cytotoxicity

Human dermal keratinocyte monolayers were exposed to various concentrations of EERB (1, 10, 50, 100, and 500 μg/mL). After 24 h of incubation, cytotoxicity was evaluated using MTT test. Table 21.2 shows mean ± standard error of percentage of MTT cleaved by mitochondrial enzymes versus negative control. The EERB did not induce cytotoxic effect for concentrations inferior or equal to 100 μg/mL. For 500 μg/mL a slight cytotoxic effect (−15%, $p < 0.05$) could be observed. Phenol (positive control, 640 μg/mL) exhibited cytotoxic effect (−85%, $p < 0.001$).

Free Radical Protection of EERB

Study Using Human Keratinocyte Monolayer

Keratinocyte monolayers are good models to realize these studies since in vivo they are the first cells to be exposed to cosmetics. Keratinocytes are the main target of UV, and play a central role in several responses of photo damage after UV [1]. In present work, human keratinocyte monolayer was treated

Table 21.3 Protection against free radicals (UVB irradiation) using human keratinocyte monolayer

Test substance		MTT (UA)	MDA (μM)	MDA/MTT (μM)/(AU)
EERB (μg/mL) + UVB	10	0.486 ± 0.008	0.77 ± 0.04	1.58 ± 0.10 ($p < 0.02$)
	50	0.508 ± 0.011	1.10 ± 0.01	2.16 ± 0.04 (NS)
	100	0.508 ± 0.024	1.68 ± 0.17	3.29 ± 0.19 ($p < 0.02$)
Negative control + UVB		0.510 ± 0.011	1.22 ± 0.09	2.36 ± 0.16
Positive control Vit E (200 μg/mL) + UVB		0.622 ± 0.006	0.75 ± 0.01	1.21 ± 0.03 ($p < 0.01$)
Absolute negative control (culture medium)		0.871 ± 0.007	0.07 ± 0.02	0.08 ± 0.02 ($p < 0.002$)

Human keratinocyte monolayer was treated with EERB and irradiated with UVB. To evaluate the protective role of EERB against UV insult to cells, we have measured cell population assessment (MTT assay) and the level of lipid peroxidation (MDA) in the culture medium under UV irradiation. Vitamin E (200 μg/mL) was used as negative control

Abbreviations AU arbitrary units, *EERB* enzymatic extract bran rice, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *MDA* malondialdehyde, *NS* statistically nonsignificant ($p < 0.05$). *p* calculated versus irradiated negative control

with EERB (preventive treatment), and irradiated with UVB. To evaluate the protective role of EERB against UV insult to cells, we have measured two parameters; first, cell population assessment was monitored by the MTT assay, thus a reduction of its cleavage is linked to cellular death and when cells die MTT value decreases. The second parameter studied is the level of lipid peroxidation by measuring MDA in the culture medium under UV irradiation. MDA is the end product of the lipid peroxidation process induced by free radical or activated oxygen species when cells are exposed to oxidative stress. So, both processes, cell viability and lipid peroxidation, are inversely correlated when cells are exposed to UV.

The results of EERB antioxidant capacity in keratinocyte monolayer are presented in Table 21.3. In all irradiated groups a decrease in cell viability was observed and this is similar to decline in the negative control and irradiated EERB supplemented groups approximately of 58%. In the positive control supplemented with vitamin E, the viability was 71% after irradiation. In order to compare the protection against free radicals in all groups, the relation MDA/MTT is used better than MDA alone.

It can be observed that in UVB-irradiated negative control the MDA was 29.5 times that of the negative control. On the other hand, the UVB-irradiated positive control showed that vitamin E (200 μg/mL) inhibited MDA/MTT generation induced by UVB irradiation (−49%, $p < 0.001$). Both the UVB-irradiated negative and positive controls validate the test system. For the EERB analysis, we observed a significant inhibition (−33%, $p < 0.001$) of MDA/MTT at 10 μg/mL. For higher concentrations of EERB, this inhibition decreased (−8.5% for 50 μg/mL) and a strong opposite effect (+39%) was observed for 100 μg/mL. This result is the consequence of an artifact: the self-absorption of the EERB at 535 nm masks the inhibiting effect of EERB. At low concentration this effect is negligible, whereas at high concentration this effect is predominant. In these conditions, with 10 μg/L, the decrease in MDA synthesis following irradiation is in the same order magnitude as that of vitamin E (200 μg/mL). However, to confirm the possible antioxidant effect of the EERB we realized a study on reconstructed human epidermis, where problems due to the self-absorption of the EERB ($\lambda = 535$ nm) at high concentrations are avoided.

Study Using Reconstructed Human Epidermis

Antioxidant capacity of EERB in reconstructed human epidermis is presented in Table 21.4. As mentioned above a decrease in viability is produced after irradiate cells, so to compare the results

Table 21.4 Protection against free radicals (UVB irradiation) on human reconstructed epidermis

Test substance		MTT (AU)	MDA (μM)	MDA/MTT (μM)/(AU)
EERB (μg/mL) + UVB	1	0.164 ± 0.002	0.82 ± 0.04	5.07 ± 0.95 (NS)
	10	0.171 ± 0.044	0.84 ± 0.01	5.14 ± 1.43 (NS)
	100	0.200 ± 0.011	0.64 ± 0.01	3.20 ± 0.23 ($p < 0.01$)
Negative control + UVB		0.149 ± 0.015	0.86 ± 0.08	5.78 ± 0.73
Positive control Vit E 600 (μg/mL) + UVB		0.280 ± 0.038	0.74 ± 0.05	2.67 ± 0.33 ($p < 0.01$)
Absolute negative control (culture medium)		0.668 ± 0.025	0.46 ± 0.13	0.69 ± 0.20 p < 0.001

Reconstructed human epidermis was treated with EERB and irradiated with UVB. To evaluate the protective role of EERB against UV insult to cells, we have measured cell population assessment (MTT assay) and the level of lipid peroxidation (MDA) in the culture medium under UV irradiation. Vitamin E (600 μg/mL) was used as negative control

Abbreviations: *AU* arbitrary units, *EERB* enzymatic extract bran rice, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *MDA* malondialdehyde, *NS* statistically nonsignificant ($p < 0.05$). *p* calculated versus irradiated negative control

about protection against free radicals the relation MDA/MTT is used. In irradiated negative control, MDA/MTT level was increased, as expected (+837%, $p < 0.001$), versus absolute negative control. In irradiated positive control (vitamin E, 600 μg/mL) MDA/MTT level was decreased, as expected (−54%, $p < 0.01$), versus irradiated negative control. These results obtained with irradiated negative and irradiated positive controls validate the test system and the experiment. When the EERB was assayed, concentrations of 1 μg/mL and 10 μg/mL did not modify significantly MDA/MTT level versus irradiated negative control, but the extract reduced significantly (−44.6%, $p < 0.01$) MDA/MTT synthesis and secretion at 100 μg/L. This protective effect against free radicals observed at 100 μg/mL was comparable to that induced by vitamin E at 600 μg/mL.

Evaluation of Sun Protection Factor

The value of sun protection factor (SPF) is expressed like the fraction of UVB and UVA that is retained by the cream layer. The SPF was 4.8 ± 0.3. This fact indicates a good photoprotection and dispersibility. This value is very elevated for a totally natural product, with absence of physical and chemical filters.

Discussion

The use of natural ingredients in cosmetics to prevent skin oxidative damage is steadily increasing. We have developed a soluble enzymatic extract from rice bran that could be used as a good new cosmeceutical. Firstly, the possible skin irritation of this extract was evaluated by using an occlusive patch test and the results showed good skin compatibility. Rice-derived ingredients generally are considered to be nonallergenic; several authors have reported no skin irritation when oils, fatty acids, or waves obtained from rice bran were tested [21].

We have found that EERB protects against free radical produced by UVB irradiation. This property may be due to several components. The enzymatic extract of rice bran is specially enriched in gamma-oryzanol [12, 22], which has antioxidant capacity [11]. This constituent is a group of ferulic acid esters that is being increasingly focused as an ingredient for drugs, nutraceutical foods, cosmetics, as well as dermatology preparations [23].

The antioxidant capacity of gamma-oryzanol has been attributed to its structure, which includes ferulic acid. Ferulic acid belongs to the family of polyphenolic compounds known as hydroxycinnamic acids, which impart cutaneous benefits [24]. This phenolic molecule has radical-scavenging activity [25] and can chelate the ferrous ion decreasing the formation of hydroxyl radical via inhibition of iron-dependent Fenton's reaction [26]. Besides, its carboxylic acid group also acts as an anchor by which it binds to the lipid bilayer providing protection against lipid peroxidation.

Ferulic acid alone absorbs some UV and therefore is itself a weak sunscreen. When mixed with vitamins C and E, it stabilizes the formulation and acts synergistically to double the photoprotection from fourfold to eightfold [27–29]. This association is particularly effective for reducing thymine dimer mutations known to be associated with skin cancer [30].

In addition, ferulic acid has anti-inflammatory properties. A number of antioxidants including ferulic acid and related ester derivatives such as gamma-oryzanol decrease the levels of some inflammatory mediators, e.g., prostaglandin E_2, tumor necrosis factor-alpha (TNFα), and interleukin 1-beta (IL-1β), and inhibit inducible nitric oxide synthase (iNOS) or cycloxygenase 2 (COX-2) [31]. It has been postulated that such anti-inflammatory activity likely involves the inhibition of the transcription factor, nuclear factor kappa-B (NF-κB). Inflammation and oxidative stress are closely linked to tumor promotion, so is probably that ferulate esters, with strong anti-inflammatory and anti-oxidative activities, act as antitumor promoters [24].

EERB is also a rich source of vitamin E (tocopherols, 100 mg/Kg; tocotrienols, 180 mg/Kg) [12]. In the skin, vitamin E is especially abundant in the stratum corneum, delivered there by sebum. The stratum corneum is first to absorb the oxidative stress of sunlight and pollution. With this exposure, vitamin E is depleted; so topical application is particularly advantageous. Besides, its lipophilic structure makes vitamin E an especially attractive cosmeceutical for application and absorption [32]. Vitamin E occurs in nature in at least eight different isoforms: α (alpha)-, β (beta)-, γ (gamma)-, and δ (delta)-tocopherols and α (alpha)-, β (beta)-, γ (gamma)-, and δ (delta)-tocotrienols. Tocotrienols differ from the corresponding tocopherols only in their aliphatic tail. Tocopherols have a phytyl side chain attached to their chromanol nucleus, whereas the tail of tocotrienols is unsaturated and forms an isoprenoid chain. Both forms of vitamin E act as antioxidants by their ability to donate phenolic hydrogens to lipid radicals [11]; tocotrienols are more efficient radical scavengers in biomembranes and penetrate more rapidly through skin than the corresponding tocopherols [33].

Tocotrienols also exhibit anticarcinogenic properties due to isoprenoid structure [34]. Tocotrienols are mixed isoprenoids, meaning that only a part, the lipophilic chain, is derived via the isoprenoid pathway. It has been reported that isoprenoids, including tocotrienols, inhibited the proliferation of human breast cancer cell lines, suppressed the growth of murine B_{16} melanomas, and induced cell-cycle arrest in the G-1 phase and apoptosis in human and murine tumor cells [35]. These effects can be observed with different isoprenoids, which are not antioxidants; so it is possible that the anticarcinogenic effects of tocotrienols are not necessarily related to their antioxidant properties. Lipid-rich plant products and vegetable oils are the main natural sources of vitamin E. Tocotrienols are found in high concentrations in palm oil and rice bran [36]. Other natural sources include coconut oil, cocoa butter, soybeans, barley, and wheat germ. Sunflower, peanut, walnut, sesame, and olive oils however contain only tocopherol [34].

The protective effect from lipoperoxidation observed when the EERB was used may be also due to the polyunsaturated fatty acid composition (35%). Other natural substances such as rose mosqueta [37], grape seed oil [38], and sea bean oil [39] are used as cosmetics because of their polyunsaturated fatty acids' content, which act as radical-scavenging agents.

Antioxidant activity of EERB may be also related with a relatively high content of sulfur amino acids [12]. These amino acids are precursors of two important natural antioxidants: taurine and glutathione. Taurine serves as an antioxidant, reacting with potentially toxic aldehydes, such as acetaldehyde and malondialdehyde, and preserves levels of GSH, which is a fundamental defense mechanism in conditions of increased oxidative stress [40].

Exposure to ultraviolet radiation (UVR) is associated with increasing skin pigmentation. One important property of gamma-oryzanol is its ability to decrease skin spots inhibiting the enzyme tyrosinase. Tyrosinase (polyphenol oxidase) is a multifunctional copper-containing enzyme that is involved in the synthesis of melanin. Melanin synthesis in mammals proceeds from the amino acid L-tyrosine through a series of enzymatic and chemical steps initiated by tyrosine hydroxylation to yield DOPA and DOPA oxidation to L-DOPA quinine. Many naturally occurring tyrosinase inhibitors have been reported and tested as cosmetics and pharmaceuticals to prevent overproduction of melanin in epidermal layers [41]. The structure of the ferulic acid moiety in oryzanol was considered to resemble tyrosine, thereby blocking enzymatic activity [21]. Vitamin E also inhibits the enzyme tyrosinase [32]; so the use of EERB as cosmetic may be important to prevent the serious esthetic problems in human beings about abnormal melanin pigmentation.

On the other hand, we have found that the EERB shows an SPF of 4.8 ± 0.3. Oryzanol has absorption maxima at 231, 291, and 315 nm and it has been proposed as a UVA filter in sunscreen cosmetics. Several patents for sunscreen formulations describe the use of rice bran-derived ingredients. A patent by Ishbashi K [42] reported that skin oil containing 3% gamma-oryzanol had an SPF of 3. Another patent by Loo CC [43] reported that rice bran oil applied in a topical formulation was an effective sunscreen against exposure to UVR at 295–315 nm [42].

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Part V
Essential Nutrients and Skin Cancer

Chapter 22
Folate Nutrition in Skin Health and Skin Cancer Prevention

Yira Bermudez, Katharine Cordova, and Joshua D. Williams

Key Points

- Folate is vital in promoting human health and preventing diseases.
- Folate nutrient levels support many biochemical processes proposed to have a particularly important impact on the maintenance and function of healthy skin.
- Folate deficiency is linked to numerous common dermatological conditions.
- Ultraviolet light radiation from sunlight, the major factor of skin cancer development, is capable of degrading folate nutrients, resulting in folate deficiencies in human skin.
- Folate supplementation has been suggested as a promising strategy for the prevention of skin cancer; however, many questions regarding folate nutrition within human skin must be answered before strategies to modulate folate nutrition may be rationally designed and safely implemented.

Keywords Folate • Skin physiology • Dermatology • Skin cancer • Folic acid

Abbreviations

5,10-Methylene-H_4folate	5,10-Methylenetetrahydrofolate
5-Formyl-H_4folate	5-Formyltetrahydrofolate
5-FU	5-Fluorouracil
5-Methyl-H_2folate	5-Methyldihydrofolate
5-Methyl-H_4folate	5-Methyltetrahydrofolate
AICAR	Aminoimidazol-4-carboxamide ribonucleotide
DHFR	Dihydrofolate reductase
dTMP	Deoxythymidylate monophosphate
dUMP	Deoxyuridylate monophosphate

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FAICAR	*N*-Formylaminoimidazol-4-carboxamide ribonucleotide
FDA	Food and Drug Administration
FGAR	*N*-Formylglycinamide ribonucleotide
FOLR1	Folate receptor
FPGS	Floyl-polyl-gamma(γ)-glutamate
FRα	Folate receptor alpha
GAR	Glycinamide ribonucleotide
GGH	Gamma(γ)-glutamyl hydrolase
H_2folate	Dihydrofolate
H_4folate	Tetrahydrofolate
HCP	Heme carrier protein
Hcy	Homocysteine
hPCFT	Human proton coupled folate transporters
MS	Methionine synthase
MTHFR	Methylenetetrahydrofolate reductase
MTX	Methotrexate
NADPH	Nicotinamide adenine dinucleotide phosphate
O_2	Atmospheric oxygen
O_3	Ozone
RCS	Reactive carbonyl species
RDA	Recommended dietary allowances
RFC	Reduced folate carrier
ROS	Reactive oxygen species
SAM	*S*-Adenosylmethionine
SHMT	Hydroxymethyltransferase
SPF	Sun protection factor
TS	Thymidylate synthase
UV	Ultraviolet
UV-A	Ultraviolet light wavelength A (315–400 nm)
UV-B	Ultraviolet light wavelength B (280–315 nm)
UV-C	Ultraviolet light wavelength C (100–280 nm)
UVR	Ultraviolet radiation

Introduction

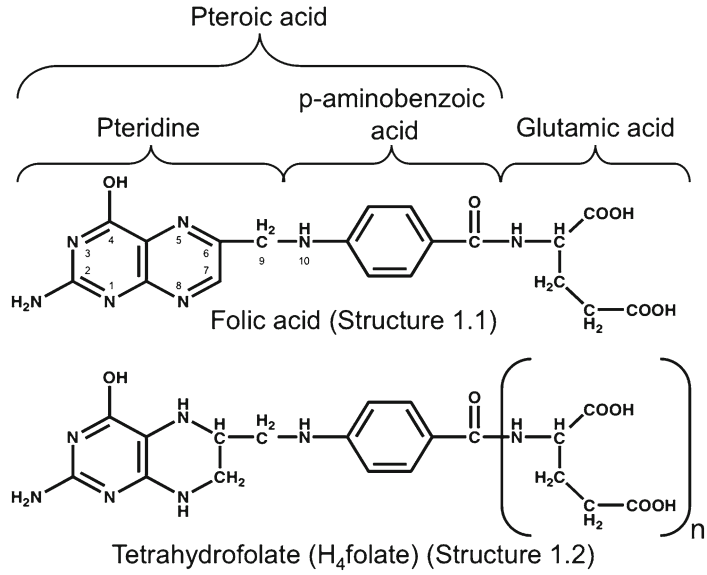
This chapter will discuss the role of folate nutrition in the unique environment of human skin. The folates are a family of structurally similar, water-soluble, B vitamins, which have been well documented as vital in promoting human health and preventing disease. Optimized folate nutrient levels support many biochemical processes important for the maintenance and function of healthy skin. This importance is underscored by potential links between folate deficiency and psoriasis, vitiligo, exfoliative dermatitis, glossitis, and skin cancers. Human skin is particularly prone to the development of carcinomas. It is established that skin cancer risk correlates with exposure to the complete carcinogen, ultraviolet radiation (UVR) from sunlight. Total avoidance of solar exposure is impractical.

Folate species are degraded by exposure to ultraviolet wavelengths making skin tissue potentially vulnerable to folate deficiencies. The potential impact of folate nutrition in cancer has been demonstrated by large-scale epidemiological and nutritional studies indicating that decreased folate intake increases the risk of cancer development. It has been hypothesized that folate supplementation may

be a promising strategy for the prevention of skin cancer, particularly cancers induced by UVR. However, many questions regarding folate nutrition within human skin must be answered before strategies to modulate folate nutrition may be rationally designed and safely implemented.

Folates: Chemical Structure and Nomenclature

The terms folic acid, folate, and vitamin B9 have been used interchangeably to describe a large family of chemically similar compounds. This family of compounds was first described in 1931, when it was demonstrated by Lucy Willis that extracts of liver and yeast, when administered orally, were effective in treating tropical macrocytic anemia [1]. The specific nutritional factor responsible for the observed therapeutic benefit was subsequently isolated from liver extracts and the chemical structure was determined to be *N*-[4-{[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino}benzoyl]glutamic acid with the name "pteroylglutamic acid" being proposed by the discovering chemists [2]. The molecular structure of the folate family is often chemically viewed in three segments, a pteridine residue, *p*-aminobenzoic acid linked by a methyl group at the 6-position of the pteridine ring system to form pteroic acid, and a single glutamic acid residue linked to the aminobenzoic acid by an amide bond (Fig. 22.1). The nomenclature describing this molecule has become somewhat ambiguous as the less cumbersome



Nomenclature	Substituent at N-5	Substituent at N-10
Tetrahydrofolate (H_4folate)	-H at 5,6,7, & 8	-H
Dihydrofolate (H_2folate)	-H at 7 & 8	-H
5-Formyl-H_4folate	-HCO	-H
5-Methyl-H_4folate	$-CH_3$	-H
10-Formyl-H_4folate	-H	-HCO
5,10-Methenyl-H_4folate	=CH-	$^+$
5,10-Methylene-H_4folate	$-CH_2-$	

Fig. 22.1 Folate structure and nomenclature. The structure, numbering conventions, and component breakdown of the synthetic monoglutamate folic acid (structure 1.1). The structure of the naturally occurring, bioactive folate tetrahydrofolate (structure 1.2). Naturally occurring folates generally exists as polyglutamates, where each glutamate residue is linked via a gamma glutamyl bond. The other naturally occurring folates are defined by their substituents at the *N*-5 and *N*-10 positions

name of "folic acid," with the word "folic" derived from the Latin—*folium* (leaf), has gained popular, albeit incorrect, usage in describing all members of this nutrient family [3]. Under more precise nomenclature conventions, folic acid is merely the parent structure of this large vitamin family created as a stable, synthetic analog that is found in supplements and added to fortified foods (Fig. 22.1 (structure 1.1)). The term "folate" in turn collectively refers to the naturally occurring forms of the vitamin which differ from folic acid in the oxidation state of the pteridine ring, with all folate mediated metabolic activity being attributed to derivatives of tetrahydrofolate (H_4folate), which is reduced at positions 5, 6, 7, and 8 (Fig. 22.1 (structure 1.2)). In addition to oxidation state, biological folates vary in the one-carbon substituent at the *N*10 and *N*5 positions as well as in the number of glutamic acid residues conjugated via gamma glutamyl bonds to form a polyglutamate tail. While biological folates generally exist as polyglutamates, the designation of glutamate chain length is unwieldy and therefore only practiced when precise differentiation between glutamation states is required. The structure of folic acid, the reduced derivative H_4folate, and the individual substituent patterns that define the other biologically relevant folate forms and the corresponding nomenclature are illustrated in Fig. 22.1.

Folate Nutrition

Humans cannot synthesize folate de novo and thus depend upon dietary sources to meet their nutritional needs. Dietary sources naturally rich in folate include liver, leafy green vegetables, broccoli, asparagus, certain types of beans, and citrus fruit. Consumption of large quantities of foods only moderately rich in folate, including potatoes and dairy products, may also contribute to meeting folate nutritional needs. The recommended dietary allowance (RDA), as set by the Food and Drug Administration (FDA) of the United States upon the recommendations of the Institute of Medicine of the National Academy of Sciences, is 400 μg/day for both men and women above age 14. The RDA for pregnant or lactating women is higher while the RDA for children and infants increases with age [4].

The National Health and Nutrition Examination Survey (1988–1994) and the Continuing Survey of Food Intakes by Individuals (1994–1996) found that most individuals surveyed did not consume adequate folate levels [5, 6]. These results prompted the Folic Acid Fortification Program, which was initiated by the FDA in 1998 to increase the folate content of commonly consumed foods such as cereals and grains through the addition of synthetic folic acid during food processing. While the overall consequences of this mandatory supplementation have been debated, it is agreed that the folate nutritional status of individuals living in the United States, as measured by folate blood levels, has been positively influenced [7, 8]. Despite fortification, it is estimated that folate deficiency still affects approximately 10% of the population as well as more than 50% of the children and elderly that live in poverty in the United States [9, 10].

Folate Nutrient Uptake and Cellular Transport

As humans are dependent on dietary sources for folate, the processes of absorption and transport are critical in determining overall folate nutritional status. Dietary folate polyglutamates must be converted to the monoglutamate forms prior to absorption. This conversion step is regulated by the enzyme γ-glutamyl hydrolase (GGH). This enzyme exists in two forms, one located on the brush borders of enterocytes and one located within intracellular lysosomes [11, 12]. The intracellular form of the folate hydrolase is uniformly expressed along the entire length of the small intestine, while the brush border form is expressed mainly in the proximal part of the small intestine where uptake is maximal [13]. The intestinal brush border form of the folate hydrolase has been shown to be upregulated

under conditions of dietary folate deficiency [14]. Conditions affecting the homeostasis of the intestines such as alcoholism, ciliac disease, and Crohn's disease are associated with folate malabsorption and diminished circulating folate concentrations [15].

In both the small and the large intestine, the uptake of the hydrophilic folate monoglutamates occurs via efficient, pH-dependent, and specialized carrier-mediated mechanisms. The uptake of dietary folate by the intestinal enterocytes is mediated by either the reduced folate carrier (RFC) or the human proton coupled folate transporter (hPCFT) [16, 17]. Following uptake, folate monoglutamates are transported from within the cells across the basolateral membrane, likely via the hPCFT, to the circulating plasma [18]. While 5-methyl-H_4folate is the most abundant folate species found in plasma, measurable levels of circulating folic acid have been observed after high-dose oral supplementation [19, 20]. Once in the plasma, the absorbed folate monoglutamates are then circulated to peripheral tissues where they are taken up by cells via transport-dependent processes.

The mechanisms of cellular folate transport vary by tissue and have yet to be fully characterized in human skin. Folate transport mechanisms can largely be classified into two groups and these mechanisms are likely similar in skin tissue. The first group consists of the membrane channels or carriers. The most ubiquitously expressed folate transport system is the RFC. This carrier is a bidirectional anionic exchanger for both natural reduced folates and folate-like pharmaceuticals. The transport kinetic properties of RFC show a poor affinity for synthetic folic acid compared to reduced folate species [21]. The second group of cellular folate transporters consists of the specific folate binding proteins anchored to the plasma membrane. To date, three folate receptor protein isoforms (FRα, FRβ, FRγ) have been identified with differential tissue expression. The most abundant and best-characterized isoform is FRα with expression generally localized to the apical membrane of epithelial cells [22, 23]. These folate receptors mediate folate uptake by an endocytotic pathway where receptor bound folate is internalized and released to the cellular cytoplasm against the concentration gradient [24]. In contrast to the RFC, FRα exhibits a greater affinity for folic acid and 5-methyl-H_4folate than for other reduced folate species [21].

Overview of Folate Metabolism

Folate taken up by cells is almost exclusively in a monoglutamate form. Cellular retention of folates is driven via polyglutamylation, an energy dependent reaction in which up to nine glutamate units are added to the γ-carboxyl group of the glutamate tail, as catalyzed by the enzyme folyl-poly-γ-glutamate synthetase (FPGS) [25]. FPGS activity has been characterized in many cell types and shows affinity for H_4folate as the primary folate substrate for polyglutamylation. As the folates obtained from the diet are naturally occurring 5-methyl-H_4folate and synthetic folic acid, the cellular enzymes which convert these folate species to H_4folate, methionine synthase (MS) and dihydrofolate reductase (DHFR), respectively, are viewed as rate limiting for intracellular folate nutrient accumulation [26]. Long chain, triglutamate or larger, reduced folate polyglutamates, in addition to being better substrates for folate-dependent enzymes than their monoglutamate counterparts, accumulate in the cell as they are no longer substrates for the bidirectional RFC and cannot be exported from cells via energy-dependent efflux transporters such as the multidrug resistance proteins [25, 27, 28]. The activity of FPGS is countered by the lysosomal activity of GGH which catalyzes the hydrolysis of these terminal γ-glutamyl residues from polyglutamylated folates [29]. The polyglutamylation cycle for the accumulation of intracellular folates is depicted in Fig. 22.2.

The metabolic roles of reduced folate polyglutamates are as cofactors in the transfer of one-carbon units of various oxidation states in numerous enzyme mediated reactions. Within cells, five major folate-dependent one-carbon transfer reactions have been characterized: the conversion of serine to glycine, the catabolism of histidine, and the synthesis of thymidylate, methionine, and purine nucleotides.

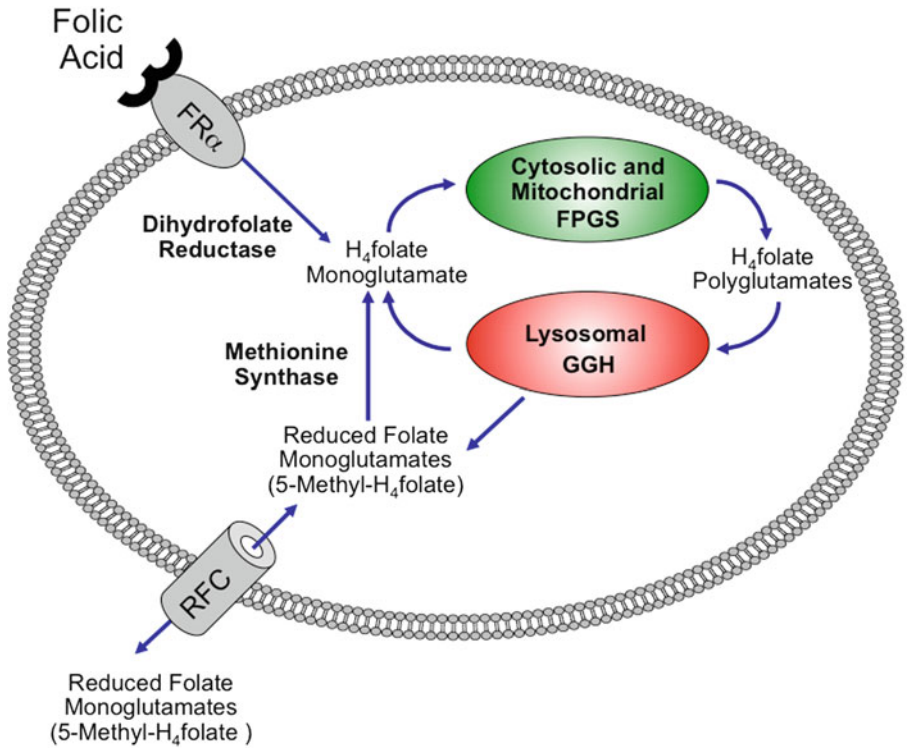


Fig. 22.2 Mechanisms of cellular folate uptake and intracellular polyglutamylation cycle for folate retention. Reduced and oxidized folate monoglutamate species are taken into the cell by specific membrane transport mechanisms (FRα—folic acid RFC—reduced folates). Once taken up, folate monoglutamates are polyglutamylated by FPGS in the cytosol or mitochondria where they then serve as optimal cofactors for folate-dependent enzymes. Terminal γ-glutamyl residues can be hydrolyzed from folate polyglutamates by GGH at which point the reduced folate monoglutamates can recycle through the polyglutamylation cycle or may be exported from the cell via the RFC

In addition to the methyl or formyl groups contributed by the dietary intake of 5-methyl-H_4folate and 5-formyl-H_4folate, the one-carbon units utilized in folate-dependent one-carbon metabolism are primarily derived from the β-carbon of serine as it is converted to glycine by the enzyme serine hydroxymethyltransferase (SHMT), which transfers the β-carbon group to a H_4folate cofactor to create 5,10-methylenetetrahydrofolate (5,10-methylene-H_4folate). An overview of intracellular folate metabolism is depicted in Fig. 22.3.

Folates serve in the biosynthesis of purine and pyrimidine nucleotides utilized in the synthesis of new DNA and RNA as well as in the repair of damaged DNA. In purine synthesis, two one-carbon units from 10-formyl-H_4folate are transferred, first to glycinamide ribonucleotide (GAR) by the enzyme GAR transformylase to form *N*-formylglycinamide ribonucleotide (FGAR) and next to aminoimidazol-4-carboxamide ribonucleotide (AICAR) by the enzyme AICAR transformylase to form *N*-formylaminoimidazol-4-carboxamide ribonucleotide (FAICAR). The transferred formate groups become carbons 8 and 2 of the purine ring. In pyrimidine synthesis, the methyl group of 5,10-methylene-H_4folate is transferred to deoxyuridylate monophosphate (dUMP) to form deoxythymidylate monophosphate (dTMP) and dihydrofolate (H_2folate) in a reductive methylation reaction catalyzed by the enzyme thymidylate synthase (TS). H_2folate is then reduced back to H_4folate by the enzyme DHFR and the cofactor reduced nicotinamide adenine dinucleotide phosphate (NADPH) [30]. Folate also serves in the remethylation of homocysteine (Hcy) to form methionine, which is subsequently converted to *S*-adenosylmethionine (SAM), the primary methyl donor for all intracellular methylation reactions

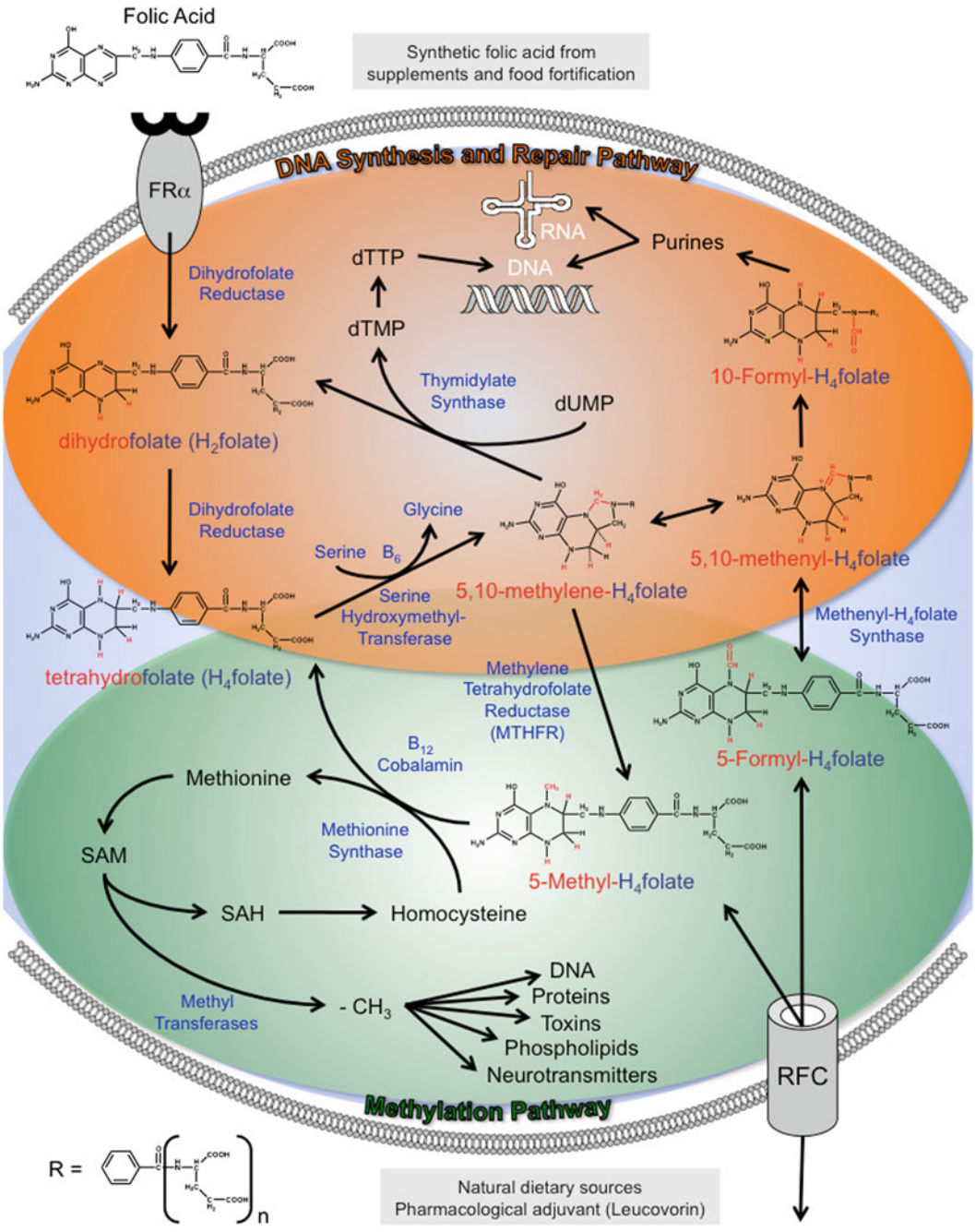


Fig. 22.3 Overview of folate metabolism

including those of DNA, RNA, proteins, lipids, neurotransmitters, and toxins. In Hcy remethylation, 5,10-methylene-H_4folate is first reduced to 5-methyl-H_4folate in a functionally irreversible reaction catalyzed by the enzyme methylenetetrahydrofolate reductase (MTHFR) [31]. The methyl group of 5-methyl-H_4folate is then transferred to Hcy to create methionine and H_4folate in a vitamin B_{12} dependent

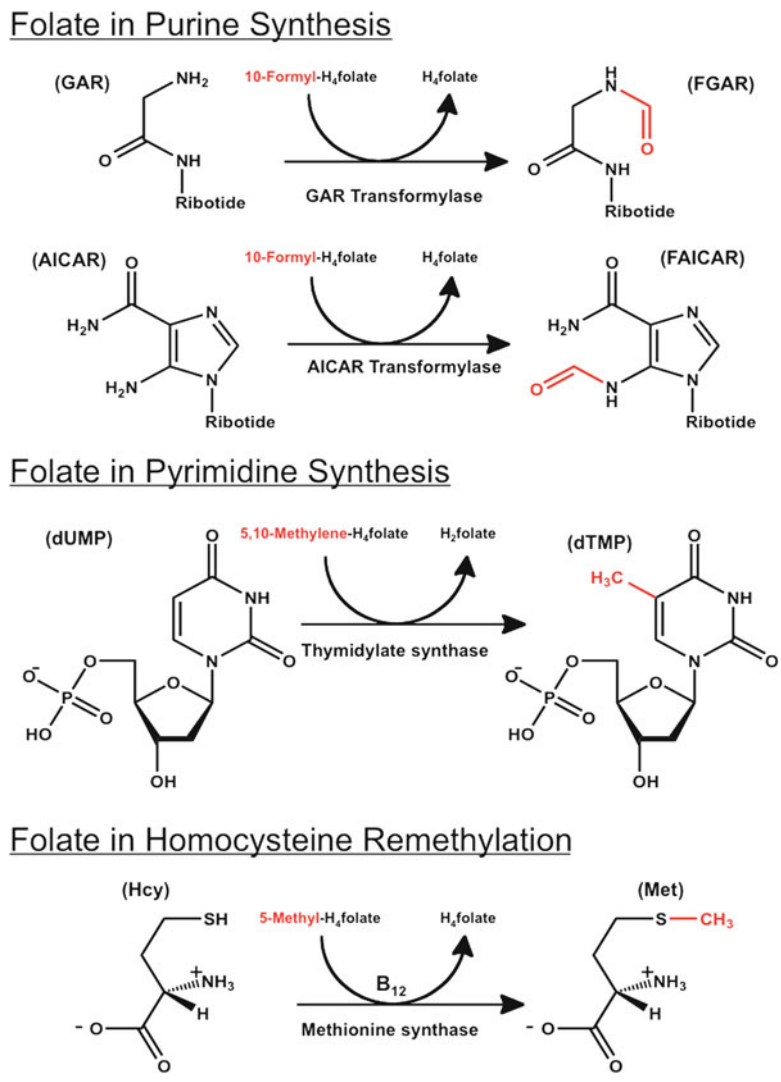


Fig. 22.4 Folate-dependent intracellular reactions. Depicted are the chemical reactions utilizing folate-dependent one-carbon transfer in purine and pyrimidine synthesis and in the remethylation of homocysteine

mechanism by the enzyme MS [32]. The folate-dependent chemical reactions in the synthesis of purine and pyrimidine nucleotides and the remethylation of Hcy are diagramed in Fig. 22.4.

The biological roles served by the various folate species require complex interconversion cycles facilitated by numerous enzymes. As these cycles intersect numerous pathways, it is now appreciated that the interconversion of folate species serves as a mechanism by which aspects of metabolism are regulated. In proliferating cells, DNA synthesis is paramount and expression of the enzyme TS peaks during the S phase of the cell cycle to ensure an adequate supply of thymidylate. Increased thymidylate synthesis results in elevated concentrations of H_2folate, which has been shown to be an allosteric inhibitor of the enzyme MTHFR. Inhibition of MTHFR ensures that intracellular pools of 5,10-methylene-H_4folate are utilized for purine and pyrimidine synthesis rather than committed toward methionine formation [33]. Methionine synthesis is regulated by the levels of both the precursor 5-methyl-H_4folate and the activation product SAM. High levels of SAM, indicating an abundant methylation capacity, inhibit MTHFR and thus the formation of 5-methyl-H_4folate. Conversely, high

levels of 5-methyl-H_4folate, indicating low levels of SAM and a reduced methylation capacity, have been shown to inhibit several non-critical methyltransferase enzymes, thus conserving limited SAM for essential methylation reactions [26, 34]. Given the complex metabolism, regulation, and interconversion of folate species in combination with the critical roles that folates play in biosynthetic reactions, it is not surprising that folate nutrition is intimately tied to human health.

Folate and Human Health

Folate was first discovered as a factor shown to be effective in the treatment of macrocytic anemia [1]. Still today, anemia characterized by megaloblastic and poorly developed red blood cells is the most common overt manifestation of severe folate deficiency. Interest in the health benefits of folate surged when it was found to play a role in preventing neural tube defects such as spina bifida and anencephaly [35]. Indeed, it was the link between folate nutrition and birth defects that prompted a folate nutritional assessment and the eventual mandatory folic acid fortification program initiated by the FDA in 1998 [5, 6]. Following the institution of folic acid fortification, a significant drop in the incidence of neural tube defects was observed across the United States, a finding that has been subsequently replicated in other countries [36, 37]. The developmental benefits of folate supplementation have continued to expand as adequate folate nutrition has now been shown to be protective against other congenital abnormalities such as cardiovascular malformations, urinary tract defects, limb deficiencies, cleft lip and palate, and Down's syndrome [38]. While the precise mechanisms remain unknown, it is clear that folate plays a large role in successful human reproduction.

Links between disease and folate metabolism continue to be discovered. An established reciprocal relationship exists between plasma Hcy and folate concentrations, and both human and animal studies show a link between Hcy levels and vascular disease. Individuals with elevated levels of plasma Hcy caused by enzyme deficiencies are observed to suffer early-onset occlusive vascular disease [39]. Even modest elevations in plasma Hcy concentration are considered to have a pathological effect on vascular endothelium and Hcy is therefore considered an independent risk factor for arteriosclerosis and venous thrombosis [40]. Folate supplementation is proposed as a preventive measure against occlusive vascular disease by facilitating remethylation to methionine and thereby lowering Hcy levels [41]. In addition to early-onset vascular disease, individuals with elevated levels of plasma Hcy exhibited cognitive impairments and there is an apparent link between occlusive vascular disease and Alzheimers disease [42]. Therapeutic intervention with 5-methyl-H_4folate is being investigated for the treatment of mood disorders and the improvement of human cognition based upon the observation that neurotransmitter metabolism depends upon methyltransferase enzymes. It has been demonstrated that 5-methyl-H_4folate is rapidly taken up from plasma at the choroids plexus where it is concentrated in the cerebrospinal fluid [26, 43].

Recently, a large amount of effort has been focused on understanding the role of folate in cancer. An increasing number of specific cancers have been linked to folate deficiencies including those of the colon, breast, pancreas, stomach, cervix, bronchus, blood, and skin [44–51]. Folate nutrition has been primarily linked to cancer through the processes of DNA synthesis and repair, with conditions of folate deficiency promoting pro-carcinogenic genomic instability. Folate nutrient supplementation has thus been proposed as a means by which to prevent cancer by promoting genomic stability. Folate supplementation has also been proposed to enhance intracellular concentrations of SAM and thus facilitate methylation-dependent reactions, where changes in methylation patterns may alter epigenetic regulation of the expression of oncogenes or tumor suppressor genes in the process of carcinogenesis. However, for nutrient modulation strategies to be successful in the prevention of any specific disease, it is necessary to first understand the specific effects of individual nutrients within the complex context of individual diseases afflicting the specific tissue of interest. Thus, the numerous aspects of folate nutrition in the context of human skin are further discussed.

Skin Physiology and Folate

Human skin is a dynamic, adapting, and interactive barrier that is uniquely poised to interface our internal biological systems with the environment. It functions as a sensory organ; as a metabolic center synthesizing vitamin D, excreting sweat, and allowing for exchange of heat and moisture; as an immune organ, surveying for potential pathogens; and as a mechanical barrier.

As our skin facilitates our detection of the ambient conditions and physical influences around us, this organ is assaulted by stresses, strains, and abuse unique from those of any other tissue. The skin is bombarded chronically by microbes from the surfaces we touch, physical extremes in temperature, swings in acidity and ionic gradients, chemicals including environmental toxins and carcinogens, and electromagnetic radiation both from the sun as well as from sources inherent in our increasingly technological society.

Evolution has produced an organ system exquisitely suited to perform this unique physiological role. Skin has three primary tissue layers, the epidermis, the outer most layer, the dermis that houses adnexal structures and provides metabolic support, and the subcutis or fatty layer. Although these are somewhat functionally and anatomically distinct, each is intimately dependent on the other.

The epidermis is a continually renewing, stratified, laminar structure comprised of four cell types, keratinocytes, melanocytes, Langerhans cells, and Merkel cells. Keratinocytes comprise at least 80% of this stratified squamous epithelium. They are characterized by keratin intermediate filaments in their cytoplasm and form attachments with their neighboring cells that allows for cell-to-cell adhesion and communication. It is mutations in the keratinocyte that causes squamous cell skin cancer. Melanocytes sit on the basal layer of the epidermis and provide melanin to the keratinocytes. This dynamic process gives our skin color and epitomizes the skins ability to rapidly adapt to environmental insults; as more UV radiation reaches the skin, the melanocytes produce more melanin protecting against UV induced DNA damage. Melanocytes are the primary cell in melanoma skin cancer. Langerhans cells are the resident antigen presenting cells that continually survey the skin and interface with T lymphocytes to trigger immune response. Merkel cells are touch receptor cells. Unregulated growth of these cells causes Merkel cell carcinoma that is a particularly rare, but often lethal form of skin cancer.

The epidermal barrier is produced and maintained by a continuous process of keratinocyte proliferation and differentiation. A subset of keratinocytes, originating in the stratum basale layer, continually divides producing new squamous cells. This endless regeneration pushes prior generations of cells outward. As these keratinocytes progress through the epidermis they undergo terminal differentiation. This coordinated series of events involves a predictable evolution of cell morphology, changes in cell adhesion complexes, and the formation of lipid products and proteins that are essential components of the skin barrier. Once near the skin surface, the enlarged, flattened, and keratin bonded cells become dehydrated and die forming a tough, durable outer layer called the stratum corneum [52]. These mummified cells are shed and replaced from beneath.

This process of continual cell division and differentiation brings significant metabolic and nutritional needs [52]. Furthermore, the direct apposition of the epidermis to the environment requires an increased utilization of stress proteins, cellular antioxidants, and an enhanced level of DNA damage repair [53, 54]. This unique and localized demand for essential nutrients creates a particular vulnerability for nutrient deficiencies.

Folate is absolutely critical in the synthesis and repair of DNA and RNA, which supports the continuous turnover of healthy, mutation-free keratinocytes. In vitro studies on dividing epidermal keratinocytes show that when cells are placed in folate deficient conditions they exhibit a rapid decline in intracellular folate levels (Fig. 22.5b) and are unable to sustain proliferation (Fig. 22.5a) [55]. Furthermore, skin cells deficient in folate halt division in an uncontrolled way, often in the middle of DNA synthesis, thus leaving their DNA particularly vulnerable to pro-carcinogenic errors and mutations (Fig. 22.5a).

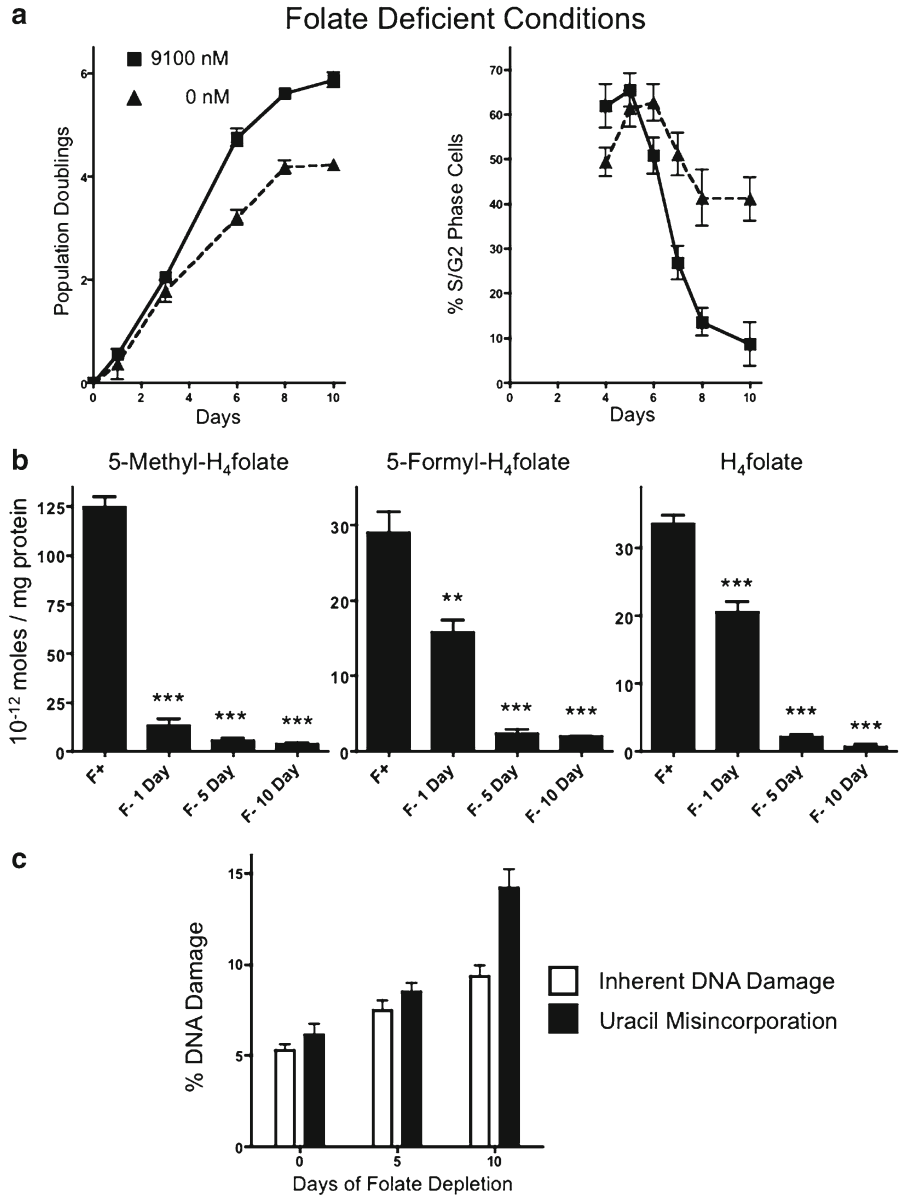


Fig. 22.5 Effects of folate depletion on human keratinocytes. (**a**) Growth curves and cell cycle data for human keratinocytes subjected to folate deficient conditions. (**b**) Intracellular levels of measured bioactive folate species in human keratinocytes following removal of folate nutrient sources. (**c**) Increasing genomic instability and uracil misincorporation in folate deficient human keratinocytes. (Adapted from [55])

With the decline in intracellular folate, skin cells are unable to support folate-dependent methylation reactions such as the 5,10-methylene-H_4folate-dependent conversion of uracil to thymidine. Increased levels of intracellular uracil have the effect of increasing both overall genomic instability as well as uracil misincorporation into the DNA of folate deficient keratinocytes (Fig. 22.5c). Folate as a cofactor in the general methylation cycle broadly impacts cell function including methylation patterns on

DNA, intracellular proteins, and signaling molecules. Indirectly this plays a role in detoxification by maintaining the cellular capacity for the detoxifying methylation of exogenous chemical toxins as well as by regulating intracellular homocysteine and subsequently glutathione levels.

Folate in Skin Diseases

Folate deficiency has been associated with a number of skin diseases. Atrophy of the oropharyngeal mucous membrane has been established as an early marker of vitamin deficiency often indicating a metabolic defect [56]. Livedoid vasculopathy, a non-inflammatory occlusive thrombotic disease, has been associated with hyperhomocysteinemia and thus decreased serum folate levels particularly in younger patients. Indeed, folic acid supplementation has been incorporated into treatment regimens for these conditions [57, 58]. Vitiligo, a disorder characterized by depigmentation of the skin, has been associated with low blood levels of both folate and vitamin B_{12} [59]. The nature of this association has not yet been defined.

A link between folate and skin disease has been consistently documented in the complex, chronic inflammatory skin disorder psoriasis. Psoriasis is a disorder of excessive growth and reproduction of keratinocytes in response to excessive signaling by the immune system. Thus, an accelerated turnover of epidermal keratinocytes increases nutritional requirements and may cause a localized functional deficiency of folate and other nutrients [60, 61]. Patients with psoriasis consistently present with mild folate deficiency and corresponding increased levels of plasma homocysteine [62, 63]. The mechanism of folate deficiency and whether it is a causative or reactionary process remains unclear; however, many of the known triggers of psoriasis including stress, skin injury, reactions to medications, fluctuations in ambient environmental conditions, hormonal fluctuations such as during pregnancy, chemical exposures, smoking, obesity, and heavy alcohol consumption are also associated with alterations in nutrition [64].

The interplay between folate, psoriasis, and the immune system is evidenced by one of the main treatments of psoriasis. Methotrexate (MTX) is an antifolate, immunosuppressant agent commonly employed by dermatologists in the treatment of psoriasis and psoriatic arthritis. Folic acid supplementation is routinely provided while on methotrexate to minimize side effects. Although folic acid supplementation has been shown to lower homocysteine levels and modulate some psoriasis associated inflammatory markers, folate nutrient supplementation alone does not improve psoriasis. [65].

Our understanding of the skin immune system is continuing to be refined in terms of the exact immunophenotypes of lymphocyte subpopulations, tissue localizations, and roles of exact immune functions as they pertain to specific pathologies. However, it is clear that a robust yet appropriately regulated immune component is vital to skin as well as overall organism health. The importance of folate nutrition to the human immune system cannot be overstated, as malnutrition is recognized as the primary cause of immunodeficiency worldwide [66].

Folate in the Treatment and Prevention of Cancer

In 1945 it was observed by Lewisohn et al. that folic acid concentrate caused regression of mammary tumors in mice [67]. Based on these results, Farber et al. added folic acid polyglutamates to the treatment regimen of children with leukemia. Unexpectedly, the administration of folic acid was observed to dramatically accelerate the disease progression [68]. Following up on these observations, Farber et al. went on to demonstrate that a folic acid antagonist, aminopterin (a precursor to MTX), was able to induce remission in children with acute leukemia [69]. From these findings, the modern era of

antifolate chemotherapy was born and with it the dogma that the antitumor effects observed with antifolate therapy are the result of folate depletion.

It was not until the 1980s that epidemiologic and clinical observations began to emerge suggesting an inverse association between folate nutritional status and the risk of human malignancies. The biological explanations for the roles of folate in the development of cancer center on the maintenance of genomic integrity and gene expression [26, 30, 70]. Laboratory studies have definitively shown that folate deficiency contributes to carcinogenesis through a diminished cellular capacity for mutation free DNA synthesis, repair of DNA damage, and maintenance of DNA methylation [71, 72]. Furthermore, an increased dietary intake of folate, whether from a naturally folate-rich diet or through supplementation, has been shown to be protective against the development of many types of cancer [44–51].

The relationship between folate status and cancer risk has been most intensely studied in colorectal cancer. Numerous small studies comparing patients who do and do not use multivitamin supplements or individuals receiving high or low dose pure folic acid supplementation have reported that increased intake of folic acid results in both a reduction in the risk of developing primary colorectal cancer or precursor adenomas and a reduction in the recurrence of resected adenomas [73–75]. However, the significance of these findings remains in question as there also exist a number of dissenting studies indicating no protective effect with folic acid supplementation [74, 76]. Amidst these conflicting results, a recent study has reported that folic acid supplementation of 1 mg/day in patients with a history of colorectal adenomas resulted in a 67% increased risk of advanced lesions, a twofold increased risk for multiple adenomas, and a significantly increased risk of cancers other than colorectal [77].

The potential tumor-promoting effect of folic acid supplementation is in agreement with numerous animal studies, which have collectively indicated a dual modality in the effects of folate supplementation on cancer development. In normal colorectal mucosa, folic deficiency seems to predispose it to neoplastic transformation, and folic acid supplementation is able to suppress this effect at moderate doses [74, 78]. However, in established colorectal tumors, folate deficiency inhibits progression and folate supplementation promotes tumor growth. Taken as a whole, results indicate a potent link between folate nutrition and cancer; however, strategies utilizing folate to treat or prevent human malignancies wield a “double-edged sword” with optimum dosing yet to be defined and where a fastidious effort must be made to understand the specific clinical context in order to maximize the beneficial effects while minimizing any associated risks.

Skin Cancer and Folate Deficiency

Extrapolating findings from other cancers, it has also been suggested that folate deficiencies may contribute to the development skin cancer. Skin cancer, or the uncontrolled growth of abnormal skin cells, represents the most commonly diagnosed cancer, surpassing all other cancers combined in the United States [79]. The National Cancer Institute estimates that more than 1,000,000 new cases of non-melanoma skin cancer were diagnosed in 2010 [80]. Although effective options for the treatment of skin cancer exist, this disease continues to exert a tremendous impact on morbidity, health, and healthcare economics [79].

The three most common forms of skin cancer are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma, all of which develop in the epidermis. The most common form of skin cancer, accounting for approximately 75% of all diagnosed cases, is BCC. This cancer arises from the dividing basal layer of the epidermis and is considered to be the least likely to metastasize. The second most common form of skin cancer is SCC. This cancer arises in the epidermal keratinocytes, eventually penetrating the underlying tissue if left untreated. Taken together, BCC and SCC are classified as nonmelanoma skin cancers (NMSC). Melanoma is less common, but is responsible for most of the skin cancer associated deaths.

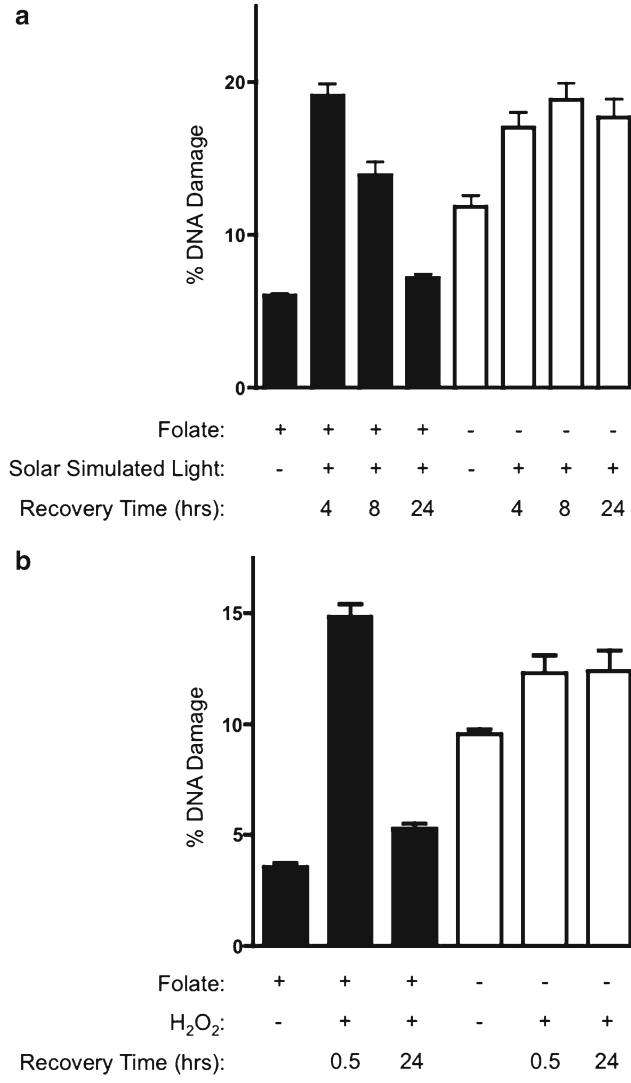


Fig. 22.6 Effects of folate depletion on DNA damage repair in human keratinocytes. (**a**) Deficiencies in DNA damage induced by exposure to solar simulated light in folate deficient human keratinocytes. (**b**) Deficiencies in DNA damage induced by exposure to reactive oxygen species in folate deficient human keratinocytes. (Adapted from [55])

Only recently have results begun to emerge looking at the role that folate deficiency may play in the development of skin cancers. Laboratory studies show human epidermal keratinocytes exhibit errors in DNA repair when grown in folate deficient conditions. These mutations manifest when cells are exposed to either simulated solar light (Fig. 22.6a) or reactive oxygen species (ROS) (Fig. 22.6b). These results highlight the potential genomic consequences of folate deficiency in skin receiving either direct solar light induced DNA damage or DNA damage from the ROS that accompanies sunlight exposure.

Epidemiologic data linking either general or skin specific folate deficiency to skin cancer is lacking and remains complicated by numerous practical chemical considerations. For now, the strongest associations between folate and skin cancer are supported by studies on genetic polymorphisms in the folate metabolic pathway, which have been shown to modify cancer risk. As discussed previously, the enzymes 5,10-methylene-H_4folate reductase (MTHFR) and thymidylate synthase (TS) are essential in folate metabolism and support the epigenetic regulation of DNA through methylation and DNA synthesis, respectively. Polymorphisms in these enzymes have been shown to be prevalent in the general population, resulting in significant individual variation in the enzymatic expression or activity levels [81–84]. Common MTHFR polymorphisms including a 677C>T A to V substitution and a

1298A>C G to A substitution, and TS polymorphisms including a six base pair insertion/deletion mutation of the 3′-untranslated region have been demonstrated to increase the risk of developing both basal and squamous cell carcinomas particularly upon a background of immunosuppression, such as that created for organ transplant recipients [51, 85, 86]. Folate metabolism gene associations indicate that factors resulting in alterations to intracellular folate homeostasis may increase the lifetime risk for developing skin cancer

Folate Antagonists in Dermatology

The use of antifolates to treat NMSC highlights the biochemical link between folate and skin cancer. Topical 5-fluorouracil (5-FU), typically formulated as a 5% cream, is routinely applied as a modality in the treatment of both pre-cancerous actinic keratosis as well as superficial nonmelanoma skin cancers. The antagonist 5-FU is an antimetabolite of uracil and acts to inhibit the enzyme thymidylate synthase (TS). The primary effect of TS inhibition by 5-FU is a depletion of thymine levels, both dTMP and dTTP, resulting in inhibition of DNA synthesis. Inhibition of TS also results in accumulation of uracil and fluorouracil monophosphates which, when converted to triphosphates dUTP and F-dUTP, are incorporated into DNA. The nucleotides are subsequently excised, causing DNA strand breaks and eventually contributing to cell death [87]. 5-FU acts as a classical cytotoxic agent where the effects are exhibited across the entire area of application but are most significant in rapidly dividing cells. The efficacy of this treatment highlights the dependence of cancerous or pre-cancerous skin cells on folate metabolism to utilize the cellular nucleotide synthesis machinery.

Another folate antagonist that is commonly employed by dermatologists is methotrexate (MTX). One of the earliest antifolates and anticancer drugs, MTX remains a mainstay in the treatment of lymphoma, acute lymphoblastic leukemia, and osteosarcoma. Within cells, MTX has been shown to inhibit the enzymes DHFR, GARFT, AICARFT, and TS, resulting in impaired DNA and RNA synthesis [88]. As with other cytotoxics and folate deficiency in general, these effects manifest most rapidly in proliferating cells. While MTX is a classical anticancer chemotherapy, dermatologists mainly utilize the immunosuppressive and thus anti-inflammatory properties of the drug in the treatment of psoriasis and psoriatic arthritis.

Interestingly, it is another treatment for psoriasis that sparked much of the current research into the relationships between folate and skin disease. Phototherapy, or the utilization of non-ionizing electromagnetic radiation, has long been an effective systemic approach for the treatment of patients with psoriasis, eczema, and cutaneous T-cell lymphoma. This therapy consists of either long ultraviolet (UV) wavelengths in combination with photosensitizing drugs or stand-alone treatments with shorter UV wavelengths [89]. In 1978, Branda and Eaton examined a small group of fair-skinned patients undergoing long wavelength UV light therapy for their psoriasis and observed a significant decrease in serum folate concentrations. This led to the conclusion that long-wavelength UV light is capable of causing the rapid and extensive photolysis of folate in vitro and may do so in vivo [90].

The biochemical necessity of folate nutrition, the link between folate deficiencies and skin disease, the efficacy of folate antagonists in the treatment of cancer, and the potential degradation of folate nutrients by UVR are intriguing relationships that are driving the direction of current research.

Skin Pigmentation and the Micronutrient Hypothesis

The impact of skin color on human history is impossible to overstate. As vision driven creatures, we readily notice differences in the skin color of individuals and skin tone is perhaps the most important physical trait used to define human groups and races [91]. As one of the most prominent human

features, the evolution of human skin color has invited many possible explanations. Debate continues between two main hypotheses explaining the origin of human skin color, one proposing sexual selection while the other favors naturally selective mechanisms. Even without a complete understanding of the mechanism, it is clear that melanin pigmentation is an adaptation to some attribute of the physical environment. The genetic characteristic of skin pigmentation is governed by numerous loci, which would require continued positive selection for its maintenance. This points to a sustained evolutionary pressure which acts to favor retention of pigmentation characteristics [92].

Toward the end of the twentieth century, our understanding of human skin pigmentation was refined with the development of geographic information and remote sensing technology. Examination of skin pigmentation and physical parameters of the environment demonstrated conclusively that human skin reflectance as a measure of pigmentation was correlated most strongly with latitude and autumn levels of UVR [93]. Establishment of UVR as a causative agent in the variation of human skin pigmentation precipitated a reinvestigation of the proposed selective mechanisms. Popular arguments framed the value of dark pigmentation in protection against sunburn, skin cancer, and overproduction of vitamin D [94, 95]. While higher levels of melanin enhance an individual protection from these UVR-induced harms, none can be considered to exert the reproductive pressure considered necessary to drive natural selection [91]. Overproduction of vitamin D has also been refuted as unlikely if not impossible due to tight biological photochemical regulation [96, 97].

It was upon this background of reevaluation that the significance of data suggesting a link between UVR and nutrients vital to reproductive success was fully appreciated [90]. The evolution of dark skin to protect against folate photodegradation meets the criteria for a direct reproductive link, as folate is essential for fetal development and fertility. Similarly, the attenuation of skin pigment in regions of low UVR may have evolved to ensure adequate vitamin D biosynthesis, which is necessary for fetal bone development and maternal bone health [95, 98]. Thus, skin pigmentation may have evolved to balance the levels of folate and vitamin D, both of which are influenced by UVR exposure and both of which are necessary for healthy offspring, (Fig. 22.7). The strength of this model is supported by recent data elucidating the importance of both folate and vitamin D in supporting human fertility and the prevalence of genetic polymorphisms in skin pigment pathways among different populations [91, 99].

The repercussions of this model are of much interest as shifting world populations result in many people residing in areas of UVR exposure that are significantly different from those to which their skin tone has been adapted. Indeed, it is well documented that the prevalence of skin cancer is highest in lightly pigmented people who experience chronic or intense episodic exposures to UVR in places far from their ancestral homelands [100]. Carried further, folate deficiency resulting from the photodegradation of folate in the skin may play a significant role in predisposing individuals toward the development of dermatological disorders including skin cancer, and this risk is greatly increased in lightly pigmented individuals residing in regions where high UVR exposure is possible.

Skin Cancer and Ultraviolet Radiation

The incidence of non-melanoma and melanoma skin cancer continues to rise [101, 102]. It is well established that DNA damage and cellular responses to DNA damage play a central role in the process of skin carcinogenesis [103]. Exposure to UVR from sunlight is the major source of skin carcinogenesis as it leads to DNA damage both directly via the formation of pyrimidine dimers and other photoproducts and indirectly via the generation of reactive oxygen species (ROS) and reactive carbonyl species (RCS) [104–107]. Thus, UVR from sunlight is a complete carcinogen.

The levels of UVR reaching the surface of the Earth are affected by numerous factors such as latitude, altitude, season, moisture content, cloud cover, and the thickness of the ozone layer. The shortest, highest energy UV wavelengths (UV-C, 100–280 nm) are essentially completely blocked or

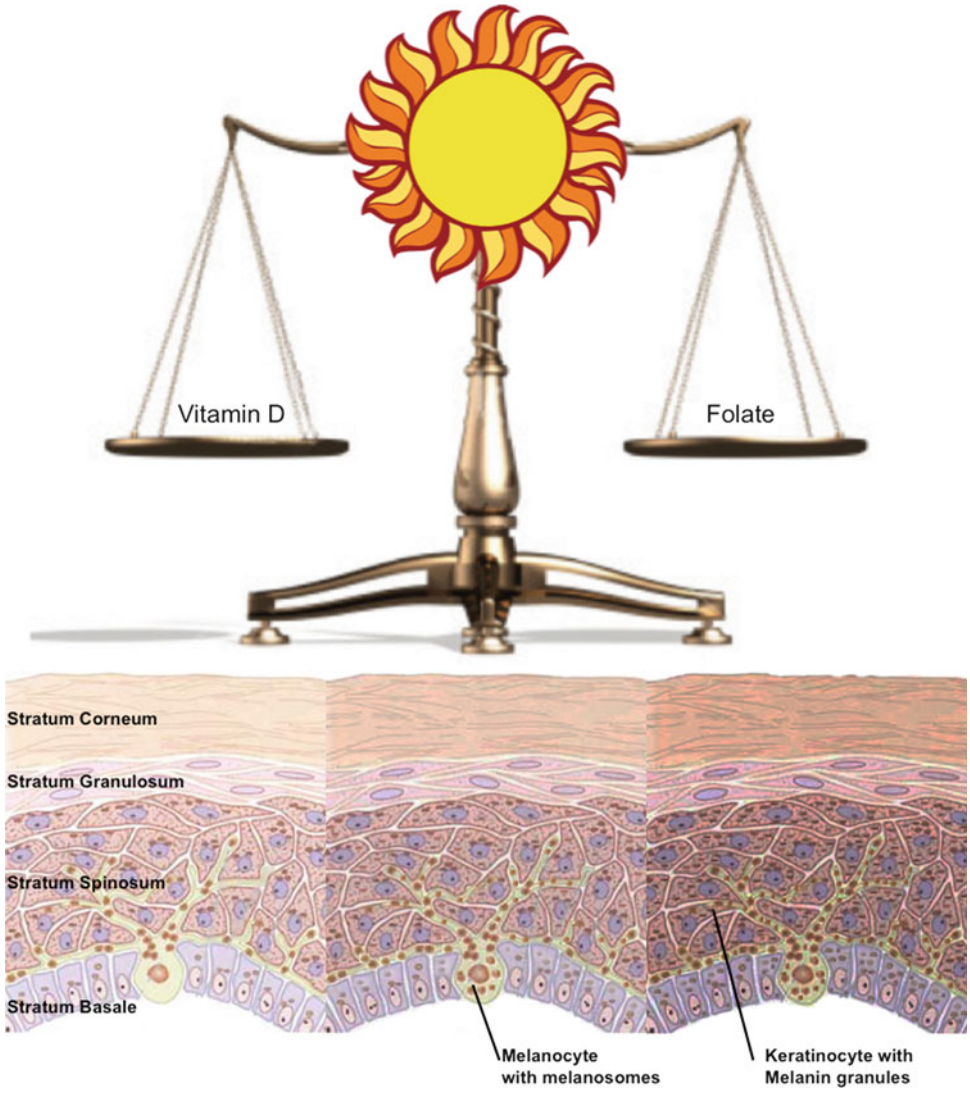


Fig. 22.7 The micronutrient hypothesis. Skin pigmentation serves to tune the balance of micronutrients that interact with solar UVR, with highly melanized dark skin serving to protect folate against photodegradation in climates of high UVR exposure and lightly melanized pale skin facilitating the production of vitamin D in areas of low UVR

absorbed by atmospheric oxygen (O_2) and ozone (O_3) and are thus not considered biologically relevant. Wavelengths in the UV-B range (280–315 nm) are only partially absorbed by O_3 and thus reach the Earth's surface at a biologically significant level, while UV-A wavelengths (315–400 nm) are only weakly absorbed by O_3 and are most easily transmitted through the atmosphere.

High energy UV-B is able to penetrate to the basal layer of the epidermis where it is powerful enough to cause direct DNA damage and is responsible for both sunburn and inflammation effects associated with solar skin damage. UV-A is lower energy than UV-B but is able to penetrate through the epidermis and deeply into the dermal layer. UV-A exposure is observed to induce skin damage and pigmentation darkening without the signs of sunburn. It is now appreciated that UV-A participates in skin photodamage and carcinogenesis through the photosensitization of endogenous chromophores in

the skin that creates ROS and RCS capable of inducing the oxidative damage of numerous cellular targets including DNA [108].

Chronic DNA damage results in progressive losses of genomic integrity and end stage skin damage. Keratinocytes with altered growth properties such as unresponsiveness to terminal differentiation signals can cause epidermal hyperplasia that progress to actinic keratosis [102, 109]. A small portion of actinic keratosis lesions progresses to in situ squamous cell carcinomas [110, 111]. Subsequent cellular changes occur, including induction of matrix proteases that allows for dermal invasion, which is the point at which the tumor is defined as a SCC.

A second major consequence of DNA damage in skin is the suppression of immune responses that would normally detect and remove damaged cells. While mechanisms of immune suppression extend beyond DNA damage, DNA damage is a major factor. The consequences of genotoxic stress include altered migration and antigen presentation by Langerhans cells as well as stimulation of cytokine release by keratinocytes that likely alters the signaling required for normal cancer suppression by immune surveillance [112].

The persistent incidence of skin cancers and their resulting economic cost to society, especially in regions where fair-skinned individuals are exposed to high levels of solar UVR such as Australia and the southern latitudes of America, represent a public health concern that continues to demand attention [113]. In addition, individuals with a history of NMSC have a tenfold increased risk for developing a second primary NMSC and twofold increased risk of developing another type of cancer [114, 115]. It is well accepted that molecular alterations due to UVR exposure is the primary pathway for carcinogenesis in the skin [116]. While limiting sun exposure and the use of protective covering and topical sunscreens have shown efficacy as primary prevention strategies, their overall effectiveness is limited by the fact that sunlight is an integral factor to our existence on this planet. Complete avoidance of UVR exposure is unrealistic and ultimately detrimental to our health. This reality coupled to the fact that the resulting damage of UVR exposure appears to be cumulative over the lifetime of an individual underscores the need to continue the advancement in our understanding of the physiological links between human health and our environment.

Skin Pigmentation, Sunlight, and Vitamin D

While it is established that exposure to sunlight is the main risk factor for the development of skin cancer, the exposure of skin to UVR is essential for the synthesis of vitamin D. Time of day, season of the year, latitude, altitude, and age are all factors that influence the ability of an individual to synthesize vitamin D; however, vitamin D levels are most strongly affected by the number of UV-B photons reaching the deep layers of the epidermis [117]. Individuals with very deep constitutive pigmentation often require 10 to 20 times longer exposure to sunlight than those of lighter pigmentation in order to promote an adequate synthesis of vitamin D [97]. The aspects of cutaneous vitamin D synthesis, nutrient levels, and skin health are expertly presented within other sections of this text; however, the UV-B facilitated reactions and epidermal localization are depicted for reference in Fig. 22.8.

Skin Pigmentation, Sunlight, and Folate

Folate metabolism is complex, with up to 150 folate vitamers existing within the cell at any one time. This complexity arises from variation in the oxidation status of the pteridine ring, type and position of one-carbon adducts, and number of conjugated glutamic acid residues [118]. In addition to the molecular complexity, folate nutritional complexity is even greater as each molecular variation confers

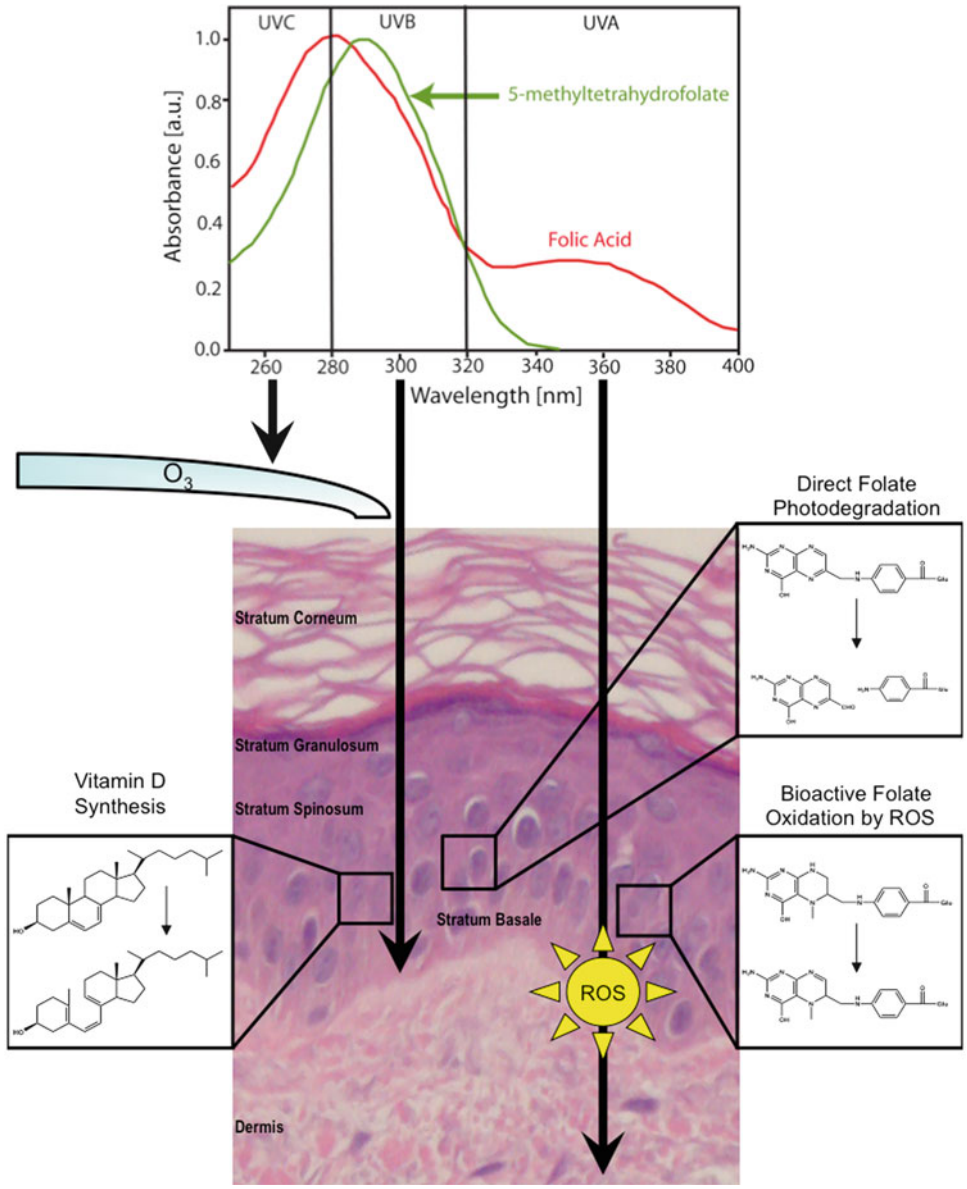


Fig. 22.8 UVR and micronutrients in human skin. UV-B is essential to the production of vitamin D in the stratum basale and spinosum layers of the epidermis and is critical to human health. However, individual folate species absorb UVR of different wavelengths within the solar spectra. Photodegradation of folates within skin occurs by oxidation of reduced folates to their biologically inactive dihydro forms, through cleavage of folates to the breakdown products 6-formyltetrahydropterin and *p*-aminobenzoylglutamate, and is accelerated in the presence of endogenous or UVR-induced ROS

upon the molecule a unique chemical stability and propensity for interconversion. Additionally, folates are present at very low concentrations in biological tissues. Taken together, the folate metabolic cycle appears particularly vulnerable to perturbation by external forces.

Folates are photosensitive compounds. Folic acid has absorption peaks at 280 and 350 nm, with the 350 nm absorption peak indicating absorbance of UV-A radiation which is very prevalent at the

Earth's surface, while 5-methyl-H_4folate has an absorption maximum at 290 nm within the UV-B range [119, 120]. Absorbance spectra for folic acid and 5-methyl-H_4folate are depicted in Fig. 22.8.

Exposure of folic acid solutions to UV-A radiation results in cleavage of folic acid to the primary breakdown products *p*-aminiobenzoyl-L-glutamic acid and 6-formylpterin [119]. Exposure of 5-methyl-H_4folate to UV-B results first in oxidation to 5-methyl-H_2folate followed by irreversible loss of the vitamin through C^9-N^{10} bond scission [119–121]. Degradation of intracellular folates can occur by oxidation of H_4folates to their enzymatic inactive H_2folate forms. The oxidized folates are much more sensitive to cleavage and both oxidation and subsequent cleavage is dramatically enhanced in the presence of photosensitizers [122]. In addition to degradation stimulated by exposure to UVR, folate has been shown to be degraded in the presence of endogenous cellular ROS, which are more abundant in proliferating cells [123].

The photosensitive nature of folate degradation implicates skin tissue as a particularly likely location for folate deficiencies. First, skin tissue, unlike other tissues of the body, is exposed to UVR wavelengths capable of direct folate photodegradation. Second, photosensitizers capable of producing ROS and RCS upon stimulation by deeper penetrating, longer UVR wavelengths exposure are abundant in skin tissue, dramatically increasing the likelihood of oxidative folate degradation [106, 108]. Finally, skin tissue is dependent upon basal cell proliferation for function and thus maintains an elevated demand for folate nutrients in addition to producing increased levels of endogenous folate-oxidizing ROS. An overview of the interactions between folates and UVR in the skin is depicted in Fig. 22.8.

Where the UVR absorbing properties of melanin are inhibitive to vitamin D synthesis, melanin protects intracellular folate. In mammals, melanins (eumelanin and pheomelanin) are produced in highly organized elliptic membrane bound cytoplasmic organelles called melanosomes which are then transferred to keratinocytes [124]. UVR exposure strongly influences the concentration, depth, and distribution of skin melanin. The tanning response largely results from redistribution of melanosomes throughout the epidermis from the basal layer melanocytes. Recent results demonstrate that folate levels regulate melanin production through the synthesis of guanosine-5′-triphosphate, a necessary cofactor in the synthesis of the tyrosinase inhibitor tetrahydrobiopterin [125, 126].

Evidence for Folate Photodegradation In Vivo

To date, in vivo studies on folate and UVR photolysis have focused exclusively on the role of UVR exposure in the degradation of folates in the blood with the endpoint consequence being adverse health effects associated with overall folate deficiencies. Within these studies, the results have been less than consistent. Including the original Branda and Eaton study, there are a small number of studies that reported finding significant decreases in serum folate levels in subjects following exposure to UVR [90, 127–129]. Significant decreases in serum folate levels were seen in these studies despite the fact that different sources of UVR were examined. These sources included narrowband UV-B (as used in the treatment of psoriasis), broadband UV-A lamps, and UV-A as received from timed solar exposure. The majority of the individuals within these studies presented with a dermatological condition, either vitiligo or psoriasis. In contrast to these studies, there are an equal number of studies in which a significant physiologic UVR exposure-induced photolytic effect on folate levels could not be supported [130–133].

The presented studies all have numerous limitations making extrapolations of the data to consistent physiological effects within the general population nearly impossible. The principle critiques are that these studies examine only a small number of subjects, the majority of the subjects studied present with various dermatological conditions which may contribute as a confounding factor, and these studies utilized different UVR sources where the UVR dose varied by experimental design. As presented previously, the photochemistry of the different folates varies depending upon the oxidation and

substitution pattern of the molecule; thus, alterations in the quality, dose, and intensity of the UVR exposure would be expected to determine the magnitude of the photolytic effect observed. Folate measurements were obtained utilizing either automated immunoassay or microbioassay techniques, neither of which is capable of discriminating between the different folate species. Thus, these experiments were not capable of detecting more subtle phenomena such as inactivating UVR-induced folate oxidation or shifts between concentrations of individual folate species, which may have significant physiological consequences in the absence of total folate degradation. It is of particular interest that in each of these studies, skin is considered as a passive barrier between the UVR source and the folate pools of the blood, despite the fact that the vast majority of UVR does not readily penetrate through the skin making localized folate deficiencies of the skin tissue itself a more likely consequence of UVR exposure.

Regardless, even with the limitations of the published studies there are a number of potentially interesting findings that may, in a cautious way, be examined to elucidate the possible links between UVR exposure, folate, and skin health. Juzeniene et al. confirmed the findings of other studies that psoriasis patients have lower blood folate levels than their normal healthy counterparts [62, 63, 131]. These consistent findings may indicate that enhanced folate loss is inherent to psoriasis pathobiology, which may in turn explain the lower folate blood levels observed in psoriasis patients after UVR exposure as they are predisposed to folate deficiency by the nature of their dermatological condition. If indeed psoriasis-associated decreased folate blood levels result from an increased utilization of folate by the epidermal keratinocytes, resulting from accelerated turnover and a concurrent nutrient loss from an increase in desquamated squames as has been suggested, then the constant turnover of normal skin must be considered a major factor in determining an individual's folate nutrient needs [60, 61]. Shaheen et al. observed that serum folate levels were significantly decreased in subjects with vitiligo after narrowband UV-B exposure [127]. The observation of UVR-induced decreases in blood folates in this population as opposed to other populations receiving similar therapies may indicate the importance of skin pigmentation in protecting folate levels. Perhaps most interesting and pertinent in this era of folic acid fortification and supplementation are the results of Fukuwatari et al. in which significant decreases in serum folate levels were observed after exposure to solar UVR in subjects deriving a major portion of their folate nutrient intake from folic acid supplements [128]. These results are consistent with the photochemistry of folic acid, which is the folate species most sensitive to direct photodegradation by the deepest penetrating UVR wavelengths.

While the presented results elicit many interesting possibilities with potentially large ramifications upon human health, the findings are far from conclusive and great caution must be used in any extrapolations from such evidence. An overall assessment is that considerable epidemiological work is needed to determine the extent of UVR-induced folate photodegradation and to gain an understanding of the potential physiological significance.

Folate in the Prevention of Skin Cancer

The unique physiology of skin makes folate supplementation a potentially promising strategy for preventing the development of skin cancer [55]. Skin is chronically exposed to UVR, which is an established carcinogen and viewed as the most important risk factor in the development of this cancer. The constant turnover of the epidermis in combination with chronic exposure to folate-degrading UVR may make skin tissue particularly vulnerable to folate depletion. This depletion may be exacerbated by the peripheral nature of the dermal capillary network, which means that the epidermal cells are last in line for micronutrient delivery behind other tissues of the body with high folate nutrient demand.

Folate is proposed as possibly beneficial in the prevention or reversal of numerous aspects of skin carcinogenesis. Optimal folate nutrition may be most beneficial in preventing skin cancer by promoting

the normal functions and the maintenance of healthy skin. The major putative relationship between cancer and folate status relates to the role of folate in supporting DNA replication, repair, and epigenetic control. This role is particularly important within skin, which when functioning properly, is an actively proliferating tissue that depends upon a balance of growth and differentiation to maintain homeostasis. Adequate folate levels allow for the high fidelity synthesis of new DNA, thus decreasing the likelihood of mutations in proliferating basal keratinocytes, which promote the vital processes of skin turnover, barrier formation, and wound healing. In this way, folate nutrition in skin may be considered primary prevention by inhibiting the formation of carcinogenic damage before it occurs. This concept is further supported, as intracellular, bioactive, reduced folate molecules are antioxidants, absorbers of UVR, and regulators of melanin synthesis, all of which serve to protect nuclear DNA from direct or indirect damage. By supporting methylation reactions, optimal folate nutrient levels may also protect against exposure to external carcinogens by promoting methylation-dependent detoxification.

Folate nutrition in skin is also proposed to have secondary cancer prevention activity in modulating or reversing the mechanisms of carcinogenesis when damage does occur. Optimized folate levels promote genomic integrity by facilitating the timely and accurate repair of DNA damage induced by exposure to environmental carcinogens or UVR, as well as DNA damage resulting from ROS and RCS. Folate may also promote genomic integrity through its role in the generation of methyl groups needed for regulation of gene expression via CpG methylation patterns. Optimized folate levels may aid in the prevention of alterations in the epigenetic regulation of oncogenes or tumor suppressor genes by facilitating adequate SAM production for the maintenance of DNA methylation.

Finally, optimal folate nutritional status may have a tertiary role in cancer prevention by supporting mechanisms constraining to the dissemination of disease once it is established, thus containing cancer to isolated locations with a better prognosis for therapy upon detection. Optimal folate nutritional status is proposed to enhance the cell growth and protein expression regulation critical to the integrity of the epidermal barrier. Studies have shown that cell populations with altered growth properties within actinic keratosis lesions can be recognized by immune surveillance and removed [112]. Alternatively, cell populations within such lesions can progress to carcinoma in situ that secrete proteases and other factors that allow escape from the epidermis. The degree of integrity of the epidermal barrier can be a deciding factor between restraining altered cellular populations from atypical differentiation or proliferation and facilitating immune removal or permitting escape from the epidermal compartment [134]. Optimized folate levels are proposed to promote a high integrity epidermal barrier both preventing carcinogenic transformation and limiting cancer progression. An overview of these interactions is depicted in Fig. 22.9.

Folic Acid Supplementation for Skin Health and Skin Cancer Prevention

Based on the wealth of information relating folate nutrition to normal skin function and possibly skin cancer prevention, it seems only logical to suggest supplementation with folic acid as a means by which to improve overall human dermatological health. Indeed, mandatory fortification of food with folic acid coupled with an increased discretionary use of dietary folic acid supplements has proven quite effective in eliminating folate deficiencies in the United States and other countries employing similar policies. However, it is the very success of folic acid supplementation that presents quite possibly the largest confounding factor within the field of folate nutrition. Folic acid is an unnatural, synthetic form of the vitamin that on its own has no biological activity until it is converted within the body, with the commercial use as a supplement driven purely due to the cost effectiveness conferred by the enhanced chemical stability of this fully oxidized molecule. As the human body is able to readily take up folic acid and reduce it to biologically active H_4folate, the impacts of ever escalating portions of our folate demands being supplied by synthetic folate in ever increasing doses have not yet

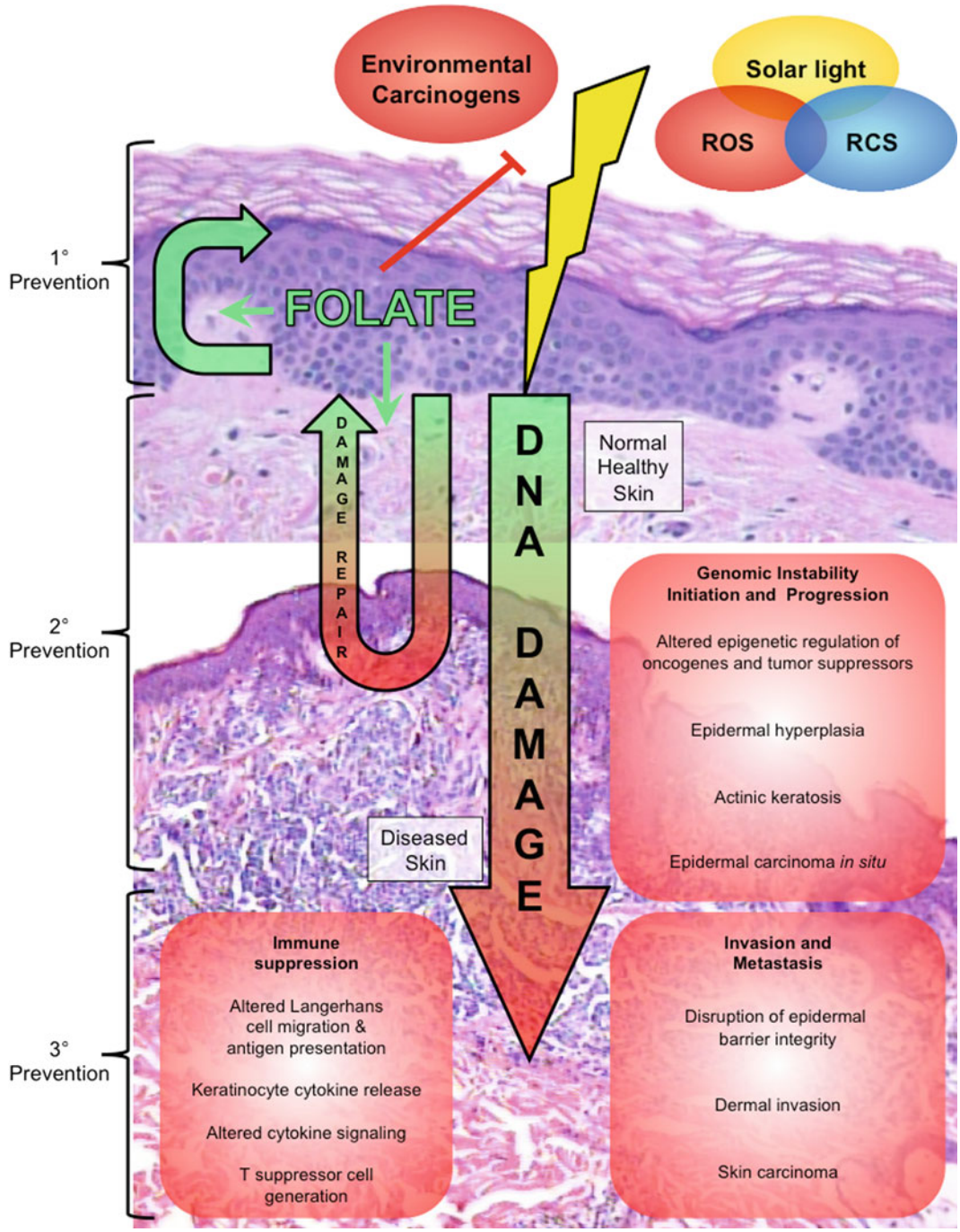


Fig. 22.9 Proposed roles for optimum folate nutrition in the maintenance of skin health and the prevention of skin cancer

been felt and may not be felt for several decades to come. For the first time detectable concentrations of un-metabolized folic acid are appearing in the bloodstreams of individuals with unknown consequences. Worrying hints of a potential storm on the horizon are now appearing linking supplementation with folic acid to increased incidence of some common cancers, metabolic and immune

deficiencies, as well as epimutations [78, 118]. At this point, prudent recommendations would be that the demands of folate nutrients be met as much as possible by the ingestion of foods rich in the naturally occurring folates to which the metabolism of our bodies are attuned. However, current dietary standards and practice are often not sufficient to support adequate folate nutrient levels causing a nutrition gap. This gap may be successfully bridged by folic acid fortification, providing that folic acid remains a supplemental rather than an essential component.

Perhaps most pertinent to skin, it is unclear how oral nutrient supplementation strategies will impact a tissue proposed to bear the brunt of the consequences of nutrient degradation mediated by external influences. Folic acid has unique chemical properties within the folate family, particularly in its sensitivity to photodegradation, and the consequences of increasing concentrations of folic acid in the skin are unknown. This has the potential for a huge impact particularly where topical strategies for modulating folate nutrition in skin are concerned. Unfortunately, the modulation of nutrients is an area where commercialism tends to outweigh scientific caution as evidence by the inclusion of folic acid in topical products despite the fact that the long-term effects of topical supplementation in the skin have not been determined. Potential concerns are evidenced by data from Hirakawa et al. demonstrating that the breakdown products generated via photolysis of folic acid participate in poly-G-specific DNA oxidation through photoinduced electron transfer [135]. Indicating that increased concentrations of folic acid in skin, which may be easily photodegraded, may participate in solar-induced DNA damage and thus may increase the risk of skin cancer, it is even plausible that folate photodegradation by either oxidation or molecular scission may be vital to more subtle aspects of biology such as circadian effects linking our physiology to the environment through the interface of our skin, which may be obscured if not obliterated by superfluous supplementation [118]. These unresolved questions and concerns seem to invoke a recurring theme in biology, that even though the molecular nature of folate and many of the biochemical mechanisms have been known for decades, there is still much to learn before strategies of modulation may be safely employed, particularly in the field of dermatology.

Summary Points

- Folate is vital in promoting human health and preventing diseases
- Folate nutrient levels support many biochemical processes proposed to have a particularly important impact on the maintenance and function of healthy skin
- Folate deficiency is linked to numerous common dermatological conditions
- Ultraviolet light radiation from sunlight, the major factor of skin cancer development, is capable of degrading folate nutrients, resulting in folate deficiencies in human skin
- Folate supplementation has been suggested as a promising strategy for the prevention of skin cancer; however, many questions regarding folate nutrition within human skin must be answered before strategies to modulate folate nutrition may be rationally designed and safely implemented

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Chapter 23
Vitamin D and Skin Cancer: Meet Sunshine Halfway

Tirang R. Neyestani

Key Points

- Vitamin D is either formed in the skin by solar UVR or is ingested with natural dietary sources (fish oils, certain fishes, egg yolk, and mushrooms), fortified foods or supplements.
- Apart from calcemic functions, vitamin D has many non-calcemic functions including antiproliferative and anticancer effects. The protective effects of vitamin D against many cancers, CVD, autoimmune disorders, diabetes, and infections have been shown.
- Though episodic intense sun exposure, especially during childhood, has an etiological association with skin cancers, notably malignant melanoma, chronic exposures seem to be protective. This effect is thought to be due to endogenous vitamin D.
- Avoidance of sun exposure may create greater risk of vitamin D deficiency-related morbidities including bone disturbances, various kinds of cancers, CVD, diabetes, autoimmune disorders, and infections.
- Exposure to sunlight of 1/4–1 MED/day can provide about 1,000–10,000 IU vitamin D to the body. Food fortification and supplementation are alternative measures to improve vitamin D status.

Keywords Vitamin D • Vitamin D endocrine system • Skin cancer • Ultraviolet radiation • Sun exposure

Introduction

The possible role of sunlight in non-skin cancer prevention emerged in 1936, when it was reported that the US Navy personnel with skin cancer had a much lower incidence of non-skin cancers [1]. Soon after, the inspection of the geographical distribution of colon cancer deaths in the United States showed that the related mortalities were highest in places where in people had the least amount of direct sun exposure (mostly big cities and rural areas with high latitudes) [2]. Later, observational

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studies added more evidence for the possible role of vitamin D in different cancers. In a cohort study on 1954 men who were followed up for 19 years for colorectal cancer, an inverse correlation between colorectal cancer risk and dietary intake of calcium and vitamin D was observed [3]. Supportive to the notion of anticancer effect of vitamin D, in a study on 176 known cases of melanoma from the US Navy personnel during 1974–1984, a protective role for short, regular exposure to sunlight was observed [4].

Skin cancers, with over one million new cases diagnosed annually in the United States and a high mortality, are virtually common [5]. Melanoma alone is the fifth prevalent cancer in males and seventh one in females in that country [6]. The contradictory role of sunshine in non-skin cancer prevention and, at the same time, induction of skin cancers, especially malignant melanoma [7, 8], is intriguing. In this article, the latest evidence on the possible association among sun exposure, vitamin D, and skin cancer are discussed. Finally, some possible measures to improve vitamin D status while protecting from harmful solar radiation are proposed.

Vitamin D

Vitamin D has a unique place among all vitamins in that it has both dietary and non-dietary sources. With a very limited natural food sources for vitamin D (mostly some fatty fishes and, in a much lesser amount, egg yolk, and certain mushrooms), man has had to rely almost solely on endogenous vitamin D to meet his needs. However, with development of urbanization which has been accompanied by a dramatic change in type of clothing and times expended for outdoor activities, endogenous synthesis of vitamin D has become inefficient in even some sunny areas [9]. Fortification of foods with vitamin D is, therefore, implemented as a preventive measure against vitamin D deficiency in many countries.

Dietary vitamin D may have either plant or animal origin (ergocalciferol [D2] or cholecalciferol [D3], respectively). Human body is equipped with an intricate system to make vitamin D3.

Biosynthesis

In the skin, vitamin D is synthesized by photoisomerization of 7-dehydrocholesterol under the influence of ultraviolet radiation (UVR) (Fig. 23.1). This chemical conversion depends on many factors such as latitude, season, clothing, using sunscreen, and even degree of air pollution [10]. Vitamin D formed in the skin must undergo two hydroxylation reactions to form the physiologically active molecule 1,25(OH)2D. The first hydroxylation step occurs in the liver by cytochrome P450 vitamin D hydroxylases, including CYP2R1, CYP2D11, and CYP2D25, at C25 to form 25-hyroxyvitamin D (25(OH)D), which is the most abundant isoform of the circulating vitamin D. 25(OH)D is transported to the kidney by vitamin D binding protein (DBP). In the kidney, 25(OH)D is taken up via magalin-mediated endocytic internalization. Magalin is a member of low-density lipoprotein (LDL) receptor superfamily [11].

Renal proximal convoluted tubule is the place of second hydroxylation of vitamin D at C1 to form the active from of the vitamin, 1,25(OH)2D. This reaction takes place under the influence of cytochrome P450 mono-oxygenase 25(OH)D 1-α-hydroxylase, which is also found in monocytes and macrophages. Hypercalcemia during malignancies, autoimmune and inflammatory disorders has been ascribed to the monocyte/macrophage 1-α-hydroxylase activation [12]. Circulating concentrations of 1,25(OH)2D is precisely regulated by homeostatic mechanisms. The main regulators of vitamin D metabolism are calcium, phosphate, parathyroid hormone, 1,25(OH)2D, and a newly identified

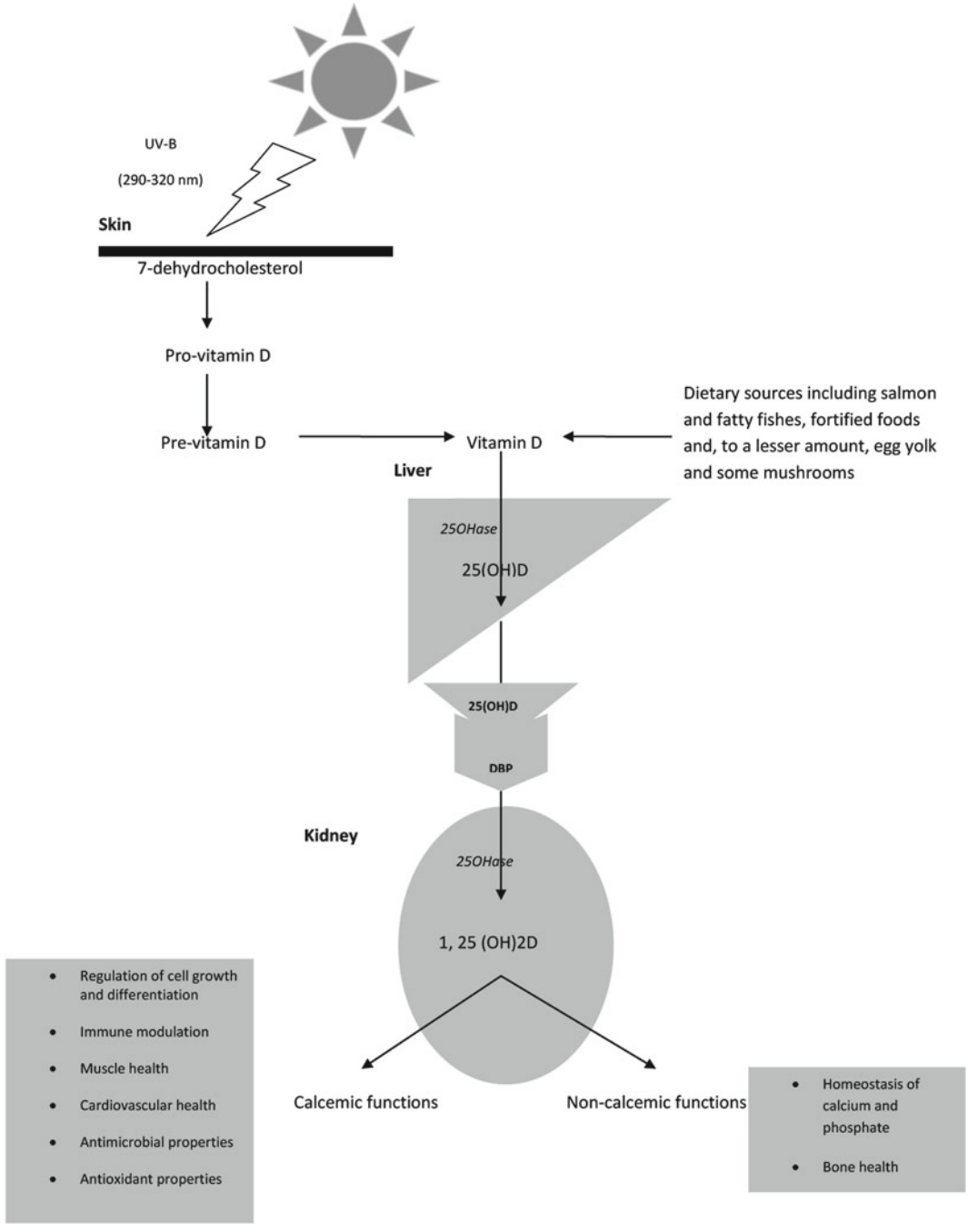

Fig. 23.1 Vitamin D biosynthesis and functions

phosphaturic factor, fibroblast growth factor (FGF)23, which suppresses renal 1-α-hydroxylase and stimulates 24-hydroxylase. FGF23 needs a cofactor for signaling, named klotho, which is a multifunctional protein. Expression of klotho gene is up-regulated by 1,25(OH)2D while in the absence of klotho, 1-α-hydroxylase is induced, suggesting a role for klotho in autoregulatory suppression of 1,25(OH)2D.

Functions

The principle function of vitamin D is to regulate intestinal calcium and phosphorous absorption to maintain bone health. Apart from these "calcemic" effects, vitamin D has also some pivotal "non-calcemic" functions including cell differentiation [13], immune modulation [14], and antimicrobial properties [15]. Of a noticeable importance in cancer prevention, antioxidant properties of vitamin D in the cell membrane have also been documented [16].

Vitamin D deficiency has been associated with many chronic disorders including type 1 diabetes, type 2 diabetes, rheumatoid arthritis, cardiovascular disease, multiple sclerosis, and cancers [17, 18].

It has been estimated that about one billion people worldwide have vitamin D deficiency or insufficiency. Factors affecting vitamin D status are many including variations in effective sun exposure due to latitude, social factors, air pollution and season, as well as age and the amount of body fat mass. Vitamin D status is also affected by some infectious and chronic diseases [10].

Mechanism of Action

As a steroid hormone, vitamin D acts through a nuclear vitamin D receptor (VDR_{nuc}), a 50 kDa protein with high affinity to 1,25(OH)2D ($K_d \approx 0.5$ nmol/L). Nuclear receptors for 1,25(OH)2D, retinoic acid, thyroid hormone and all steroid hormones are members of the steroid hormones gene superfamily [19]. Following binding to 1,25(OH)2D, VDR attaches to retinoid-X receptor (RXR) to form a heterodimer. Reaction of this heterodimer with a vitamin D response element (VDRE) on the promoter region leads to either up- or down-regulation of specific genes.

Genomic actions of vitamin D, though substantiated by several lines of researches, cannot explain the rapid response to 1,25(OH)2D like vitamin D-induced intestinal calcium absorption [20]. It has been suggested that the rapid responses are mediated through membrane vitamin D receptor (VDR_{mem}), a 64.5 kDa protein initially isolated from chick epithelium [21, 22].

Requirement

The initial recommended daily intake for vitamin D was set mostly on the basis of prevention and treatment of clinical rickets [23, 24]. With improvement of understanding of diverse non-calcemic functions of vitamin D, there has been less general agreement on vitamin D requirement [25]. Setting recommended daily intake of vitamin D has been based on optimum concentrations of circulating 25(OH)D. The proposed desirable blood levels of 25(OH)D vary from 50 [26] to 75 nmol/L [27] and above [28]. Based on these cutoffs, the proposed daily intake varies between 7.2 and 41.1 μg/day in 20–40 year people [29] to 17.5–25 μg/day in adults [30] to achieve circulating 25(OH)D of 25 nmol/L to above 80 nmol/L and 75–100 nmol/L, respectively. To attain 25(OH)D of 50 nmol/L and to diminish winter drop of it, Nordic Nutrition Recommendations 2004 increased the recommended intake for the age group of 2–60 years from 5 to 7.5 μg/day [31].

The current recommended daily intake (RDA) of vitamin D is 5 μg for 19–50 years, 10 μg for 51–70 year adults, and 15 μg for over 70 [25]. Assuming the minimum desirable concentration of 25(OH)D as 70 nmol/L and considering that 1 μg/day of vitamin D would raise 25(OH)D about 1 nmol/L, the current recommended intakes would not be efficient to meet the body need [32]. Daily intake of 50 μg has been recommended, especially for ageing population, to keep 25(OH)D levels in the range of 75–100 nmol/L, which is regarded adequate [33].

In dietary recommended values for vitamin D, the endogenous photosynthesis is not regarded. Interestingly, UV lamps can be an efficient source of dermal vitamin D3 synthesis, especially in the subjects with fat malabsorption syndromes [34]. According to Holick's rule, sun exposure to 1/4 of a minimal erythemal dose (MED) over 1/4 of the body is equivalent to oral intake of 1,000 IU of vitamin D3 [35]. Unlike oral vitamin D intake, excessive exposure to sunlight will not cause vitamin D excess, i.e., hypervitaminosis D, as both previtamin D3 and vitamin D3 are photolyzed to the non-calcemic by-products [34].

Vitamin D and Cancer

Despite the apparent causative role of UVR, the major source of vitamin D in the body, in development of skin cancer notably cutaneous malignant melanoma (CMM) [8], epidemiological studies demonstrated a significant inverse association between circulating 25(OH)D and some other cancers like breast and colorectal malignancies. It has been estimated that elevating blood concentrations of 25(OH)D to 100–150 nmol/L would lead to prevention of about 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer annually, and 75 % of the related deaths only in the United States and Canada [36]. About 20 types of cancers have been inversely correlated with solar UV exposure including cervical, esophageal, and rectal malignancies [37]. Notwithstanding, the protective effect of vitamin D against cancer development has been questioned recently [38].

Sunlight UVR causes dermal photosynthesis of vitamin D and also induces DNA damage, inflammation, and immune suppression leading to melanoma [8] (Fig. 23.2). Interestingly, the protective effect of vitamin D compounds against DNA photodamage has been demonstrated in vivo [39]. The net health effect of sun exposure, therefore, is still controversial.

Solar UV-spectrum may be divided in several bands with different biological properties, specifically UV-C (below 280 nm), UV-B (280–315 nm), and UV-A (315–400 nm). It has recently been proposed that increased UVA exposures and inadequately maintained cutaneous levels of vitamin D promote CMM [40].

It should be noted that not all types of sun exposure carry the same risks. Severe sunburns, especially in multiple episodes and during the childhood, are strongly associated with CMM. Episodic intense exposures, compared to chronic exposures without sunburns, convey a greater risk for skin cancers [41].

The anatomic areas mostly exposed to sun may not be preferentially affected. The incidence of CMM is seasonal, with more cases reported in summer than winter [42].

In a study on evaluating the net effect of sun exposure on general health in the UK and Scandinavia, it was observed that despite an increasing trend in all major forms of skin cancer as well as all major internal malignancies from north to south, there was an improving cancer survival also from north to south, indicating the more positive than adverse health effects of direct sun exposure [43]. Supportive to this finding, in a study on 26,916 Dutch skin cancer patients, standardized incidence ratios were calculated. Lower risk of colorectal cancer but higher risk of breast cancer was observed among the patients with cutaneous malignant melanoma (CMM) [44].

Though in a population-based case–control study, an inverse association was found between vitamin D intake and CMM risk [45], in a huge cohort study on 68,611 subjects for over 10 years, vitamin D intake was not associated with CMM risk [46].

Some data shows the protective effect of adequate vitamin D status against skin cancer. For instance, in a nested case–control study on ambulatory elderly men from Osteoporotic Fractures in Men (MrOS), highest quintile of 25(OH)D (>75 nmol/L) was associated with 47 % lower odds of non-melanoma skin cancer (NMSC), compared to the lowest quintile [47]. Instead, personal history of NMSC has been associated with higher risk of developing of other malignancies [48].

Some of the discrepancies observed among different studies on vitamin D and skin cancers may be attributed to the genetic variations of the study populations. In this regard, VDR variants have been

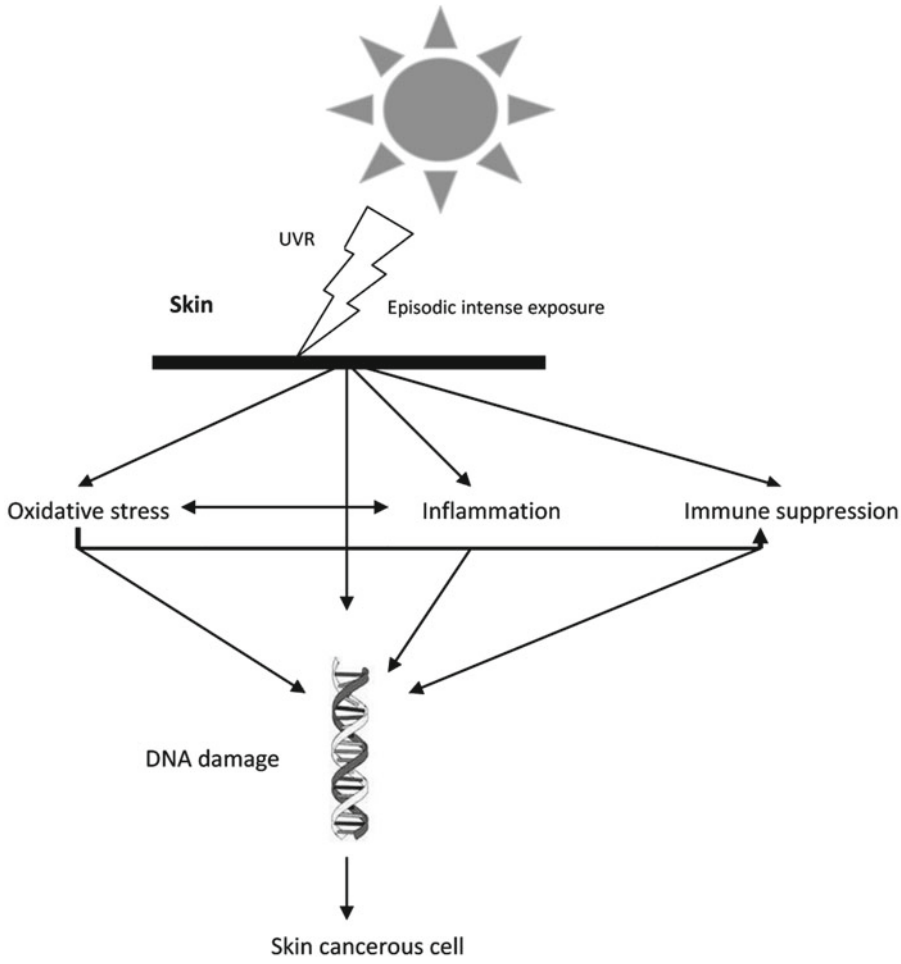

Fig. 23.2 The pathophysiological effect of intense sun exposure on development of skin cancers

the core of some studies. In a nested case–control study within the Nurses' Health Study, for instance, the possible associations among the variants of methyltetrahydrofolate reductase (MTHFR) gene (C677T and A1298C) or VDR gene (Fok1, Bsm1, and Cdx2) and skin cancers in 805 patients (219 melanoma, 286 squamous cell carcinoma [SCC], and 300 basal cell carcinoma [BCC]) and 873 apparently healthy controls were evaluated. Though no significant associations among the two MTHFR polymorphisms and skin cancers were found, a significant link between VDR Bsm1 BB genotype and increased risk for SCC was reported (OR = 1.51; 95 % CI = 1.01–4.50) [49]. The associations among VDR polymorphisms, vitamin D status, and skin cancers merit further researches.

Vitamin D, Insulin Resistance, and Skin Cancer: Is There a Link?

There are many diverse and complex molecules involved in carcinogenesis, one of which is p53 whose genetic alterations have been demonstrated to have an association with cancer development. Mutations of p53 are particularly observed in skin malignancies and it has been shown that UVR induces oncogenic transformation in this molecule [50].

"Cellular senescence," irreversible cell growth arrest, may be induced by many stimuli including oxidative stress. Tumor suppressor genes like p53 normally control this response. Recently, it has been shown that up-regulation of p53 in adipose tissue may lead to inflammatory response and consequent insulin resistance in a murine model of type 2 diabetes (T2D) [51]. Insulin resistance-inducing effect of p53 is mediated through activation of TIGAR, TP53-induced glycolysis and apoptosis regulator [52], which inhibits glycolysis via lowering cellular concentrations of fructose-2,6-bisphosphate [53].

The importance of these findings lies in the newly found link among insulin resistance, T2D, and malignancies. Patients with cancer those who have diabetes compared to the patients without diabetes, have a weaker response to chemotherapy, more complications, and poorer prognosis [54]. Moreover, elevated fasting serum insulin within the normal range has been associated with higher risk of prostate cancer in men [55]. The links between insulin resistance and cancers of colon, liver, pancreas [56], endometrium [57], breast [58], and lung [59] have been reported. The association between insulin resistance and thyroid cancer has also been hypothesized [60].

The possible effect of insulin resistance on skin cancer can be a new argument. The effect of metabolic status on skin has been recognized long ago. A well-known example is acanthosis nigricans (AN), a dermatosis that presents as thickened, hyperpigmented plaques predominantly of the flexures. It can be warty or velvety when advanced. AN may be seen in obese individuals with insulin resistance and in some elder patients with malignancies, especially gastrointestinal tumors [61].

In an in vitro study on expression and signaling of the vitamin D receptor (VDR) and peroxisome proliferator-activated receptor (PPAR) alpha, delta, gamma in the melanoma cell line MeWo, regulatory roles for PPAR and VDR in melanoma cells growth and a functionally relevant cross talk between these nuclear signaling pathways were indicated [62].

The relation between vitamin D status and insulin resistance has been shown in case–control [63] as well as clinical trial [64] studies. The possible relationship between insulin resistance and skin cancers deserves further considerations. A positive association between diabetes and NMSC in women (who usually have poorer vitamin D status than men) has been reported long ago [65]. In a large historical cohort study on subjects with T2D, the occurrence of NMSC in patients receiving exogenous insulin was significantly lower than the control group. The authors attributed this effect of insulin to activation of skin IGF-1 R [66]. In contrast, analyses of data obtained from diabetes cohorts during1963–1998 and 1999–2008 from southern England showed a reduction in risk of NMSC [67]. In none of these studies, however, glycemic control was noted. The ameliorating effect of vitamin D replenishment on insulin resistance and glycemic status has been shown recently [64]. Whether this effect can reduce the risk of skin cancers needs to be clarified with future studies.

Skin Cancer and Vitamin D: Situation in Iran

Both skin cancers and vitamin D deficiency are noticeably prevalent in Iran. In a study on 40,690 known cases of cancer in Tehran, the capital of Iran, skin cancer (comprising 23 % of all cases) was found to be the most prevalent type of malignancy with a male–female ratio of about 2:1 [68].

The occurrence of skin cancer was studied over a 15-year period (1987–2000) in Yazd, a desert area in the central Iran. A total of 1,124 cases of skin cancer were identified, approximately 11 in 100,000. The most common type of skin malignancy was BCC (76.69 %), followed by SCC (18.1 %), CMM (2.7 %), and other types of skin cancers (2.3 %). In 92 % of cases, the affected sites were face, head and neck that are more exposed to the sun. The male–female ratio was 1.6 [69]. It is noteworthy that in Iran, women usually cover their heads and necks (and in some areas even their faces) due to religious beliefs. This may, at least to some extent, explain the higher occurrence of skin cancer in men than in women in Iran.

It is noteworthy that several studies have documented a high prevalence of vitamin D deficiency in children [70] as well as adults [71] living in Tehran. Though there is no published report of the occurrence of vitamin D deficiency in Yazd, some studies have shown the high prevalence of vitamin D deficiency in both children [72] and adults [73] in Isfahan, a big city in central Iran adjacent to Yazd. It should be noted that there is no vitamin D fortification program carrying out in Iran at the moment. The vitamin D status, therefore, can be considered mostly due to direct sun exposure.

Assuming the etiologic relationship between sun exposure and skin malignancies, the prevalence of vitamin D deficiency must logically shows an inverse association with the occurrence rates of skin cancers. This notion is not supported, at least, by aforementioned Iranian data. However, there is no study on skin cancers and their relationship with sun exposure reported from Iran. Moreover, the vitamin D status of the subjects affected by the malignancies was not assessed. Therefore, more information on the possible association among sun exposure, vitamin D, and skin cancers is still needed.

Conclusions

A growing body of evidence suggests a protective role of vitamin D against a wide spectrum of chronic diseases including autoimmune disorders, diabetes, CVD, infectious diseases, and several kinds of cancers [18]. Some in vitro studies have shown the antiproliferative effects of vitamin D against melanoma cells. Poor vitamin D status, therefore, may not only increase chronic disease risk but worsen the prognosis of skin cancers as well [74]. Pandemicity of vitamin D deficiency has made both scientists and stakeholders to seek for a proper intervention. In many countries fortification of foods with vitamin D is performed as a public health policy [75, 76]. However, there are many barriers to the effective food fortification and supplementation [77]. For instance, according to the regulations of the Iranian Ministry of Health, vitamin D is categorized as "group B fortificants," which are potentially harmful in large doses and, therefore, only 10 % of the related RDAs are allowed to be added to the foods. Needless to say that with the current opinion on the desirable concentrations of circulating 25(OH)D, i.e., 75–100 nmol/L, this can hardly be effective to control vitamin D deficiency at the population level. In some countries, despite obligatory fortification of foods, vitamin D intake of the population is not adequate [78, 79]. It seems that man has to still rely, for the most part, on sunshine to meet his vitamin D needs. Deprivation of sun exposure, though may prevent skin cancer, will be at the cost of greater morbidity and mortality from various non-skin cancers, infections, and chronic diseases all resulting from vitamin D deficiency. Chronic sun exposure without severe sunburns equaling 1/4–1 MED/day, instead of episodic intense exposure, seems to provide enough endogenous vitamin D and convey minimum risk of skin cancer, if any (Fig. 23.3). Meanwhile, there is an urgent need for feasible ways for improving vitamin D status at the community level.

To improve vitamin D status, the following measures may be considered:

- Food fortification with vitamin D still seems to be the most feasible way to improve vitamin D status of the community at the moment. Using dietary pattern and consumption survey data, a "food basket of fortified foods" may be defined, instead of fortification of a single food (like milk or butter alone). The inevitable technological problems in food fortification need to be solved with multidisciplinary research programs.
- Especially in the regions with high rates of vitamin D deficiency where in foods are not currently fortified with vitamin D, it is highly recommended to educate the population on both the benefits of chronic moderate sun exposures and the hazards of intense episodic exposures.

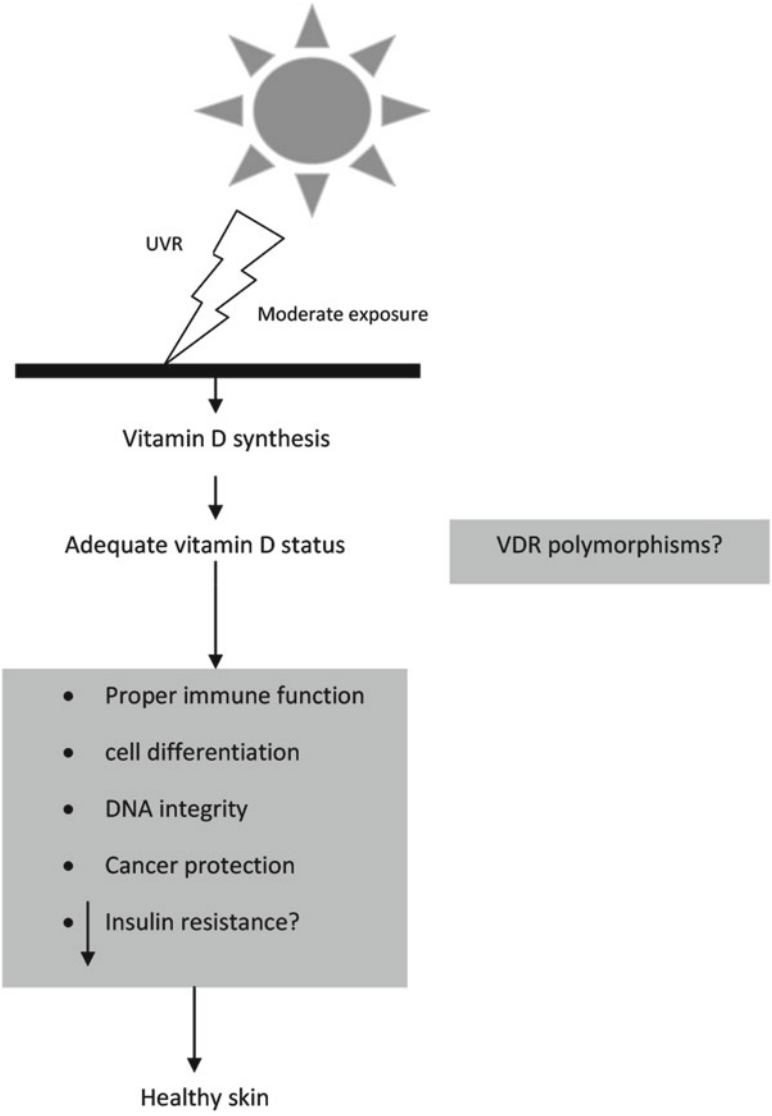

Fig. 23.3 Chronic moderate sun exposure by providing adequate vitamin D can be protective against several kinds of cancers and chronic diseases. In this case, the risk of skin damage would be minimal. The roles of insulin resistance and VDR variants in skin cancers still remained to be clarified

- In some cases, vitamin D supplementation can be employed to protect special groups (like school children) from deficiency, at least during the cold seasons. People who have to work under intense direct solar radiations (like farmers of paddy fields) must be instructed to protect themselves from sunburns using appropriate sunscreens and coverings and, at the same time, to use vitamin D supplements.
- UV lamps can be used as an alternative measure to improve vitamin D status in individual cases.

Summary Points

- Vitamin D is either formed in the skin by solar UVR or is ingested with natural dietary sources (fish oils, certain fishes, egg yolk, and mushrooms), fortified foods or supplements.
- Apart from calcemic functions, vitamin D has many non-calcemic functions including antiproliferative and anticancer effects. The protective effects of vitamin D against many cancers, CVD, autoimmune disorders, diabetes, and infections have been shown.
- Though episodic intense sun exposure, especially during childhood, has an etiological association with skin cancers, notably malignant melanoma, chronic exposures seem to be protective. This effect is thought to be due to endogenous vitamin D.
- Avoidance of sun exposure may create greater risk of vitamin D deficiency-related morbidities including bone disturbances, various kinds of cancers, CVD, diabetes, autoimmune disorders, and infections.
- Exposure to sunlight of 1/4–1 MED/day can provide about 1,000–10,000 IU vitamin D to the body. Food fortification and supplementation are alternative measures to improve vitamin D status.

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Chapter 24
Vitamin E in Skin Cancer and Aging Skin

A. Chloe Meltzer and Ronald Ross Watson

Key Points

- Vitamin E acts as an antioxidant in the body
- Vitamin E has been used in drug trials to see if it can be used to prevent skin cancer and other cancers
- Most anticancer trials use α-tocopherol
- There have been contradicting trials where vitamin E has had effects in preventing cancer
- Many cancer prevention trials that show no effect on vitamin E reducing incidence of skin cancer

Keywords Vitamin E • Tocopherol • Antioxidant • Supplementation • Skin cancer • Skin health • ROS • Free radicals • Cancer prevention

Introduction

There are many different forms of vitamin E. Little is known about β-tocopherol and its role in the cell cycle [1]. γ-Tocopherol is the most common form of vitamin E in American diets [1]. α-Tocopherol is the most commonly used form of vitamin E in drug trials. Vitamin E is an antioxidant that may be useful in dermatology [2]. Due to many experiments that showed skin benefits from its use, vitamin E has become a popular ingredient in skin products [2]. Vitamin E has proven to show some positive effects in improving skin health in many of the recent trials. However, optimum dosages are not well defined since very few trials have been published studying dose–response [2] and more trials focus on supplementation towards preventing cancer. Much research is being done to see how vitamin E supplements have effects on skin health and cancer prevention. Many factors affect skin aging and risk for cancer. Sun exposure, smoking, and genetic factors may increase problems associated with skin health [2]. Vitamin E may improve some of the issues associated with skin health, such as appearance,

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and even reduce the risk for some types of cancer [2]. Vitamin E has many functions that could affect the process of aging, as well as reduce risks for cancer, and improve skin health overall. α-Tocopherol is the focus of this review and is referred to as vitamin E.

Actions of Vitamin E

Vitamin E, as an antioxidant, is necessary for skin health because it prevents reactive oxygen species (ROS) and other free radicals from reacting with dermal tissues and damaging them [2]. Human bodies do not produce enough antioxidants on their own, so we must provide it through nutrition and dietary supplementation [3]. Antioxidants are useful in preventing oxidative damage in our cells, which causes problems biologically [4]. ROS are a group of chemically reactive ions, radicals, and molecules derived from oxygen. Free radicals, which are similar to ROS, are molecules with one or more groups of unpaired electrons, which makes the molecule more reactive [5]. Free radicals have negative consequences since they remove electrons from other molecules [5], creating more free radicals. One example of free radical damage occurs in the lipid membrane of cells. When lipids are damaged by free radicals, they cannot regulate what goes in and out of the cell, allowing toxins into the cell more readily [4]. The spectrum of functions these damaging compounds participate in ranges from hormone biosynthesis to cell signaling and aging to microbial killing [6]. Thus, ROS also contribute to aging skin. And skin must have layers of antioxidants to protect it from these harmful compounds [2]. Vitamin E is one such antioxidant that helps block the formation of ROS [7].

Vitamin E is also useful in regulating cell signals and gene expression [7], the immune system [7], and in helping to repair damaged cells [4]. α-Tocopherol can fulfill the biological requirements [7] set forth by the human body and is stored in the adipose tissues in our bodies [4]. Vitamin E is routinely used in cosmetics to protect the skin [2]. Vitamin E works in the body by stabilizing free radicals, thereby halting the chain reaction of cell-membrane injury [4]. Vitamin E has many actions in the body and can be found naturally in most of our foods, as well as many multivitamins.

Vitamin E and Skin Health

Many cosmetics use antioxidants in order to reduce the appearance of aging skin [8]. Oral supplementations of vitamin E can protect skin from external damage [8]. Sebaceous glands secrete α and γ-tocopherol, which are the outermost defenders against environmental damage [3]. This also relates to the antioxidant properties of vitamin E and its ability to protect the skin from free radicals and UV damage.

Supplementation of vitamin E may help improve some signs of dermal aging, such as dryness and wrinkling by preventing damage caused by environmental factors [2]. However, it has not been proven definitively that vitamin E can actually reduce the signs of aging [3]. The process of reducing free radicals improves aging, which is aided by vitamin E [3]. As well, having oily skin may decrease appearance of aging versus someone with dryer skin because of the overproduction of vitamin E in the sebaceous glands as a by-product of the increase in oil production [3]. Vitamin E is also common in skin care products for its photoprotective factors and moisturizing capabilities [3]. Vitamin E also improves the actions of sunscreen, reducing damage [3]. Vitamin E seems to have some positive effects on skin appearance and skin protection. This is due to its antioxidant and photoprotective properties on the skin, which reduces UV damage, which causes aging.

Animal Skin Health and Trials

As animals offer various study advantages, the role of vitamin E in skin cancer can be more preciously evaluated. There have been many animal tests to see if vitamin E use, topical or oral, can reduce the incidence of various types of cancer, including cancer of the skin. Using a topical form of vitamin E on mice helped reduce the incidence of ultraviolet-based skin cancer in mice [9]. The authors concluded that vitamin E helped protect the skin against UV-induced damage because of antioxidant and UV absorptive properties [9]. Another trial showed that vitamin E improved healing in mice [2], which also shows vitamin E's properties in skin health. The antioxidant properties of vitamin E promote regulation of cell signals, which can help promote the signals between cells that are telling the cells to heal [7].

When vitamin E was inhaled by mice in a skin cancer trial, the increase in antioxidants promoted apoptosis and cell division in the skin cancer tumor cells was also decreased [10]. The same study found that α-tocopherol could be effective in both a late intervention in cancer treatment, as well as a preventative tool in preventing the formation of UV-induced tumors [10]. An early trial performed in the 1990s found that topical application of α-tocopherol in mice before UV exposure reduced the incidence of skin cancer from 81 to 42% [11]. However, when a similar trial was performed later on, vitamin E seemed to increase the change of UV-related cancers [11]. Another trial showed that when tocopheryl succinate was injected into the skin of hairless mice, it stopped melanoma cells from growing and increased survival time [12]. This is because tocopheryl succinate suppresses the expression and secretion of vascular endothelial growth factor in these melanoma cells, which stops them from growing [12]. Another trial showed that oral α-tocopherol increases melanoma cell apoptosis in mice [12].

Vitamin E and Cancer

Free radical formation is linked to cancer [4]. Vitamin E helps prevent cell destruction by partially reducing the creation of free radicals [4]. Like other antioxidants, vitamin E also helps regulate the immune system, which allows it to better control foreign pathogens and cancerous cells [4]. Free radicals damage DNA, facilitating development of cancerous cells [4]. DNA damage, the cell's response to DNA damage, and cancer are related [9]. Vitamin E, as an antioxidant, can help prevent the cell damage that may cause cancer. There is clear correlation between vitamin E intake in the form of supplementation and decreased incidence of cancer, such as prostate cancer and colon cancer [1]. Are vitamin E supplements useful in preventing skin cancer?

Vitamin E Supplementation in Humans: Role in Skin Cancer Prevention

Some vitamin E supplementation trials have shown some benefit, while others have shown adverse reactions to the supplements, like increased overall mortality [13]. Vitamin E slows the progression of melanoma by promoting apoptosis in tumor cells [2]. The Preventative Services Task Force of the US Agency for Healthcare Research and Quality found that there was insufficient evidence to recommend either for or against the use of vitamin E supplements for the prevention of all cancers [4]. Several studies have found that vitamin E helps in prevention of some cancers, such as prostate and colon cancers, and other studies have shown that the supplement did not have any actions in comparison to the placebo [4]. Skin thickness, skin color, and other unpreventable factors are confounding factors [8]. According to Thompson and Manore, dietary supplements cannot be recommended for cancer prevention in the general population [4]. However, genetics, environmental factors, and behavioral factors must also be

taken into account when looking at cause and prevention of cancer. In some tests in the topical application of α-tocopherol, it inhibits the formation of some of the chemicals that cause cell damage from UV radiation, which leads to skin cancer [9]. Thus, vitamin E could play a role in skin cancer prevention.

α-Tocopherol is depleted by UVB radiation [9]. As vitamin E is a fat-soluble molecule, it absorbs easily into the skin [9] to easily infiltrate the skin and increase its efficacy. However, vitamin E did not have an effect in reducing exposure to UVR, which is ultraviolet radiation [8]. One case–control test performed in Washington in the mid 1990s showed an inverse relationship between vitamin E and melanoma incidence [12, 14]. Another earlier trial also showed that there was a decreasing risk of melanoma as food supplementation of vitamin E increased [14, 15]. Neither the Washington trial nor the earlier trial showed any association between supplementation, food, and melanoma risk [14, 15]. Although another study showed that high doses of vitamin E, of more than 400 IU, had an association with all-cause mortality [12, 16]. This could also be associated with the idea that too much of any substance will have adverse effects on the body.

The potential of supplementation of vitamin E to prevent skin cancer are taken from studies on other types of cancer, such as studies on prostate cancer. In prostate cancer, the use of vitamin E as a preventative aid showed no definitive results [17]. In the α-tocopherol, β-carotene Cancer Prevention (ATBC) Study, subjects were given supplements to see if the effects on mortality from cancers of the upper digestive system [1]. This test found that there was no reduction in incidence of cancers of the upper digestive system when vitamin E supplementation was in use [1]. However, there was a 32% decrease in incidence of prostate cancer in men who were taking vitamin E supplements [1]. Some further studies also found that vitamin E may slow progression of prostate cancer [1]. Other tests showed that vitamin E supplementation could help reduce risk from smoking, and that it was useful in protecting against rectal and colon cancer [1]. However, others saw an increase in overall mortality with vitamin E when compared to other antioxidants [18]. Another study showed that as the number of years of vitamin E supplementation increased, there was a decrease in the risk of total overall mortality [13].

A recent test performed found that oral daily supplements with combinations of daily supplements of antioxidants with 120 mg of vitamin C, 30 mg of vitamin E, 6 mg of beta-carotene, 100 μg of selenium, and 20 mg of zinc for a median of 7.5 years actually increased the incidence of melanoma in women [18, 19]. This may mean that regular intake of these substances may be associated with harmful effects [18], especially since there may be differences in nutrient metabolism between genders [19]. There may also be differences in how different genders metabolize antioxidants in the skin, which could also explain why women had higher incidences of skin cancer with antioxidants [19]. Another trial looked at approximately 38,000 men and 40,000 women in Washington to examine these findings of the trial previously described [18]. Participants were asked to answer a questionnaire about diet, lifestyle, health history, diet, supplement use, etc. [18]. The trial used the same supplements and dosages, meant to mimic most multivitamins, from the previous trial and looked at use for the past 10 years [18, 19]. Approximately two-thirds of the users in the trial were using multivitamins, which allowed the people performing the trial to isolate for supplement use [18]. This trial showed that vitamin E had no increased risk of melanoma, even with continuous follow-ups [18]. Another trial performed by the Nurses' Health Study also showed no correlation between antioxidant supplementation and melanoma risk [18, 20], although other tests did not show any reduction in UV-induced oxidative stress with oral supplementation of vitamin E [12, 21].

Conclusion

While there are many trials that show vitamin E has a positive effect on preventing skin cancer, as well as other forms of cancer, human trials still have not been able to show a consistent relationship between vitamin E and reduction of melanoma [12]. However, animal trials seemed to have shown

some sort of benefit in protecting the skin and in slowing down tumor growth. Because of all of the mixed data on human trials, dietary vitamin E may not actually give an adequate benefit [12].

There have also been some trials that show an increased mortality in users who take vitamin E over time [22]. As well, the suggested dose of vitamin E given by the FDA may not actually correspond to what may be the most beneficial dose since there have not been enough trials to test dosages [2]. However, one trial did show that dosages of vitamin E of 400 IU showed adverse health reactions [12]. This may have also been because multivitamins like Centrum have 30 IU of vitamin E [23], which is more than ten times the supposed recommended daily dose, according to what is considered the recommended daily intake [7]. There is not enough data to support use of vitamin E in skin disorders [2]. However, some tests using oral vitamin E supplements saw some improvement in appearance of skin disorders [2].

With all of the information given, it is still inconclusive whether or not vitamin E is useful in preventing cancer. However, more tests should be performed to see if conclusive answers could be given. Vitamin E does seem to be beneficial in promoting and protecting skin health.

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Part VI
Dietary Components and Skin Cancer

Chapter 25
Dietary Plant Extracts and Foods in Prevention and Skin Cancer: An Overview

Chelsea Lynn Carey and Ronald Ross Watson

Key Points

- Dietary supplements are defined as products that are intended for ingestion to supplement the diet, not as conventional foods or sole item of a meal
- Consuming fruits, vegetables, and/or dietary supplements provide the body with antioxidant protection
- Polyphenolic compounds provide anti-inflammatory and anticarcinogenic properties
- Administration of extracts to mice test subjects resulted in protection against UV-skin carcinogenesis by reducing tumor incidence, tumor multiplicity, and tumor size

Keywords Skin cancer • Polyphenols • Antioxidants • Grape seed proanthocyanidins • Resveratrol • Green tea polyphenols • Silymarin/silibinin • D-Limonene • Lycopene

Perceptions on Skin Cancer

The skin is the largest sensory organ of the body. This first layer of defense acts as a barrier against various pathogens, regulates body temperature, and is relatively impermeable to water [1]. Due to increased UV exposure from sunlight, the addictive use of tanning beds, inadequate diets, and skin health ignorance among young and old, the incidences of skin cancer have increased and it is currently one of the most common types of cancers in both the male and female populations [2]. As a result, cancer prevention and skin health has become a topic of great discussion among health care providers.

Skin cancer is generally divided into two categories: melanoma and non-melanoma. Non-melanoma skin cancer includes basal cell carcinoma and squamous cell carcinoma [3]. Basal cell carcinoma is

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the most common form of skin cancer which originates in the keratinocyte cells in the epidermis, and rarely spreads to other tissues. Squamous cell carcinoma also originates from keratinocytes in the epidermis, but appear more scaly and red relative to the smoother, lighter surface of basal cell carcinomas. Although rare, squamous cells have the ability to invade other tissues of the body. Both types are often present in areas of skin frequently exposed to sunlight, such as the head, neck, or shoulders. Although melanoma is not as common as the former, it is the most lethal form of skin cancer. Melanomas also reside in the epidermis, but originate from the melanocyte cells. Its lethality is derived from its ability to metastasize and spread cancerous cells throughout the body [3]. Melanomas are identified by strange changes in shades and color, abnormal borders, asymmetrical shape, or noticeable growth or enlargement. It is essential to have melanoma detected and treated as fast as possible [2].

Perceptions on Dietary Supplements

Chemotherapy or surgery are common treatments in the removal of cancerous lesions; however, fruits, vegetables, and dietary supplements exemplify a precautionary tactic to help decrease the risk of future occurrences, or use as primary prevention. Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), dietary supplements are defined as products that are intended for ingestion to supplement the diet, contain dietary ingredients such as vitamins, minerals, herbs, amino acids, metabolites, enzymes, or extracts, and can be distributed in various forms of capsules, liquids, powders, or nutritional bars [4]. They are not advised to be used as a conventional food or as sole item of a meal or diet. Dietary supplements are not required to be registered through the FDA; however, labels must be accurate and must not make specific claims of health improvement [5]. In using plant extracts in supplements, as well as preparing meals with fresh fruits and vegetables, the body is provided with antioxidant protection against free radicals found in the environment.

In this chapter, the general molecular mechanisms and preventative properties of dietary extracts are discussed as an overview. A variety of common extracts are briefly reviewed to demonstrate their preventative attributes against solar damage and skin cancer in animal or human models.

Grape Seed Extracts (*Vitis vinifera*)

For centuries, grapes have been one of the most widely consumed fruits due to their distinguished taste and great benefits to health. The grape seeds are removed as byproducts during the production of wine and grape juice, and are then utilized as extracts [6]. Grape seeds are comprised of compounds such as vitamin E, flavonoids, linoleic acid, and oligomeric proanthocyanidin complexes, more commonly referred to as grape seed proanthocyanidins (GSPs) [7]. Proanthocyanidins are classified as compounds with polyphenolic structure and serve as potent antioxidants [8], and aid in the removal of free radicals from the body [6]. Grape seeds are rich in proanthocyanidins, and are known to provide anti-angiogenic, anti-inflammatory, and anticarcinogenic properties [9]. In aiding skin photoprotection, these polyphenols target molecular mechanisms such as inflammation, antioxidant defense enzymes, and inhibition of various cellular proteins [10].

Various studies on the preventative effects of GSPs have been performed on mouse subjects. In the cases examining UV-induced photocarcinogenesis, dietary feeding of GSPs to SKH-1 hairless mice revealed strong inhibitory effects on promotion/progression stages of tumors [9]. The GSPs inhibited UV-induced malignant conversion of papillomas to carcinomas and reduced tumor incidence, tumor multiplicity and tumor size in the mice. Topical treatments of GSP's had similar results [9]. On a more

molecular level, studies conducted using a culture of human epidermoid carcinoma A431 cells have found that in vitro treatment of GSPs inhibit cell proliferation and activate various cascades that lead to the induction of apoptosis, or cell death [9]. These in vitro and in vivo observations provide valid statements of the photoprotecitve and anti-photocarcinogenic benefits of grape seed proanthocyanidins; however, further studies should be conducted on the benefits of dietary grape seed extracts and human skin [6].

Grape Skin

Like grape seed extracts, the skin of the grape also has compounds that are beneficial to health. Vitamin E, flavonoids, linoleic acid, and proanthocyanidins are found in lesser amounts in the skin compared to the grape seeds, but the polyphenol primarily examined is resveratrol [7]. Resveratrol is a phytoalexin, an antimicrobial substance synthesized by the respective plant in response to a pathogen [1], and has been found in more than 70 different plant species, such as grapes, peanuts, fruits, red wine, or mulberries [6]. Fresh grape skin in particular are abundant in resveratrol and affect selective processes in tumor initiation, promotion, and progression, as well as suppress supplementary angiogenesis or metastasis [1]. In regard to the chemopreventive actions, resveratrol works as an antioxidant, anti-inflammatory, and anti-mutagen [6].

A number of in vitro and in vivo studies have been conducted to observe the effects of resveratrol in relation to skin cancer. In vitro studies were selected to observe the anti-proliferative and photoprotective effects of resveratrol in cell cultures. Studies found that resveratrol induced cell cycle arrest and apoptosis in human epidermoid carcinoma (A431) cells, as well as apoptosis in human melanoma cell lines [11]. It is also suggested that resveratrol fights oxidative stress by decreasing intracellular reactive oxygen species in melanoma cells, as well as bind to specific polyphenol receptor sites on the skin, providing a protective effect against certain free radicals [11]. The in vivo mice studies observed both the topical and oral consequences of resveratrol. Topical treatments resulted in effects such as inhibitions of UV-induced skin edema, inflammation, and generation of hydrogen peroxide or lipid peroxidation in the skin, as well as reduce tumor incidence and onset. Oral administration to SKH-1 mice also hindered tumorigenesis and the transformation of benign papillomas into squamous cell carcinomas [11]. Based on the results of various studies, resveratrol offers promising outcomes in photoprotection and anticarcinogenic effects; however, due to a low in vivo bioavailability, continued efforts are needed to enhance availability and study its supplemental effects on humans [11].

Green Tea

Originating in Asia, tea has been one of the largest exports around the world for centuries and its use has become integrated into many cultures. Green tea differs from black tea in appearance and production. In contrast to black tea, green tea is manufactured by carefully drying and steaming the leaves and buds of the plant at high temperatures after harvesting, avoiding oxidation and polymerization of the plant's polyphenols [12]. In avoiding oxidation, larger amounts of polyphenols are able to be present. The major polyphenolic compound found in green tea is catechins, flavanol compounds with antioxidant properties, including (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate, and (−)-epicallocatechin-3-gallate (EGCG) [12]. These catechins are found to have antioxidant, anti-inflammatory, and anticarcinogenic properties [12]. Many studies have found that EGCG in particular is a potent antioxidant and is a major contributor to the anticarcinogenic effects [13]. In aiding skin photoprotection, these polyphenols target various molecular mechanisms such as inflammation, enhancing antioxidant defense enzymes, inhibition of DNA damage [10], anti-angiogenesis, and DNA repair [12].

Experimental evidence from several in vitro and in vivo studies supports the claim of chemopreventative activities of green tea polyphenols (GTPs) against photocarcinogenesis [12]. Administration of GTPs both orally and topically to mice test subjects resulted in protection against UV-induced skin carcinogensis by reducing tumor incidence, tumor multiplicity, and tumor size [6]. Mice that were administered GTPs via drinking water developed fewer tumors than in ones who were not, and the administration induced partial regression or inhibition of tumor growth in established skin papillomas. Dispensation of green tea as the only source of beverage to the mice also showed a decrease in papillomas, keratocanthomas, and squamous cell carcinomas induced by UV exposure, suggesting that various forms of green tea or GTPs contain beneficial properties [12]. Topical administration of EGCG also resulted in a large decrease of total tumor volume and incidence; however, studies have found that it has exceptionally high protection from photocarcinogenesis in form of hydrophilic ointment [6]. These findings suggest that applying EGCG or GTPs in this topical formulation may increase penetration or absorption capacity of the skin layers, and the presence of high concentrations of GTPs can be responsible for higher photoprotection [6]. In combination with sunscreens or skin care lotions, GTPs can provide an effective and nontoxic strategy in reducing the risk of skin cancer; however, clinical trials are needed to evaluate the usefulness of GTPs or EGCG alone or in combination with existing therapies for skin cancers in the human populations [12].

Milk Thistle

Native to Europe, the fruits and seeds of milk thistle (*Silybum marianum*) have been used to treat a range of conditions, from relieving various liver conditions to treating boils or other skin diseases [14]. Milk thistle extract is presently sold as a dietary supplement throughout Europe, Asia, and the United states as a remedy for liver-related ailments; however, the current topic of study among researchers is the anticancer properties of various compounds found in milk thistle [15]. Silymarin, the primary flavonoid or subclass of polyphenol found in milk thistle, is composed of the major constituent silibinin and smaller amounts of silibinin stereoisomers [6]. Silibinin has been identified as the anticancer agent within silymarin; however, both silymarin and silibinin are identified as strong antioxidants that are capable of scavenging free radicals and reactive oxygen species [16].

Similar to other botanical extracts, studies on the photoprotective effects of silibinin and silymarin on skin cancer have been conducted using mouse models and cell cultures. On a cellular level, different mechanisms of actions that silibinin exerts on malignancies include enhancement of pro-apoptotic molecules, expression of cell cycle inhibitors, tumor suppressors and growth inhibitory proteins, and inhibition of cell proliferation, cell cycle regulators, pro-angiogenic factors, transcriptional factors, growth factors and receptors, anti-apoptotic molecules, and matrix metalloproteinase [15]. Silibinin's strong anti-photocarcinogenic effects derive from the actions of these cellular mechanisms. Results of topical and oral silbinin treatments include decrease in tumor incidence, increased latency period (appearance of first tumor) and decreased tumor volume [16]. Although the combination of both dietary feeding and topical application convincingly present a strong protective effect against UV-induced tumorigenesis, additional studies are in progress to assess clinical use among human populations [16].

Citrus Peel

Citrus fruits are praised as tasty, healthy foods and can be often used to enhance the flavor of foods and beverages [17]. Although citrus fruits such as oranges, grapefruits, and lemons contain a lot of vitamin C, potassium, antioxidants, and bioflavonoids in the flesh or juice, the grated lemon peel, or

zest, also contains compounds beneficial for health [17]. The compound D-limonene is only found in the oil of the peel and has been found to inhibit skin carcinogenesis and yield photoprotective activity. One such study on the effects of D-limonene was based out of southern Arizona tested the correlation between citrus fruit consumption and carcinogenesis [18]. Since the patients are exposed to lots of sunlight throughout the year living in sunny Arizona, the risk factor for skin cancer increases greatly. Participants were distributed questionnaires that sought information on things such as orange or grape fruit juice consumption, skin characteristics such as sunburns, tanning history, or use of sunscreen, past family medical history, alcohol and tobacco use, and diet [18]. The results of the study revealed that although there is no direct evidence that consumption of citrus fruit or juice has adverse or beneficial effects on squamous cell carcinomas, a strong correlation between citrus use and squamous cell carcinoma risk was determined. The individuals without skin cancer had a higher percentage of citrus consumption than those with cases of cancer [18]. D-limonene may be responsible for chemoprevention of the initiation phase in carcinogenesis and provide a low cost, easily available, nontoxic supplement in preventing cancer or during chemotherapy for the future. As this is a fairly new study, more research is needed to further evaluate the protective effects of D-limonene from citrus peels.

Tomatoes

Tomatoes (*Solanum lycopersicum*) are largely cultivated crops that are consumed all around the world. They can be prepared in several ways, from eaten fresh to processed into tomato juice, paste, ketchup, salsa, soup, etc. [13]. Tomatoes and tomato products are rich in antioxidant compounds and are a great source of carotenoids. Carotenoids are pigments that have an important role in protecting plants against the photo-oxidative process; important carotenoids found in tomatoes include phenolic compounds (flavonoids and phenolic acids), ascorbic acid, and lycopene [19]. The acyclic carotenoid lycopene is the natural pigment responsible for the red color and exemplifies the most effective antioxidant among various carotenoids [20]. In regard to bioavailability, lycopene consumed in the natural *trans* form is poorly absorbed, whereas the cutting, heating, and processing of tomato products induces the isomerization from *trans* to *cis*, which in turn promotes absorption and bioavailability [13]. Several studies have suggested that lycopene is an effective free radical scavenger and potent antioxidant, and prevents carcinogenesis and atherogenesis by protecting critical biomolecules including lipids, low-density lipoproteins (LDL), proteins, and DNA [13].

Topical application of lycopene in mouse models has been used to demonstrate the chemopreventative effects of lycopene against photo-induced tumors [13]. Application prior to UVB exposure resulted in reduced photoinjury, as measured by decreases in inflammatory response [13], and Ornithine decarboxylase (ODC) enzymatic activity, whose over-expression is associated with tumor promotion [20]. This application also helped maintain normal levels of epidermal markers associated with proliferation [13], which hinders the progression to malignant transformation [20]. Other studies involving humans observed that supplementation of oral lycopene was shown to protect against UV-induced erythema, a redness or rash of the skin [19]. Although many extensive studies have focused on the effect of lycopene with other cancers and cardiovascular diseases, further tests should be conducted on lycopene's effects on photocarcinogenesis and photoaging of the skin [20].

Conclusion

The preceding extracts were only just a sample of a large variety of products that demonstrate valuable anti-photocarcinogenic effects against skin cancer. Based on the experimental evidence examined from in vivo and in vitro tests, it is suggested that daily consumption or application of various

dietary botanicals may be beneficial by providing protection against the harmful effects of solar UV radiation [6]. Continued research is recommended to determine which compounds have sufficient bioavailability and how much of the extract is needed to receive the desired protection. However, dietary supplements alone will not diminish the risk of skin cancer or the growth of cancerous cells. It is important to indulge in healthy habits including wearing protective clothing, using sunscreen, avoiding excessive sun exposure, and maintaining a balanced diet and exercise.

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Chapter 26
Promising Plant Extracts with In Vivo Anti-melanoma Potential

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Key Points

- Melanoma is a highly aggressive and therapy-resistant skin cancer.
- Plants have been used in the treatment advanced stages of various malignancies.
- *Silybum marianum*, *Prunus persica*, *Momordica charantia*, and *Silybum marianum* extracts might be used as anti-melanoma potential.

Keywords Melanoma • Phytotherapy • Pharmacology • Emerging bioactive phytochemicals • Plant extracts • Traditional and alternative medicine

Abbreviations

CO	*Calendula officinalis*
COX	Cyclooxygenase
DITC	Dacarbazine

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FDA	Food and drug Administration
IC-50	Half maximal inhibitory concentration
LACE	Laser activated calendula extract
MC	*Momordica charantia*
miRNA	micro-RNA
SM	*Silybum marianum*
UV	Ultraviolet

Introduction

Melanoma is a highly aggressive and therapy-resistant skin cancer that originates from melanocytes, photoprotective melanin pigment-producing cells derived from multi-potent and highly migratory progenitors in the neural crest [1]. The global incidence of this life-threatening disease, ranging between 2.3/100.000 and 2.6/100.000, represents an increasing worldwide health problem [2]. The risk factors involved in initiation and/or development of the melanoma may include genetic (e.g., $V_{600}E$ *B-RAF* mutated gene, DNA repair variants) [3, 4], epigenetics (e.g., hypermethylation of specific miRNAs and/or promoters) [5, 6], cellular (e.g., melanoma stem cells) [7], and/or environmental ones (e.g., UV radiation) [8]. Unfortunately, the current treatment options, including bio-chemotherapy, for metastatic melanoma provided modest results, notably because of their relative high toxicity and low specificity which consequently are due to the complex etiology (e.g., genetic heterogeneity) and physiopathology of melanoma (e.g., potent drug resistance systems) [7]. Thereby, the complete response rates with dacarbazine (DITC), the only drug approved by the US Food and Drug Administration (FDA) for the treatment of metastatic melanoma, rarely exceed 5% [9]. Then, current development of new targeted therapeutics such as PLX4032 (RO5185426), acting as a potent *B-RAF* mutant inhibitor, are intended to provide an alternative option for melanoma tumor regression and enhancement in patient's survival [10]. Besides, intense research for natural anti-melanoma compounds is definitively required. Indeed, herbal plants and plant-derived medicines have been used as the source of potential anticancer agents in traditional cultures all over the world and, are becoming increasingly popular in modern society. Over 50% of today's available anticancer drugs (e.g., vinca alkaloids, etoposide, taxanes, irinotecan, roscovitine, flavopiridol) are originated from natural products, providing a great confidence to investigate more sources for the development of effective anticancer agents [11].

We here briefly and mainly review the pharmacological, molecular mechanisms and toxicological aspects of four most promising plants extracts that have displayed potential anti-melanoma effect in vivo: *Momordica charantia*, *Calendula officinalis*, *Silybum marianum*, and *Prunus persica*. Current and future directions with regard to phytotherapy of melanoma are also discussed.

Momordica charantia L. (*MC*)

MC plant (Cucurbitaceae), commonly known as bitter gourd or bitter melon, grows in tropical areas of Asia, Amazon, East Africa, and the Caribbean. *MC* has been used in traditional medicine in developing countries such as Brazil, China, Ghana, Haiti, India, Mexico, and Peru. Its pharmacological actions and potential uses include anticancer, antidiabetic, anti-inflammatory, antioxidant, antibacterial, antiviral, anthelmintic, abortifacient, antifertility, immune-modulatory activities [12].

Pharmacological Anti-melanoma Activity

In vitro and in vivo studies using different MC extracts (e.g., fruits, leaves) have shown antitumor activity, including anti-melanoma potential [13]. The anticarcinogenic activity of *MC* was maximal when its peel was used [14]. Among biologically active *MC* chemicals including saponins, glycosides, alkaloids, triterpenes, and steroids, experimental studies showed that saponin called α(alpha)-momorcharin is toxic to melanoma cells [12].

Molecular Mechanisms of Anti-melanoma Activity

The precise molecular actions of *MC* phytochemicals that render melanoma cells toxic have not been established yet. Nevertheless, it has been reported that *MC* exert anticancer activities through various mechanisms such as inhibition of DNA, RNA and cellular protein synthesis, cell cycle arrest in G2 and M phases, repression of guanylate cyclase activity, activation of natural killer (NK) cells, induction of apoptosis as well as modulation of biotransformation and detoxification enzymes [12].

Toxicity

MC was shown to be safe in experimental animals when ingested in low doses up to 2 months. However, toxicity and even death, in laboratory animals, have been reported when extracts were administered at high doses intravenously or intraperitoneally [12]. The fruit and seeds demonstrated greater toxicity than the leaf or aerial parts of the plant [12]. Documented adverse effects of *MC* were hypoglycemic coma and convulsions in children, headaches, a favism-like syndrome, reduced fertility as well as increase levels of gamma-glutamyltransferase and alkaline phosphatase in animals [12].

Calendula officinalis L. (*CO*)

Flowers of the plant *CO* (Asteraceae), commonly known as "Marigold," are used in the West and in Asia for their anti-inflammatory properties [15]. Phytopharmacological studies of different *calendula* extracts have shown antitumor, anti-genotoxic, antiviral activities along with high efficiency in the prevention of acute dermatitis in cancer patients undergoing postoperative irradiation [16].

Pharmacological Anti-melanoma Activity

Its cytotoxic effect on tumor cell lines in vitro and its anticancer efficacy in vivo was briefly outlined more than two decades ago [17]. The principal chemical constituents of *CO* include triterpenoids, carotenoids, saponins, and flavonoids [16].

Interestingly, an aqueous extract of the plant called *LACE* (*laser activated calendula extract*) demonstrated a potent in vitro growth inhibition of several tumor cell lines, including B16 murine and

ANDO-2 melanoma cells for which 76 and 100% of growth inhibition has been noticed respectively [18]. This growth inhibition of LACE extract was similar to that reported for taxol in tumor cell lines [18]. Furthermore, this extract displayed an anti-melanoma activity in vivo, using athymic nude mice injected subcutaneously, orally or intraperitoneally with human melanoma ANDO-2 cells [18]. Indeed, 60% of tumor regression was observed, which was similar to the effect obtained with the commonly used chemotherapy drug, paclitaxel, but with the advantage to display higher prolongation of life span of tumor bearing mice than paclitaxel [18].

Besides, another investigation indicated anti-metastatic effects of alcohol extract of the *CO* flowers on lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice, through the inhibition of key molecules involved in processes of metastasis [16]. This anti-metastatic effect would be explained by the presence of carotenoids (e.g., lycopene, β (beta)-carotene) in *CO* flowers which further reduce transcript levels of pro-inflammatory cytokines [16].

Molecular Mechanisms of Anti-melanoma Activity

The mechanisms of growth inhibition were explained by cell cycle arrest in G0/G1 phases as well as by a caspase-3 dependent apoptosis in a LACE dose-dependent manner [18]. However, the specific molecular induction of apoptosis in melanoma cell lines is not determined yet [18]. Also, further research is required to identify the active phytochemical(s) of such activity [18]. Besides, the inhibition of the metastasis process initiated by injection of B16F-10 melanoma cells into C57BL/6 mice was explained by the repression of the metalloproteases MMP-2, MMP-9, prolyl hydroxylase, lysyl oxidase, pro-inflammatory cytokines (e.g., TNF-α (alpha), IL-1β (beta), IL-6, and GM-CSF) expression and the activation of tissue inhibitor of metalloproteases, TIMP-1 and TIMP-2 [16].

Toxicity

LACE extract displayed a low toxicity in vivo. Notably, the anti-proliferative activity of LACE was not accompanied by systemic toxicity in mice or rats at a dose of 50 mg/kg body weight [18]. Simultaneous combined oral administration of *CO* flower extract with tumor inoculation showed significant reduction in the number of lung colonization along with significant increase in the life span of metastatic lung tumor bearing C57BL/6 mice [16] indicating that the extract is rather safe and beneficial.

Silybum marianum L. (*SM*)

SM (Asteraceae), *aka Carduus marianus* or milk thistle, is primarily an indigenous plant of mediterranean region and southwest Europe and, the seeds of this plant have been used for the last 2,000 years to treat liver disorders (e.g., hepatitis, cirrhosis) thanks to its antioxidant and anti-inflammatory properties [19].

Pharmacological Anti-melanoma Activity

One of the most bioactive compounds present in the fruits and seeds of *SM* is called silymarin, a flavanoid represented by a mixture of mainly three flavonolignans, silybin (silibinin), silydianin, and silychristin [20]. Silibinin is the major (70–80%) and most active biological component of silymarin

[21]. This later has demonstrated efficacy in animal models related to protection against UV radiation-induced inflammation, oxidative stress, immunosuppression and skin cancers (e.g., basal cell and squamous cell carcinoma as well as melanoma) [22, 23]. Based on the antioxidant and anti-inflammatory activity of silymarin, the chemopreventive effect of silymarin has been tested and determined, using notably animal models of chemical carcinogenesis and photo-carcinogenesis [22, 24]. The antioxidant nature of silymarin and its role in photoprotection of oxidative stress-associated skin disorders including skin cancers has been recently confirmed [21].

Molecular Mechanisms of Anti-melanoma Activity

Antioxidant activities demonstrated the ability of silibinin or silymarin to protect the skin from the adverse biological effects of UV-B radiation via modulation of the MAPK and NF-(kappa)B signaling pathways, providing a molecular basis for the anticarcinogenic effect of silymarin/silibinin in an in vivo animal model [21, 25, 26]. Laboratory studies, conducted in several in vitro and in vivo cancer models, suggest that there was no significant difference between silymarin and silibinin in terms of chemo-preventive or biological activities [27]. Thereby, topical treatment of silymarin in UV-exposed mouse skin significantly reduced myeloperoxidase activity, a marker of leukocyte infiltration, as well COX-2 expression and its prostaglandin metabolites [23, 28]. These studies suggested that anti-photocarcinogenic activity of silymarin is associated with the inhibition of UVB-induced inflammation and inflammatory mediators in the mouse skin. It was then suggested that silymarin may prove to be a useful chemopreventive agent against UV-B radiation induced inflammation associated skin diseases including melanoma and non-melanoma skin cancers in humans [21].

Toxicity

In animal models, silymarin is nontoxic even at higher physiological doses, which suggests its safe use for humans [29].

Prunus persica L. (Ku-35)

Ku-35 (Rosaceae), commonly known as “peach tree,” originates from China before being introduced to Persia and the Mediterranean region [30]. Besides the edible juicy peach fruit, it has been shown that the flowers of Ku-35 contain polyphenols, including flavonoids and antocyanins, which can contribute to the antioxidative activity and potentially protect against UV-induced cellular DNA damage [31].

Pharmacological Anti-melanoma Activity

Ku-35 flowers ethanol extract showed high antioxidative activity and protective effects of Ku-35 against UVB- (50 mJ/cm^2) and UVC- (5 mJ/cm^2) induced DNA damage in NIH/3T3 mouse fibroblasts (IC_{50} of 162 and 141 μg/ml, respectively) as well as against UVB-induced skin carcinogenesis in SKH-1 hairless mice [31]. Comparatively to the control group of mice, the application of high dose

Ku-35 cream (25%) prior to UVB exposure resulted in a considerable delay of tumor development (~76%) as well as potent protection in tumor incidence and tumor multiplicity throughout the treatment period [31].

Molecular Mechanisms of Anti-melanoma Activity

It has been suggested that the protection of UVB-induced skin carcinogenesis of Ku-35 may partly be due to the inhibition or prevention of DNA damage (mutations, DNA strand breaks…) by enhancement of DNA repair mechanisms [32] and/or reduced lipid peroxidation through its potent antioxidant activity [31]. Furthermore, a recent ex vivo studies showed that silymarin is able to inhibit melanoma cell migration/invasion by targeting β (beta)-catenin signaling pathway [33].

Toxicity

In opposite to DL-alpha-tocopherol, Ku-35 did not possess any pro-oxidant activity in addition to its antioxidant activity, suggesting that Ku-35 is a safe antioxidant for applicable to clinical use [31].

Discussion

Melanoma is the main cause of death that is related to skin cancer, and its incidence is increasing worldwide [2]. At early stage, melanoma is cured in most cases by surgical removal of the tumor but it is almost always fatal at advanced stage once the metastatic phase is developed. Systemic chemotherapy is often the only viable treatment, but the lack of selective cytotoxicity often leads to intolerable side effects. Indeed, melanoma has one of the worst response rates to chemotherapy of all neoplasias [34].

Therefore, the search for alternative drugs that are both effective and nontoxic in the treatment of cancers is an important research line. Increased efforts are being made to isolate nontoxic bioactive products from medicinal plants for their possible utility in preventing or treating cancer in an effective and specific fashion. Plants have a long history of use in the treatment advanced stages of various malignancies, including cancer (e.g., *Angelica gigas*, *Catharanthus roseus, Podophyllum peltatum*, *Podophyllum emodii*, *Taxus brevifolia, Ocrosia elliptica*, *Camptotheca acuminata*) [35].

During the last decade, some ex vivo studies reported interesting plant extracts with anti-melanoma potential (e.g., *Inula viscosa*, *Allium sativum*, *Rabdosia japonica*, *Phyllanthus urinaria*) with anti-melanoma potential [36, 37]. Those beneficial effects are mainly due to phytochemicals (e.g., flavones, flavanols, isoflavones, catechins, taxanes) [36–38], while their mode of action in a particular malignancy is not always elucidated.

In the present work, we have reported from the literature what we consider as the four most promising plant extracts tested in vivo for potential anti-melanoma activity and their low toxicity. The main bioactive phytochemicals, alpha-mormocharin and silymarin, from *M. charantia* and *S. marianum* respectively, would be responsible for the anti-melanoma potential. Nevertheless, further research is required to isolate the ones from *C. officinalis* and *P. persica.* Besides, the in vivo molecular mechanism of phytochemicals isolated from *S. marianum* is getting relatively well understood, which is encouraging for future clinical use.

Conclusions and Future Prospects

Our selected four plant extracts with anti-melanoma potential show good promise for their future use in treating melanoma patients (case of *M. charantia*, *C. officinalis*) or preventing photocarcinogenesis in the global population (case of *S. marianum* and *P. persica* extracts that might be used in combination with sunscreens or skin care lotions). When the plant is comestible, it might be interesting to conduct an epidemiological survey with regard to incidence of malignancies among population that consumes this plant. Moreover, and according the new model of tumorigenesis and carcinogenesis involving chemo-resistant and elusive cells such "cancer stem cells," testing of plant extracts or new promising phytochemicals in "melanoma stem cells" and in tumor-bearing animals induced by "melanoma stem cells," could be important to enhance efficiency in tumor regression and avoid relapse of the tumor. Eventually, further studies are required in human system to determine cellular uptake, distribution as well as long-term effect of the isolated phytochemicals in the skin and against melanoma.

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Chapter 27
Molecular Sensors and Mediators of Skin Cancer Preventative Phytochemicals

Andrea Aquilato, Barbara B. Doonan, Tze-chen Hsieh, and Joseph M. Wu

Key Points

- Skin cancer, a leading cause of cancer deaths in the US population, lacks treatment options when the disease relapses at local or distant sites.
- Therapies for melanoma cases are particularly challenging owing to metastasis and resistance to currently available treatment modalities.
- Lay and scientific literature both suggest that diet and specific dietary ingredients may reduce the incidence of skin cancer. Recent focus has been directed at the consumption of fruits and vegetables.
- Phytochemicals abundant in vegetables and fruits have been shown to have therapeutic and preventative potential against skin cancer and melanoma.
- Phytochemicals exert pleiotropic effects, molecular synergies and affect a multitude of cellular targets in skin cancer and melanoma.
- An approach and mechanistic framework aiming to identify fruit/vegetable-derived phytochemicals with potential for generating a chemopreventive index marked by increased distinct anticancer properties and a decreased spectrum of untoward effects capable of optimally targeting multiple genetic and epigenetic derangements found in skin cancer and melanoma are presented, and they serve as a foundation for a combinatorial dietary strategy for future animal models and clinical studies.

Keywords Skin cancer • Melanoma • Dietary phytochemicals • Combinatorial diet-based anti-skin cancer strategy

Abbreviations

8-OHdG	8-Hydroxy-2-deoxy-guanosine
AK	Actinic keratosis
AK	Activated kinase
AL	Acral lentiginous

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ARE	Antioxidant response element
BCC	Basal cell carcinoma
CD11b+	A surface marker of monocytes/macrophages and neutrophils
CHS	Contact hypersensitivity
CPDs	Cyclic pyrimidine dimers
DAC	Differential displacement affinity chromatography
DMBA	Dimethylbenzanthracene
EGF-R	Epidermal growth factor receptor
EREs	Estrogen response elements
GSH	Glutathione
GTP	Green tea polyphenol
IL	Interleukin
JNK	c-Jun N-terminal kinase
LOH	Loss of heterozygosity
MNNG	*N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine
MNU	Methylnitrosourea
MSH	Melanocyte-stimulating hormone
NER	Nucleotide excision repair
NMSC	Nonmelanoma skin cancers
NO	Nitric oxide
NOS	Nitric oxide synthase
NQO2	Quinone reductase 2
ODC	Ornithine decarboxylase
PCNA	Proliferating cell nuclear antigen
PD	Pyrimidine dimer
PI3K	Phosphatidylinositol 3-kinase
PUVA	Psoralen plus UVA
ROS	Reactive oxygen species
RTKs	Receptor tyrosine kinase
SCa	Skin cancer
SCC	Squamous cell carcinoma
SGTE	Standard green tea extract
SS	Superficial spreading
TGF-β	Transforming growth factor
TPA	12-*O*-Tetradecanoylphorbol-13-acetate

Skin Cancer and Melanoma as a Growing Public Health Problem in the United States

The fascination and trepidation of mankind with the sun and human exposure to sunlight is readily evident in review of historical records in Western and Eastern cultures. Considered as the largest of human organs, the skin functions as a biological barrier that protects the inner organs from environmental stressors such as exposure to UV from sunlight and xenobiotic agents, as a thermoregulator, as a frontline defense for the immune system, and as a detector/responder to fluctuations in sensation. To maintain its physical integrity, the skin requires an intact epidermis and underlying dermis overlaying a subcutaneous layer. The epidermis is composed of squamous epithelial cells called keratinocytes, which are further partitioned into layers of cells differing in activity and capacity for proliferation and differentiation.

The innermost basal layer of keratinocytes shows active proliferation, and is overlaid by cells robustly committed and engaged in differentiation. In contrast, metabolically inert cells form the outermost epidermal layer (stratum corneum), while fibroblasts characterize the underlying dermis of the skin. This cellular organization represents a tight, dynamically regulated process with cells in the basal layer continuously dividing, differentiating and migrating upward into the apical layer, destined for eventual cell death as they replenish the outermost stratum layer required to maintain the barrier integrity of the skin. Integrity of the skin is also bolstered by the process of melanogenesis, which is regulated by the dynamic interplay between skin keratinocytes and the pituitary gland. Exposure to UV light damages the keratinocytes, which stimulates the natural repair mechanism in UV-exposed damaged cells through the release of chemicals into the blood stream that travel to the pituitary gland resulting in release of melanocyte-stimulating hormone (MSH). Specific binding of MSH to melanocytes triggers a cAMP-dependent signal cascade leading to an increase in the activity of tyrosinase, which catalyzes the conversion of tyrosine to melanin. The melanin is accumulated in granules called melanosomes, which are moved along to the end of the melanocyte dendrites and taken up by nearby keratinocytes; an increase in melanin levels confers protection to keratinocytes preventing further damage from UV exposure and restoring the intactness of the skin [1] (Fig. 27.1). It is therefore evident that any breach in the skin architecture or in the control of melanogenesis may lead to skin aberrations and even cancer, while changes in proliferation and differentiation patterns, e.g., excessive or deficient proliferation not counterbalanced by differentiation, may also compromise the integrity of the skin and diminish its protective capacity.

What challenges and signals might trigger such imbalances and possible disruption in melanogenesis? Disproportionate proliferation relative to differentiation or sub-optimal melanogenesis may result from exposure to UV radiation from sunlight, in particular, its UVB component (280–320 nm). This has been reported to cause erythema, sunburn, hyperpigmentation, immunosuppression, hyperplasia, photoaging, and SCa [2]. With global warming and its accompanying progressive depletion of the ozone layer, it is reasonable to expect that exposure to sunlight and UV levels will increase. Such could be a significant factor in the observed prevalence for skin disorders with an associated sustained, persistent inflammation, hyperactive pigmentation and proliferation, which may well contribute to an increase in risk and incidence rate for SCa.

SCa may be classified according to their cellular origin; melanomas deriving from melanocytes or their precursors and nonmelanoma skin cancers (NMSC) mostly from keratinocytes, the major cell type of the epidermis. In many Caucasian populations NMSC is the commonest malignancy. In the United States it is estimated that up to 1,000,000 new cases are diagnosed each year [3]. Common NMSC include basal cell carcinoma (BCC) which accounts for 80% of NMSC and the remainder is mostly attributed to squamous cell carcinoma (SCC). Both BCC and SCC show infrequent fatality rates owing to their low aggressiveness, and patients are easily treated by surgery [3]. The low metastasis of NMSC may relate to the ubiquitous exposure of skin to UV radiation, a major causal factor for NMSC. Exposure to UV may impose an evolutionary gain-of-advantage and constraint on keratinocytes that renders them insensitive or resistant to the adoption of an aggressive malignant phenotype. Susceptibility to Sca is inversely correlated with the degree of melanin pigmentation, as vividly illustrated by the fact that in the US whites are many times more likely to have Sca than blacks [4]. Also, populations living nearest to the equator, at the lower degrees of latitude, appear to be at greatest risk for development of SCa, probably due to the greater amount of UVB irradiation reaching the earth's surface there. Notably, the risk for SCa has been on an upward trend owing in part to an overall increase in the amount of UVB reaching the earth's surface as a result of depletion of stratospheric ozone.

Compared to NMSC, malignant melanomas account for only 4% of SCa and yet rank as the fifth most common cancer in men and the sixth in women. Significantly, the frequency of melanoma has increased by a factor of 15 in the past 60 years and the incidence rate has been

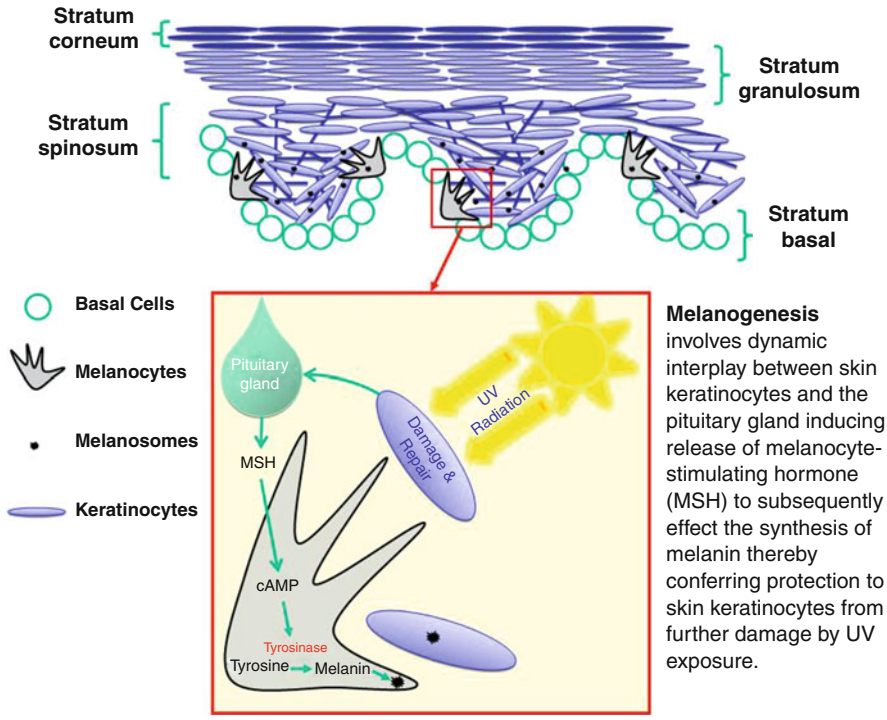
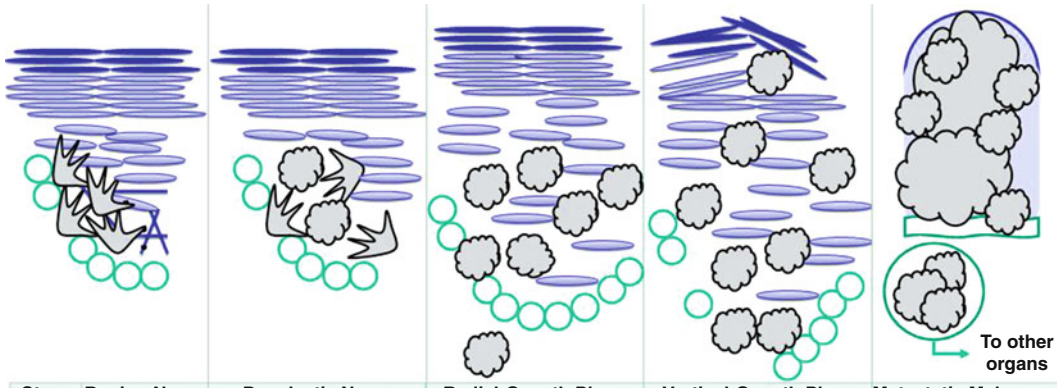


Fig. 27.1 Depiction of structural organization of normal skin, required to maintain its physical integrity. Overall, the skin is organized into an intact epidermis and underlying dermis overlaying a subcutaneous layer. The epidermis is composed of squamous epithelial cells called keratinocytes, which are further partitioned into layers of cells differing in activity and capacity for proliferation and differentiation. Stratum basale is the deepest epidermal layer, attached to the underlying dermis that contains a single row of stem cells (precursors to the keratinocytes) and melanocytes (10–25% of the cells). Stratum spinosum consists of several layers of keratinocytes, containing a web-like system of intermediate filaments. Melanosomes are present in stratum spinosum as well. Stratum spinosum is overlaid by stratum granulosum, comprised of three to five layers of flattened cells in the process of keratinization, in which the cells fill with keratin. The outermost layer is called stratum corneum, characterized by many layers of dead cells: flat membrane sacs filled with keratin. Shown also is the dynamic interplay between skin keratinocytes and the pituitary gland for the regulation of melanogenesis. Exposure to UV light damages the keratinocytes, which stimulates the natural repair mechanisms within the cells. The repair mechanism is initiated by UV-exposed damaged cells releasing chemicals into the blood stream that travel to the pituitary gland eliciting the release of melanocyte-stimulating hormone (MSH), which binds specifically with melanocytes. MSH binding triggers a cAMP-dependent signal cascade leading to an increase in the activity of tyrosinase, which is the enzyme responsible for the conversion of tyrosine to melanin. As melanin is synthesized, it is accumulated in granules called melanosomes, which are moved along to the end of the melanocyte dendrites and taken up by nearby keratinocytes

steadily increasing at ~5% yearly, particularly in fair-skinned individuals in the United States and among populations of European origin living in sunny regions [5]. Furthermore, risk for melanoma is also associated with latitude of residence lending credence to exposure to sunlight as having paramount causal importance [6]. A significant proportion of the disease is familial, suggesting that specific genes regulate susceptibility [7]. Potentially deleterious effects of sunlight exposure are largely averted through photoprotection by melanin, which is synthesized in specialized dendritic cells called melanocytes [1].

Melanoma is considered the most common fatal SCa. Melanoma arises from malignant transformation of melanocytes and has an aggressive course once the tumor has spread beyond the superficial skin. Like all tumor types melanoma shows considerable heterogeneity in outcome and molecular



Stage	Benign Nevus	Dysplastic Nevus	Radial-Growth Phase	Vertical-Growth Phase	Metastatic Melanoma
Events	Proliferation of structurally normal melanocytes.	Developement of aberrant growth.	Cells acquire the ability to proliferate intraepidermally, and can penetrate the dermis singly.	Lesions acquire the ability to invade the dermis and form an expansile nodule.	The successful spread of cells to other areas of the skin and other organs, where they can successfully proliferate.
Genetic Changes	BRAF mutation	Tumor suppressors Loss of CDKN2A & Loss of PTEN	Promoter of cell proliferation Increased CD1	Cell adhesion molecules Loss of E-cadherin N-cadherin expression $\alpha\gamma\beta3$ integrin expression Degradation of collagen in basement membranes MMP-2 expression An inbibitor of apoptosis Survivin Reduced TRPM1 (expression is inversely correlated with melanoma aggressiveness)	Absent TRPM1

Fig. 27.2 Different melanoma stages and some of the key molecular aberrations observed. Stage I: Benign nevus, accompanied by proliferation of structurally normal melanocytes, characterized by BRAF mutation. Stage II: Dysplastic nevus in which aberrant growth starts to develop, accompanied by loss of CDKN2A and tumor suppressor PTEN. Stage III: Radiant growth phase where cells acquire the ability to proliferate intraepidermally and can penetrate the dermis. In this stage cells show increased expression of CD1. Stage IV: Vertical-growth phase melanoma. Here lesions acquire the ability to invade the dermis and form an expansile nodule. Genetic changes associated with this phase include loss of E-cadherin accompanied by expression of N-cadherin, $\alpha\gamma\beta3$, MMP-2, surviving, and reduced expression of TRPM1. The final stage of melanoma involves its ability to metastasize. Parts of Fig. 27.2 were modified from Miller and Mihm [73]

pathogenesis [8, 9]. Clinically, distinct patterns of melanoma are observed; these include acral lentiginous (AL) presenting in the distal extremities and also superficial spreading (SS), lentigo maligna, and nodular types. Almost all histological and clinical patterns of melanoma are increased in patients with a history of heavy sun exposure, particularly marked by discrete serious sunburn episodes [10]. Mucosal and soft tissue presentations of melanoma while rare appear to have a distinct pathogenesis. A variety of different histological appearances in melanoma has also been observed, including the typical epithelial forms as well as desmoplastic (spindle cell) and anaplastic variants [11]. Different stages of melanoma and some of the key molecular aberrations observed are illustrated in Fig. 27.2. When the disease is caught early and there is no associated evidence of metastasis (lesion <1.5 mm), melanoma can be easily treated by surgery/local excision with a 5-year survival rate greater than 90%. Comparatively, melanomas greater than 1.5 mm but less than 4 mm in thickness with no evidence of metastases have an overall 5-year survival rate of 70–80% while patients with melanomas 4 mm or greater in thickness or with lymph node invasion have a survival rate that decreases to approximately 35–48%. However, once the cancer spreads and the depth of invasion is >4 mm, the prognosis is dismal even with adjuvant therapy, e.g., administration of interleukin-2 (IL-2) and interferon

(IFN), and the 5-year survival rate is reduced to less than 2% [12–14]. The standardized chemotherapy for stage IV metastatic melanoma is administration of decarbazine, which acts by chemically modifying DNA [15]. Unfortunately, only 10% of melanoma patients show any response to the drug, primarily as palliative therapy without evidence of benefit for overall survival. Given that decade long exposure to photocarcinogens is involved in initiation of melanoma, and melanoma readily recurs from malignantly transformed melanocytes, often not easily detected once released from the primary tumor, treatment and management of these cancers is a real public health concern and challenge in need of novel therapies.

Cellular and Molecular Events Induced by Exposure to UV Radiation

Exposure to UV elicits multiple alterations in the structure and function of the skin; exposure to UVA (320–400 nm) results in deeper penetration in the skin whereas more damage is incurred during exposure to the greater-energy-per-photon UVB (280–320 nm). Studies have shown that acute and chronic exposure to sunlight can absorb wavelengths in the UVB region sufficiently as to causally induce skin disorders including sunburn (likely source of apoptotic cells), actinic keratosis (AK) lesions, and skin cancer [3]. In the case of AK and SCC, UVB exposure effectively replaces epidermal keratinocytes with poorly differentiated atypical squamous cells. Therefore, treatments that promote or facilitate epidermal cell differentiation in photodamaged skin can re-establish the homeostatic balance between proliferation and differentiation and in principle, counteract the development of AK lesions and SCC by UV exposure.

UV Exposure and Formation of DNA Photoadducts

Exposure to UVB and UVA both can cause DNA damage, resulting from the absorption of photons and production of reactive oxygen species (ROS). The importance of ROS in the UV signaling pathway is underscored by extensive evidence showing that exposure to enzymatic and nonenzymatic antioxidants decreases UVB-induced apoptosis in keratinocytes; in contrast, agents that elevate levels of ROS stimulate UVB-induced signaling pathways involving MAP kinases (JNK and p38 kinase), thus augmenting the induction of apoptotic cell death.

UV exposure primarily induces the formation of cyclic pyrimidine dimers (CPDs) and 6–4 photoadducts in cellular DNA; these aberrations occur more frequently in tandem-repeated pyrimidine sequences, possibly causing overall distortions in DNA structure and restricting or facilitating the accessibility of proteins to the modified DNA, thereby affecting both structure and function [16]. CPDs and 6–4 photoadducts typically cause transition mutations from C to T and CC to TT. These are potentially mutagenic if not recognized and repaired by the nucleotide excision repair (NER) complex targeting altered DNA structures resulting from exposure to UV [17]. Individuals harboring malfunctional DNA repair machinery are at greater risk for skin carcinogenesis.

As mentioned previously, risk for development of melanoma is associated with area of geographical residence and has been observed to be increasing in fair-skinned individuals. Melanin, the pigment synthesized by melanocytes, which are specialized dendritic cells has been shown to confer photoprotection by its ability to absorb UV photons and ROS; poorly melanized skin is more vulnerable to injury caused by UV radiation. Compared to epidermal keratinocytes, melanocytes have a more limited proliferative potential and capacity; as such, UV exposure is more likely to damage and destroy keratinocytes than melanocytes. In addition, melanocytes display a higher level of expression of anti-apoptotic proteins, e.g., bcl-2 and survivin which render melanocyte resistance to UV-induced

apoptosis [18]. These properties suggest that damaged melanocytes are protected from apoptosis and have an increased capacity for incorporating and retaining mutations, which can be clonally expanded during cycles of melanocyte division. Therefore, UV-exposed melanocytes are refractory to undergoing apoptosis and are programmed to survive, divide and thrive in the mutated state induced by UV exposure with increased potential for ultimate manifestation as melanoma. Fair-skinned people with a potential to freckle appear at greater risk for melanoma as freckles are believed to represent clones of mutated melanocytes. Further, a correlation has been identified between intermittent, intense UV exposure and the development of melanocytic nevi and ultimately melanoma.

UV Exposure and Oxidative Stress

UV radiation can generate ROS in exposed skin cells and induce oxidative stress, which may cause damage at the cellular, molecular and genomic levels. Oxidative stress has been shown to induce inflammatory response, modifications in lipids and proteins, damage to DNA, as well as activation or inactivation of enzymes and signaling mediators. Collectively such could contribute to UV-induced photodamage of the skin [5]. As an example, 8-hydroxy-2-deoxy-guanosine (8-OHdG) is an ROS-induced DNA adduct known to change base-pairing properties of guanine to thymine and can therefore be a potential source of transition mutations. Recent evidence suggests that ROS, at appropriate doses, may actually function as intracellular mediators in signal transduction and effectively increase the expression of genes playing a role in the development/establishment of pathological conditions including immunosuppression and also affect different stages of photocarcinogenesis. Similarly, several transcription factors, e.g., nuclear factor NFκB and activator protein AP-1, playing pivotal roles in regulation of expression of proinflammatory genes and cell cycle regulatory proteins, are controlled by ROS-mediated redox mechanisms, and may serve as markers of inflammation and tumor transformation in photocarcinogenesis.

UV Exposure and Immunosuppression

UV exposure of skin keratinocytes induces the secretion of cytokines including IL-10 which produces an altered local and systemic immune response conducive to antigen-elicited hypersensitivity and predisposition to infections by microorganisms by a SOCS-3-mediated transcription program (Fig. 27.3) [19, 20]. Exposure to UV also induces isomerization of *trans*-urocanic acid, a deamination product of histidine found in abundance in the stratum corneum of the skin. This isomerization reaction occurring in skin keratinocytes effectively changes *trans*-urocanic acid from its normal role in stratum corneum differentiation as an integral part of the homeostatic mechanism, to its *cis*-isomer with a participatory function in immunosuppression [21–23]. This occurs through alteration of the activity of the antigen presenting cells, possibly via the secretion of IL-10, and other immune cytokines and factors, e.g., TNF-α and IFN-γ that collectively establish a chronically immunocompromised state. A schematic showing the effects of IL-10 is presented in Fig. 27.3.

Photocarcinogenesis

The development of SCa arising from exposure to UV may be envisaged as a multistage process encompassing a cascade of sequential events whose establishment depends on the duration, intensity and the wavelength at which skin exposure occurs. There is ample evidence showing that UV exposure

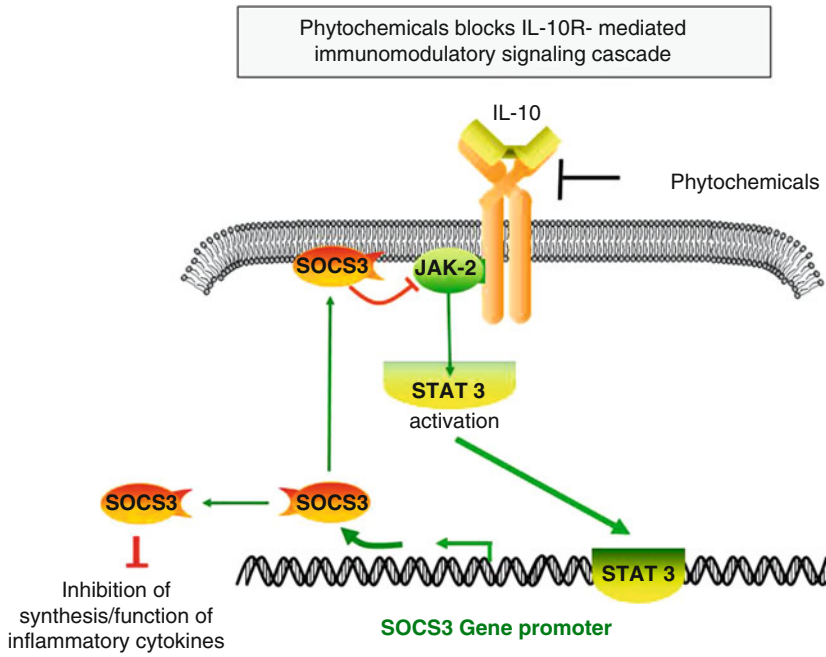


Fig. 27.3 A schematic showing the effects of IL-10 in inhibiting the JAK/STAT3 mediated SOCS3 transcription synthesis and function of inflammatory cytokines. Phytochemicals act by blocking IL-10R-mediated immunomodulatory signaling cascade

alone suffices to induce the three stages of carcinogenesis: initiation, promotion, and progression [1, 24]. In photocarcinogenic initiation, irreversible DNA damage accompanied by altered expression of several genes likely occurs in the epidermal cells. This is then followed by tumor promotion whereby cells harboring the damaged DNA and subsequently altered genes undergo clonal expansion producing in time a pre-neoplastic state with capacity for generating malignant lesions. A significant time span (latent period) often will elapse between initiation and the promotion stages of photocarcinogenesis. The final staging to carcinoma in situ and the conversion of the lesion into a clinically invasive and potentially metastatic malignant tumor is comparatively rapid.

C-H-Ras and Initiation and Progression of Skin Carcinogenesis [25]

The proto-oncogene c-H-ras is considered to be a major molecular determinant in initiation and progression of skin carcinogenesis, as evidenced by studies using dimethylbenzanthracene (DMBA) [26]. This commonly used initiator of carcinogenesis reproducibly causes an A→T transversion at codon 61 of the c-H-*ras* gene resulting in a Gln→Leu substitution. Other evidence has shown the methylating agents *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and methylnitrosourea (MNU) to both induce a G→A transition at the second base of codon 12 of the c-H-*ras* gene. It should be noted that tumorigenesis in the skin can also be initiated by topical skin application of a retrovirus encoding a mutant c-*ras* gene, or in transgenic mice carrying an activated skin-expressed c-*ras* gene. Notably, transgenic mice carrying the mutated c-Ha-*ras* gene under the control of differentiation-specific keratin promoters develop hyperkeratosis of the skin which become papillomas simply by mild stimuli invoked by biting or scratching and are predisposed to subsequent progression to SCC [27, 28].

Transforming Growth Factor TGF-β and SCa

TGF-β genes are multifunctional secretory growth effectors that affect cellular proliferation and differentiation, modulate extracellular matrix (ECM), and have dual tumor-suppressor and oncogenic effects [29, 30]. TGF-β is a potent inhibitor of keratinocyte proliferation; loss of the growth response to TGF-β is a frequent occurrence in human and mouse skin tumorigenesis, and is considered to be one of the earliest events of premalignant progression associated with the high-risk papilloma phenotype.

TGF-β is known to act by initiating signals through membrane-associated kinase receptor complexes to effect phosphorylation of cytoplasmic mediators called SMADs. Once phosphorylated, SMADs undergo cytosol-to-nucleus translocation where they locate and activate transcription of pertinent genes by interacting with coactivators or corepressors of the RNA synthesis machinery to transcribe the expression of genes whose protein products drive the multitude of phenotypically diverse changes in TGF-β-responsive cells. In tumorigenesis, malignant cells escape from the tumor-suppressive effects of TGF-β by mutational inactivation or dysregulated expression of the molecular components in the TGF-β signaling pathway. Interestingly, although melanoma cells are resistant to the tumor-suppressive effects of TGF-β, they show no detectable defects at the receptor/SMAD level. Therefore, in these lesions, it is likely that TGF-β effects occur independently of the TGF-β receptor/SMAD-mediated signaling pathway.

Hedgehog

The hedgehog (SHH)-GLI signaling pathway is functionally active in the matrix of human hair follicles, and appears to be required for the proliferation of normal cultured human melanocytes. The gene product of hedgehog is a protein that rests on the cell membrane and sends signals to the nucleus of the cell. Hedgehog signaling also regulates proliferation and survival of human melanomas: growth, recurrence, and metastasis of melanoma xenografts in mice are prevented by local or systemic interference of hedgehog signaling function [31]. It is noteworthy that oncogenic Ras-induced melanomas in transgenic mice are functionally linked to the hedgehog signaling activity both in vitro and in vivo [32]. Moreover, Ras-MEK and AKT signaling also regulates nuclear localization and transcriptional activity of hedgehog signaling in melanoma cells.

Among genes under the control of the hedgehog protein is the patched gene serving as a feedback regulatory control feature to limit the propagation of hedgehog signaling events [33]. Patched proteins, in response to hedgehog initiated signaling cascades, accumulate in the cell to serve as a check and balance, interrupting the signals emanating from the hedgehog protein. This dynamic regulatory mechanism implies that mutations in either the patched and/or hedgehog gene may result in the induction or inhibition of skin tumorigenesis.

p53

p53 plays an important role in the control of the cell cycle, cell division, and DNA repair. A functionally competent p53 is also pivotal in surveying, sensing and repairing mutated genes in skin cells as a result of exposure to UV rays and other environmental challenges that adversely affect the integrity of the genome. Mechanistically, p53 acts by stalling DNA replication and cell division so as to allow repair to occur. If, however, as a result of exposure to UV radiation, mutations occur in p53 itself, thus affecting its DNA repair sensing and functioning activity, cell division could proceed unabated resulting

in establishment of a cancerous cellular state [34–36]. Given that skin cells are in a constant state of cell division and differentiation, inheritance of a functionally compromised, mutated p53 has been shown to increase the risk for SCa by 50% at age 30 and by 90% at age 70.

Existence of Melanoma Susceptibility Locus

In the case of cutaneous malignant melanoma, due to a dramatic worldwide increase in the numbers of fair-skinned individuals, and the poor prognosis for individuals with advanced disease, much effort has been spent in identifying genes that increase risk in UV-exposed individuals. A candidate high-risk gene is MDM2, which when mutated in women may increase risk for melanoma, at younger ages, in concert with estrogens [37, 38]. Understanding of melanogenesis and its potential therapeutic targeting have also come from analysis of genes involved in the cell cycle in hope of discovering compounds that attenuate or nullify the effects of a given mutated gene which may present opportunity for targeted therapy. In this context, studies of somatic lesions in melanoma tumors and cell lines have provided leads and clues to clinicians and molecular geneticists in the identification of several chromosomal locations that exhibit loss of heterozygosity (LOH) and in some cases homozygous deletions. These efforts have led to discovery of 9p21, the site of a potential tumor-suppressor gene involved in melanoma susceptibility [39, 40]. This locus, also called MLM, is inherited as a dominant allele with penetrance that range upwards from 50%, and has been shown to encode a negative growth regulator, p16. p16 expression is known to cause cell cycle arrest, by targeting cyclin-dependent kinases, cyclin, and the retinoblastoma gene product pRb. Importantly, p16 inactivation may occur in nearly half of all advanced human cancers. Other genes possibly playing a part in melanoma formation include cdk4, a target of p16 biochemical inhibitory activity.

BRAF

Another mutation frequently found in melanomas developing on intermittently sun-exposed skin is BRAF. BRAF, a serine/threonine kinase, signals downstream of receptor tyrosine kinase (RTKs) and Ras proteins. The BRAF gene is the most frequent mutation (30–70%) observed in human melanoma. BRAF mutations are most common in the nodular and SS types, and rare in AL (5–10% of cases) and non-cutaneous melanomas. BRAF mutation correlates with distinct histopathologic features, such as intraepidermal melanoma nest formation and a larger rounder border of the tumor with surrounding skin. Moreover, the presence of a BRAF mutation in metastatic melanoma is associated with poorer prognosis from time of first metastasis, or time from first resected metastasis [41, 42].

Eighty percent of the BRAF mutations are found at exon 15, at a single amino acid residue, usually a Val→Glu substitution (referred to as V600E). This mutation alters the autoregulatory activation of the kinase causing increased kinase activation and signaling through the MAP kinase pathway and subsequent activation of the Brn-3 transcription factor. In studies of metastatic melanomas, the exact role of BRAF mutation in disease maintenance, progression and outcome remains equivocal [43]. A specific signature has been identified with the activating BRAF mutations that could be useful therapeutically. Studies of mutated BRAF have also led to the development of a drug called PLX4032, designed to target the V600E BRAF mutation [44].

Dietary Phytochemicals and Prevention of Skin Cancer and Melanoma

Skin Cancer Photoprotection and Photochemoprevention by Preventative Phytochemicals

Photoprotection and photochemoprevention are steps, activities and approaches designed to forestall and reduce the adverse exposure to factors that increase the risk for SCa. In photoprotection, actions are taken to primarily prevent overexposure to UV. These include activities such as avoidance of excessive exposure to the sun, use of protective clothing to cover areas of the neck, arms and legs, use of hats and sunglasses, topical application of sunscreens and blockers containing an appropriate sun protection factor for efficient absorption of UV light, minimized practice of tanning in tanning beds, and regular and timely self-examination for suspected early malignant lesions. Many of the recommended photoprotective measures require close adherence to lifestyle practices that are not easily implemented. Some, such as use of photoprotective sunscreen chemicals, may introduce side effects such as irritation, allergy, photosensitivity, and formation of ROS. Not surprisingly, photoprotective approaches have limited efficacy and success and should be supplemented with measures and considerations designed towards the prevention of SCa.

Photochemoprevention is a primary or adjunctive strategy that recognizes the formidable challenges required for complete destruction of cancer cells, even in the face of successful and apparently complete surgical removal of SCa cells or their state-of-the-art-test-confirmed eradication using toxic chemicals or radiation. Surgery often proves to be impractical in cases of metastatic carcinoma and conventional chemotherapy is well noted for its unintended toxic side effects and lack of specificity. Among the cancer therapeutic armamentarium considered to achieve increased specificity and improved efficacy without added cytotoxicity is chemoprevention, which is a concept pioneered by Sporn that refers to the use of pharmacologic or natural agents to impede development or progression of invasive cancer.

As suggested by Hong and Sporn, efficacy of chemopreventive candidates may have multiple mechanistic bases: reversal of abnormal differentiation, suppression of cell replication/growth, and induction of apoptosis. Additionally, prevention of activation of carcinogens and their increased removal (detoxification) are also considered prudent chemoprevention strategies. Recently a large number of dietary chemopreventive agents, including phytochemicals, have been reported as being capable of decreasing carcinogenesis. Several studies have evaluated the protective effect of natural products against UV induced damage in cells, tissues, animals and humans. Thus, chemopreventive phytochemicals may target oncogenic proteins that lead to the dysfunctional proliferation of cancer cells, act as intracellular modulators of apoptosis, induce transformation of normal epidermal keratinocytes to their carcinomic counterparts, as well as diminish the effect of protein molecules that are anti-apoptotic and enhance the ones that are pro-apoptotic. Since cancer is viewed as a multifaceted disease, in order to simultaneously target more mutagenic pathways, a variety of agents with different modes of action are needed.

Evidence in Support of Skin Cancer Preventative Phytochemicals

Phytochemicals

As mentioned, primary prevention of SCa has been the use of sunscreen or the wearing of protective clothing, but these approaches have had limited success. Several prospective trials show that sunscreens have little or no effect on NMSC incidence. Furthermore, some studies have indicated that sunscreen use may cause undesirable side effects, as mentioned earlier [45].

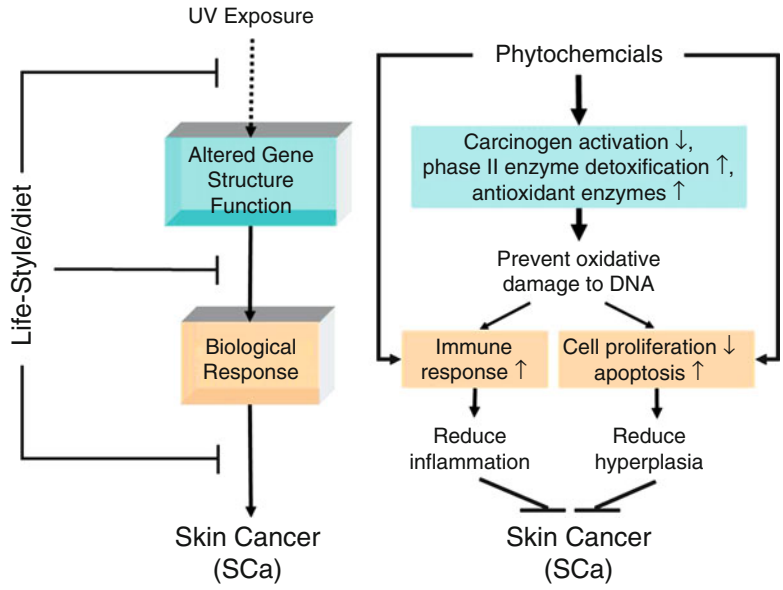


Fig. 27.4 Mechanism of chemoprevention by phytochemicals. Phytochemicals may act as chemopreventive agents to modify carcinogen activation, enhance phase II enzyme detoxification and modify antioxidant enzymes to prevent UV exposure induced DNA damage which prevents the occurrence of SCa. Phytochemicals may also modulate immune response or antiproliferative activity to reduce inflammation or decrease hyperplasia resulted in anti-SCa

The apparent inadequacies of current primary prevention methods have prompted researchers to search for other ways to protect against various types of cancer. A significant correlation between diet and many types of cancer has been shown in epidemiological data throughout the world. Additionally, in vitro and in vivo experiments suggest that there are a number of phytochemicals that display anti-cancer properties. The word phytochemicals means plant chemicals found in fruits, vegetables, beans, grains, and other plants. Use of phytochemicals as chemopreventive agents appears to have practical implications in reducing the risk of SCa because chemoprevention can be achieved by modification of diet and lifestyle.

While it is believed that these chemicals have protective properties, researchers are still studying how they work. There are several possible mechanisms through which these phytochemicals may act as chemopreventatives: they may modify carcinogen activation, enhance phase II enzyme detoxification, modify antioxidant enzymes, prevent oxidative damage to DNA, decrease inflammation, cell proliferation, and hyperplasia, modulate immune response, or induce apoptosis (Fig. 27.4) [46].

Phytochemicals exist in many forms. One of the main classes of phytochemical is the polyphenols, which can be divided into flavonoids and non-flavonoids. Polyphenols constitute one of the most abundant groups of plant metabolites. They range from simple phenolic molecules to highly polymerized compounds with molecular weights approaching 30 kDa. Recent interest in food phenolics and other phytochemicals has increased owing to their roles as antioxidants, antimutagens, and scavengers of free radicals in prevention of carcinogenesis. Studies have shown a correlation between an increased intake of phenolic antioxidants and a reduced risk of SCa. These antioxidants function as terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid peroxidation. To decrease the risk of prolonged oxidative damage, they rapidly donate a hydrogen atom to radicals to disrupt the oxidation of lipid and other molecules. Several representatives richly found in fruits and vegetables with such phenolic antioxidant capacity are concomitantly demonstrating anticancer effect through induction of apoptosis.

Some flavonoids that have been shown to have chemopreventative properties include apigenin, genistein, silymarin, and quercetin. Non-flavonoid polyphenols with chemopreventative activities include resveratrol, curcumin, and EGCG.

Isoflavones (Phytoestrogens)

Genistein. Genistein is an isoflavone present in soy, ginkgo biloba extract, Greek oregano, and Greek sage. It has been shown to possess antioxidant and anticarcinogenic effects in skin [47]. Genistein has potent anticarcinogenic effects that are largely independent of its estrogenic activities. For example, genistein is a potent and relatively specific inhibitor of tyrosine protein kinases, which are involved in almost all of the cell growth and proliferation signaling cascades [48]. In hairless mice, genistein, either topically applied or orally supplemented, dose-dependently inhibited UVB-induced skin carcinogenesis, in terms of reduction of tumor incidence and multiplicity, and was shown to arrest the growth and induce the differentiation of malignant melanoma in vitro by modulating TPK expression [49].

There is compelling evidence in support of genistein exerting photoprotective and photochemopreventive activity to minimize the detrimental effects of UVB irradiation in skin: genistein significantly inhibits UV light-induced oxidative damage in purified DNA and cultured cells, and pretreatment of hairless mice with genistein prior to UVB exposure inhibited UVB-induced generation of H_2O_2, lipid peroxyl radicals, and 8-OHdG in the epidermis [50]. Topical administration of genistein before UVB radiation reduced the expression of c-fos and c-jun, components of the activator protein AP-1, in SENCAR mouse skin in dose dependent manner [51]. It also dose-dependently inhibited the formation of CPD and restored the PCNA (proliferating cell nuclear antigen) expression in both mouse and reconstituted human skin [49, 52]. PCNA is profoundly expressed in proliferating and metastatic tumors and serves as a marker of DNA repair. The suppression of UVB-induced damage and PCNA expression restoration suggests that genistein may modulate UVB-mediated initiation and promotional activities. In human keratinocytes, genistein dose-dependently inhibited the UVB-induced phosphorylation of epidermal growth factor receptor (EGF-R) and MAPK [49]. Phosphorylation of EGF-R and activation of MAPK are thought to be involved in promotional activities, including the release of inflammatory mediators, such as prostaglandins, and the stimulation of cell proliferation.

Genistein inhibited in vitro UV-induced DNA oxidation and reduced erythema and histological inflammation induced by psoralen plus UVA (PUVA) in mouse skin, and inhibited UV-induced apoptotic changes, including caspase-3 and p21 activated kinase 2 activation in A431 human epidermal carcinoma cells [49].

In a clinical study examining the effect of genistein on UV-induced sunburn, genistein applied before UV exposure was found to significantly block sunburn and inhibit the induced photodamage [49].

Quercetin

Quercetin is a flavonoid found in fruits (citruses, apples, red grapes, and berries), vegetables (onions, green leafy vegetables), grains, and beverages (red wine, and black and green teas). Animal model studies regarding the cancer-preventing activities of quercetin have yielded mixed results: tumor incidence and multiplicity were reduced in one in vivo study of mice, and in another similar study, no inhibitory effects were observed [50]. In another example of mixed results, it was found that oral intake of quercetin does not prevent UVB-induced carcinogenesis, but it does restore the skin-associated contact hypersensitivity (CHS) response. Included here are results indicating quercetin's potential as a chemopreventative agent.

Quercetin inhibited the in vivo and in vitro growth of B16-BL6 cells (a murine melanoma variant highly metastatic to the lungs) in a concentration-dependent manner. It also potently inhibited in vitro cell invasion and metastasis to the lung in mice models [53]. In another study, quercetin almost completely blocked MEK1 activity, the effect being greater than a specific MEK inhibitor [54]. A component of the MAPK pathways, MEK plays a critical role in linking extracellular signals associated with Ras activation of nuclear transcription events associated with various types of tumors.

Quercetin has also been reported to induce apoptosis as a result of phosphatidylinositol 3-kinase (PI3K) inhibition and through other mechanisms including ROS generation. The PI3K signaling pathway represents an important target for the prevention of NMSC because it activates, via phosphorylation, Akt (a protein kinase involved in the inhibition of apoptosis) and mediates a cell survival response to UVB. Quercetin significantly inhibited Akt phosphorylation in mock-irradiated and UVB-irradiated HaCaTs, and stabilization of quercetin with ascorbic acid increased its efficacy as a PI3K inhibitor, indicated by a further reduction in phosphorylated Akt levels [55].

Resveratrol (*Trans*-3,4′,5-Trihydroxystilbene)

Resveratrol is a phytoalexin which has been found to mitigate age-related diseases such as neurodegeneration, carcinogenesis, and atherosclerosis. It is present in grapes, especially the skins, red wine, peanuts, fruits, and mulberries [47]. It is a potent antimutagen, antioxidant, anti-inflammatory, antiproliferative, an inducer of phase II drug metabolizing enzymes, and an inhibitor of COX and hydroperoxidase. Overexpression of COX-2, an enzyme involved in the conversion of arachidonic acid to prostaglandins, plays a role in carcinogenesis.

Studies have shown that resveratrol acts as a chemopreventative agent by inhibiting cellular events at all three stages of carcinogenesis (initiation, promotion, and progression) [56]. In the SKH-1 hairless mouse model, topical application of resveratrol significantly inhibited tumor incidence and delayed the onset of tumorigenesis in both pre- and post-UVB irradiation. Tumor multiplicity was also significantly reduced [57]. If applied before UVB radiation to mice, resveratrol can significantly inhibit UVB-induced increase in skin thickness, hyperplastic response, leukocyte infiltration, and COX-2/ornithine decarboxylase (ODC) activities [56]. In SKH-1 hairless mouse skin, topically applied resveratrol prior to UVB-irradiation resulted in a significant inhibition of UVB-induced cellular proliferation, the expression of survivin (a tumor promotion biomarker), and the phosphorylation of survivin [57].

Resveratrol is a potent antioxidant: pretreatment of human epidermal keratinocytes with resveratrol inhibited UVB-induced H_2O_2 generation and lipid peroxidation, which is a stable source of oxidative stress. Treatment of HaCaT cells with resveratrol prior to UVB irradiation resulted in an increase in cell survival, associated with the reduction of ROS production [56]. The antiproliferative effects of resveratrol were shown to be modulated by cell cycle regulatory proteins [58]. Resveratrol inhibits UV-mediated increase in PCNA, cdk, and MAPKK in mice [45].

The inflammatory, growth-modulatory, and oncogenic effects of many chemicals are modulated through NF-κB. Resveratrol has been shown to block the activation of NF-κB in certain cell types. Treatment of keratinocytes with resveratrol shows a significant dose- and time-dependent inhibition of UVB-induced activation of NF-kB [59]. At high doses, resveratrol acts as a pro-apoptotic compound, by inducing the loss of the mitrochondrial membrane potential. This leads to the release of cytochrome C and Smac/Diablo, and subsequent activation of caspase-9 and caspase-3 (key enzymes in apoptosis). Resveratrol also inhibits protein kinases, related to growth inhibition and apoptosis in various cancer cell models.

Several phase I clinical trials are currently under way to determine the pharmacokinetics and safety of resveratrol. Several reports suggest that although resveratrol has been shown to inhibit cancer cell growth in vitro, it has been ineffective in animal studies.

Epigallocatechin Gallate

Epidemiological studies have shown that high consumption of green tea decreases the frequency of various types of malignancies, including SCa. Green tea is a rich source of polyphenols (GTP), mainly of EGCG, as they constitute 30–35% of the dry weight of the leaf. The GTP is mainly derived from catechin.

In studies with Balb/c and SKH-1 hairless mice, it has been determined that both topical and oral administration of EGCG decrease the incidence, multiplicity and the volume of tumors induced by UV. The application of EGCG induces partial regression of papillomas in mice, as well as inhibiting the malignant transformation of UVB-induced papillomas to carcinomas. Furthermore, chronic administration showed no visible signs of toxicity.

Several studies in experimental models in vitro and in vivo indicated that the GTP prevents photocarcinogenesis through several molecular mechanisms. EGCG acts as potent antioxidant and can scavenge ROS, as superoxide radicals, hydroxyl radicals, H_2O_2, and singlet oxygen. The topical application of EGCG decreases the production of H_2O_2 and nitric oxide (NO) as well as the expression of nitric oxide synthase (NOS) in epidermis and dermis in C3H/HeN mice. EGCG also protects human skin from oxidative stress induced by UV and restores the level of glutathione (GSH) and antioxidant enzymes in the skin.

In the same way, the EGCG pretreatment inhibits UVR-induced infiltration of inflammatory leukocytes, particularly CD11b+ cells (a surface marker of monocytes/macrophages and neutrophils), which are the main producers of ROS in the skin. EGCG produces a balance in the relationship between cytokines IL-10/IL-12. This can be mediated by antigen presenting cells in the skin and lymph nodes or blocking the infiltration of CD11b+ cells that secrete IL-10. This suggests that application of EGCG on the skin reduces inflammation and inhibits the signals involved in cellular processes such as inflammation, proliferation and cell transformation that play an important role in the development of SCa [60].

UV-radiation causes an increase of active transcription factors such as NF-κB and members of the complex AP-1, c-Fos and c-Jun. GTP regulate signal transduction in human keratinocyte cell lines and in SKH-1 hairless mice (topically applied): they inhibit the expression of c-fos, the activity of AP-1 and modulate MAPK signaling and NF-κB induced by UV. Treatment of cultured cells with EGCG inhibits the expression of MMPs, which are involved in the degradation of collagen fragments and the collagen of the basement membrane. EGCG also regulates cell cycle progression and induces apoptosis in p53-dependent transformed cells. The oral administration of GTP in SKH-1 hairless mice increases the number of cells positive for p53 and induces the DNA repair process.

While the anticancer activities of tea and GTP have been observed in animal models of carcinogenesis, such activity has not been convincingly demonstrated in humans. This could possibly be due to the fact that the conditions in experimental studies can be carefully controlled, but epidemiological studies of humans cannot.

Curcumin

Curcumin is a polyphenol obtained from the turmeric rhizome, and possesses antitumoral, anti-inflammatory, and anti-infectious activities. Several studies have suggested antimutagenic activity, inhibition of radiation (and chemical carcinogen-induced) neoplastic lesions in many tumor models probably by an antioxidant mechanism [45].

Protection of cultured human cells from radiation-induced DNA damage may be due to its strong antioxidant properties [45]. It has been found that topical application of curcurmin in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-pretreated epidermis of CD-1 mice significantly inhibited UVA-induced ODC activity and ODC gene expression. Application significantly inhibited TPA- and UVA-induced ODC activity in mouse epidermis, which may be due to its role as a ROS scavenger.

The molecular basis of anticarcinogenic and chemopreventive effects of curcurmin is attributed to its effect on several targets, including transcription factors, growth regulators, adhesion molecules, apoptotic genes, angiogenesis regulators and cellular signaling molecules. Curcurmin inhibited UVB-induced AP-1 transcriptional activation in HaCaT cells, and strongly inhibited COX-2 mRNA and protein expression in UVB irradiated HaCaT cell.

Curcumin can prevent UV irradiation-induced apoptotic changes in human epidermoid carcinoma A431 cells, including c-Jun N-terminal kinase (JNK) activation, loss of mitochondrial membrane potential, mitochondrial release of cytochrome c, caspase-3 activation, and cleavage/activation of PAK 2 [45].

As with the tea polyphenols, the poor bioavailability of curcumin limits its cancer-preventing activity.

Silymarin

Silymarin is a flavonoid derived from the fruits and seeds of milk thistle. Silymarin is a mixture of mainly three flavonoids—silibinin, silydianin, and silychristan, with silibinin being the major and most biologically active component [61].

In mice models, topical application of silymarin inhibited carcinogenesis in terms of tumor incidence, multiplicity and growth of the tumors, as well as increased the latent period of tumor appearance. Silymarin administered in the diet of mice inhibited tumor multiplicity and tumor volume, but had only a moderate effect on tumor incidence [61].

Topical application has also shown a substantial protection against UVB-induced DNA damage, and inhibits sunburn cell formation, edema, apoptotic cell death, and COX and ODC activities [62]. There was also inhibition of UV-induced intracellular production of ROS and reactive nitrogen species.

Topical application of silymarin significantly inhibits UV-induced infiltration of leukocytes. Its application resulted in significant reduction in myeloperoxidase activity both in epidermis and dermis of UV-exposed mouse skin, which suggests the inhibition of UV-induced infiltration of inflammatory leukocytes and therefore the anti-inflammatory effect of silymarin [56]. Silymarin inhibits UVB-enhancement of IL-10 levels both in the skin and in the draining lymph nodes of mice. IL-10 is considered as an immunosuppressive cytokine. IL-12 production was also increased in the skin and draining lymph nodes of the mice. It has been shown that IL-12 has the ability to stimulate the immune system by stimulating the development and function of T-cells. It is possible that silymarin treatment enhances the levels of IL-12 in the draining lymph nodes by increasing the number of antigen presenting cells that migrate from the skin to the regional lymph nodes in UVB-irradiated mice [61].

Apigenin

Apigenin is a flavonoid present in herbs (endive, cloves), fruits (apples, cherries, grapes), vegetables (beans, broccoli, celery, leeks, onions, barley, parsley, tomatoes), and beverages (tea, wine). One of the most commonly consumed sources of apigenin is chamomile tea. It has been shown to be an anti-mutagen, antioxidant, free radical scavenger, anti-inflammatory, and anticarcinogen [45]. Apigenin's antioxidant effects and its role as a free radical scavenger have been studied extensively in in vivo and in vitro mammalian studies [63].

Application of apigenin prior to UV-induced tumorigenesis resulted in an inhibition of UV-induced increase of ODC activity and increased tumor-free survival in the mice [63]. Similarly, a DMBA-induced, TPA-promoted increase of ODC activity was inhibited by topically applied apigenin [64].

It has also been found that apigenin exhibits a number of molecular signaling effects, including modulation of the MAPK cascade. In many cancers, including skin cancer, defects in the MAPK cascade lead to uncontrolled growth.

Yet another effect of apigenin is on the cell cycle. Treatment of mouse keratinocytes by apigenin has been found to induce a reversible G2/M and G0/G1 arrest by inhibiting p34 and p21 kinase activity, respectively [65]. p34 and p21 are cyclin dependent kinases essential for the progression of the cell cycle. Cell cycle arrest was accompanied by increased p53 stability (p53 is a tumor suppressor protein) and accumulation of p53 [65].

Recent studies of mouse keratinocytes, as well as human keratinocytes, suggest that apigenin suppresses the UV-induced increase in COX-2 expression by inhibiting its transcriptional activity. While the effects of apigenin on human SCa have been studied in vitro, there have been no human clinical trials.

Identification and Characterization of Proteins Targeted by Dietary Phytochemicals in Prevention of Skin and Melanoma Carcinogenesis

As mentioned, in recent years epidemiological and experimental studies have focused on a wide variety of natural products that provide protection to the development of SCa because they can alter or correct a variety of cellular functions induced by UV. The use of natural products as photochemopreventive agents can contribute to reduction of SCa risk in combination with changes in lifestyle, diet and products for skin care. Epidemiological studies have suggested an inverse association between the consumption of fruits and vegetables and risk of cancer at several anatomical sites. This associated protection has been attributed in part to intake of phytochemicals, which are structurally diverse, polyphenolic compounds present in abundance in fruits, vegetables, and plant-derived beverages such as tea, red wine, and fruit juices.

It may be surmised that phytochemicals, by virtue of their functional attributes, interact and bind to specific proteins in responsive cells. To identify and isolate such phytochemical target proteins, a ligand affinity chromatography approach may be envisaged where phytochemicals of interest are immobilized individually on epoxy-activated agarose generating a platform that enables the detection, selection, and capture of target proteins in responsive cells and tissue specimens. Target proteins displaying the most pronounced qualitative differences between different stage melanoma cells will be further analyzed by mass spectrometry to verify whether distinct target proteins already exist in the human genome database, or are yet to be identified. Proteins successfully identified by mass spectrometry will be cloned and expressed. This is an innovative strategy that can probe low abundance proteins, reveal mechanisms of chemoprevention of phytochemicals of interest, provide insights accompanying the progression of SCa and melanoma at different stages, and generate SCa stage specific target protein profiles subserving as a mechanism-based repository for drug discovery and patient selection for responsiveness to treatment by phytochemicals.

The aforedescribed strategy may be readily applied to define the molecular targets that determine or contribute to reduction of an individual's risk of melanoma recurrence by resveratrol [66], on assumption that the plethora of bioactivities attributed to this dietary polyphenol is in part due to the ability of this grape-derived phytochemical to interact and bind specific target proteins [67, 68]. In contrast to many current studies on chemopreventives, where the mechanistic explanatory themes are largely focused on attributes of cancer cells [69]—such as (1) elaboration of autocrine growth signals, (2) non-responsiveness to growth inhibitory signals, (3) non-responsiveness to apoptotic signals, (4) unlimited proliferative potential, (5) recruitment of blood vessels to vascularize the incipient tumor, and (6) the ability to move through tissues and proliferate at sites distant from the site of the primary tumor—this approach relies on the specificity and affinity of resveratrol to define molecules and

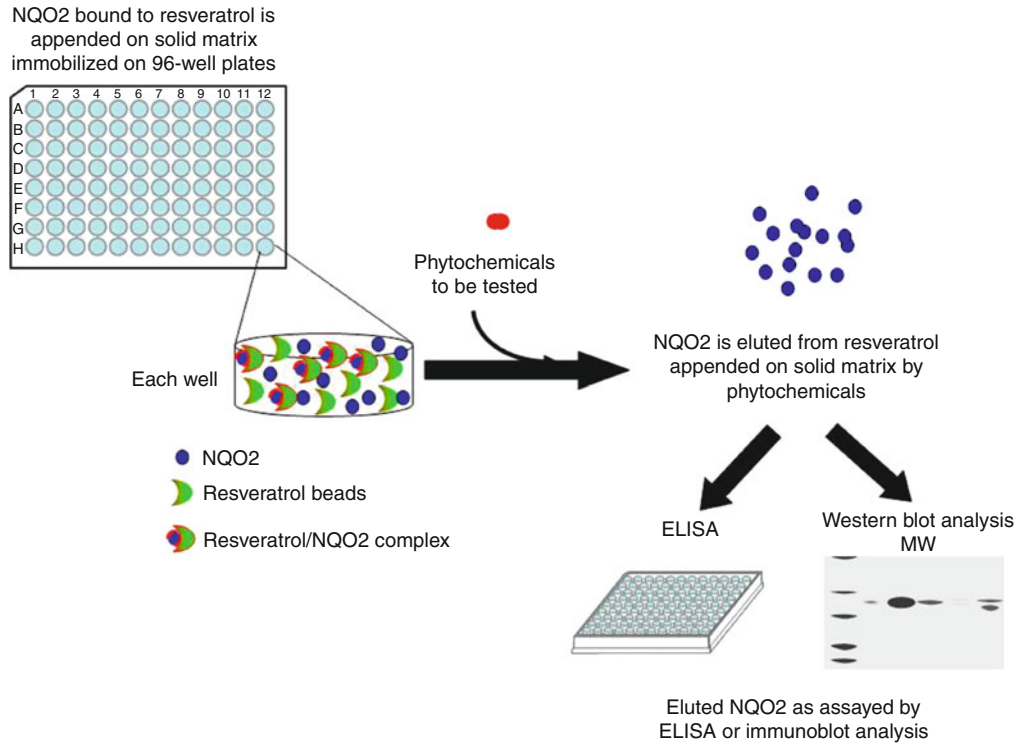


Fig. 27.5 Anti-SCa and melanoma drug screening by NQO2 displacement affinity chromatography. This method is used to screen lead compound from sets of chemically synthesized small molecules, based on displacement of NQO2 from resveratrol/NQO2 complex. The differentially displaced NQO2 can be further analyzed and quantified by ELISA and western blot analysis. This strategy provides the basis of a novel screen for uncovering phytochemicals from existing chemicals and from uncharacterized hits in plant extracts with potential anti-melanoma activities

molecular pathways that are relevant to the biology of melanoma. Unlike proteomics appearing in much of the current scientific literature, discovery and identification of target proteins on the basis of retention on resveratrol affinity columns offers a more narrow and defined scope since proteins being analyzed are selected based on interaction with a molecular bait (in this case resveratrol) chemically coupled to a solid support (epoxy-activated agarose). This constitutes a ligand-specific platform for affinity capturing of targeting proteins with possible direct or indirect involvement in the chemopreventive properties of the ligand under consideration. This strategy could add a new perspective in the analysis of melanoma that is independent of advances made in disease diagnosis and management, and could lead to identification of novel cellular targets different from those based on known malignant features of melanoma.

Towards the goal of discovering novel single or grouped phytochemicals for an anti-melanoma intervention initiative and eradicative efforts in the future, the aforedescribed approach can be further adapted as a cost-effective method for identifying novel anti-melanoma chemopreventive agents from existing chemicals and from uncharacterized hits in plant extracts. We have named this method differential displacement affinity chromatography (DAC). In its fundamental operation, a ligand is covalently attached to a solid matrix forming an immobilized high specificity and binding ligand-matrix platform suitable for capturing rare proteins from complex mixtures. The aspect of differential displacement of a known phytochemical, e.g., resveratrol, target protein called quinone reductase 2 (NQO2) bound to resveratrol (a high affinity inhibitor) coupled to agarose [70–72] provides the basis of a novel screen for uncovering phytochemicals with potential anti-melanoma activities (Fig. 27.5).

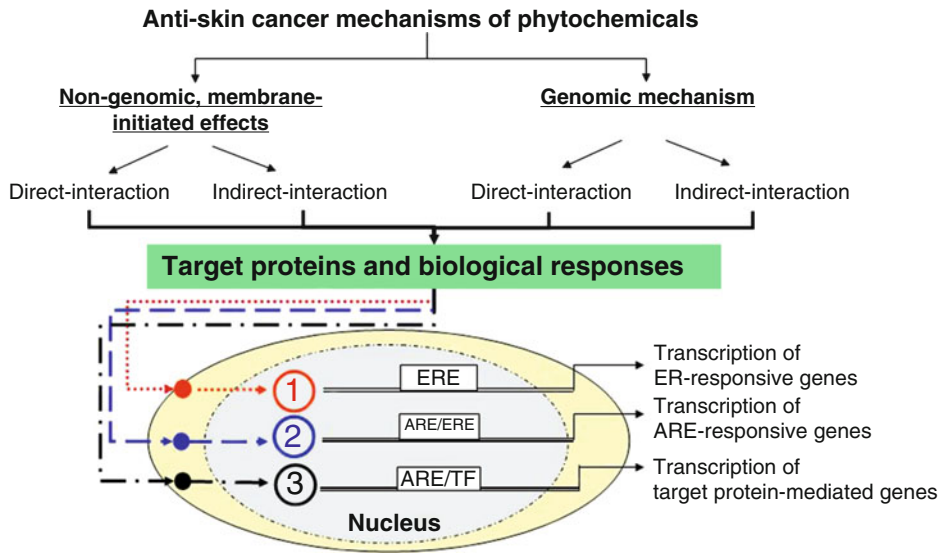
Competition and displacement of quantifiable NQO2 by known phytochemicals and unknown hits from plant extracts provides an unbiased, robust and comprehensive strategy for small molecules with anti-melanoma potentials. Marginally active and highly potent NQO2 displaceable molecules may be combined to generate anti-melanoma cocktails less likely to produce phytochemotherapeutic resistance and tolerance. Efficacy of grouped anti-melanoma phytochemicals may be validated using in vitro assays and systems that evaluate their selectivity on different aspects of carcinogenesis.

DAC is supported by observations from laboratory studies. Studies on the cardioprotective and chemopreventive activities of the grape-derived polyphenol resveratrol have shown using resveratrol coupled to an epoxy-activated agarose affinity column combined with mass spectrometry and cloning, that NQO2 binds to resveratrol. High-resolution X-ray crystal analysis of a NQO2-resveratrol complex shows that resveratrol is anchored to the active site of NQO2 through hydrogen bond formation with all three hydroxy groups of resveratrol, generating a molecular conformation of NQO2 that provides enthalpy for its high affinity binding to resveratrol (K_D, 35 ± 15 nM) [72]. Importantly, cloned NQO2 produced by recombinant technology can be quantitatively complexed to the resveratrol affinity matrix and be displaced using known and yet-to-be characterized chemicals. We propose the quantitative release of resveratrol-complexed NQO2 by increasing doses of phytochemicals as a novel paradigm for discovering new anti-melanoma chemicals. First, the displacement affinity approach offers simplicity—a single step release may reveal low-abundance, high potency anti-melanoma chemicals. Second, an identified chemical highly efficacious in displacing NQO2 from bound resveratrol is likely to have a functional link to the role NQO2 plays in phytochemical–cancer cell interaction. Third, the displacement strategy may enable a connection to be established between the candidate anti-phytochemicals, the displaced NQO2, and the biology of melanoma carcinogenesis. Finally, screens based on differential displacement of recombinant NQO2 should generate hypotheses-driven experiments for future studies.

Mechanism of Action of Dietary Phytochemicals in the Control and Management of Skin Cancer and Melanoma

Companion questions relevant to the described approach to discover candidate phytochemicals with anti-SCa and anti-melanoma potentials is the basis of efficacy of the identified chemicals and how they might exert their photopreventive mechanisms. Phytochemicals, many of them polyphenolic in chemical composition, are considered one of the most abundant groups of plant metabolites and also constitute an integral component of both human and animal diets. Unquestionably phytochemicals as a group display amazingly diverse complexity in chemical structure, molecular size, and solubility. Many of the simpler ones are low-molecular-weight phenolic derivatives, e.g., flavonoids that may display significant solubility under physiological settings depending on their structural features and polarity. It is therefore not unreasonable to propose that they may exhibit potential photopreventive activities that target many of the hallmark aspects of cancer, namely, unrestricted cell proliferation; autonomous growth and refractoriness to antigrowth signals; aversion to programmed cell death or apoptosis; capacity for adaptative, non-fastidious angiogenesis, tissue invasion, and metastasis (Fig. 27.6).

Our current hypothesis regarding anti-SCa activities of phytochemicals is that they act by non-genomic and genomic mechanisms, both directly and indirectly. The non-genomic photoprotective activities of phytochemicals may encompass its direct binding and interaction with cellular target proteins, as well as occur indirectly via its ability to function as effective modulator of enzyme activities. In the former direct mechanistic theme, for instance, resveratrol has been shown to bind to integrins on cell membrane, leading to integrin-coupled signaling cascades and subcellular protein



1. **Classical mechanism of phytochemicals.** Phytochemicals act as phytoestrogens and interfere with binding EREs located in promoters of ER-responsive genes.
2. **ARE/ERE dependent genomic activity.** Phytochemicals modulate Nrf2/Keap1 interaction, promoting translocation of Nrf2 and leading to activation and/or expression of ARE-responsive genes.
3. **ARE-independent, target protein-mediated genomic activity.** Target proteins mediate protein-protein interactions which in turn affect promoter and transcription of phytochemical-responsive genes.

Fig. 27.6 Pleiotropic anti-SCa mechanisms of phytochemicals. Proposed non-genomic and genomic activities of phytochemicals contributing to its suppression of skin and melanoma activities, both directly and indirectly, impinging on cell proliferation, inflammation, oxidative stress, and SCa-specific gene expression

distribution. Additionally, in the case of NQO2, it is conceivable that binding of resveratrol to NQO2 induces a conformational change, which directly alters its molecular fate in cellular trafficking and gene expression; a conformationally altered NQO2/resveratrol complex may facilitate additional interaction with NQO2-binding proteins to effect subsequent changes in gene expression. By contrast, in the latter indirect non-genomic mechanism of action, resveratrol has been reported to potently inhibit several enzymes [70].

Photoprotection by phytochemicals mediated by genomic mechanisms is postulated to involve multiple transcription regulatory features including (1) their phytoestrogenic effect where they effect transcription via the classical mechanism of binding to estrogen response elements (EREs) and (2) ability of phytochemicals to regulate antioxidant response element, ARE-dependent transcription, by promoting translocation of transcription factor Nrf2 from the inactive, cytosol-located Nrf2/Keap1 complex to the nucleus to facilitate transcription of antioxidant response element, ARE-responsive genes, and (3) ERE/ARE-independent, RTP-dependent, or mediated transcriptional events. We therefore set forth the hypothesis that SCa and melanoma are preventable using a diet-based strategy, and that suboptimal doses of phytochemicals present in aggregates in “healthy diets” may display optimized photoprotective potential via synergistic interaction to establish functional complementation, thereby creating a broadened anti-Sca/melanoma preventive index with potentiated anti-tumorigenic activities and ideally reduced untoward side effects. Importantly, this hypothesis can be readily tested and verified experimentally based on knowledge already available in the scientific literature and in advances continuously revealed by ongoing scientific studies.

Conclusions

In keeping with clues and insights revealed and suggested by epidemiological studies, dietary factors, specifically the consumption of fruits and vegetables, appear to play a protective role in skin carcinogenesis. Studies have repeatedly shown that an increase in intake of vegetables and fruits helps protect against SCa. Particularly noteworthy in this consideration are the phytochemicals which are abundant in fruits and vegetables. As has been reviewed as the focus of this chapter, it is evident that while phytochemicals are not essential for normal functioning of the body and are not key contributors to metabolic optimization in physiological settings, they nevertheless have beneficial health effects and play a significant role in the prevention and amelioration of diseases. In this role they act as anti-inflammatory agents and as scavenger of free radicals in addition to conferring protection via a variety of other mechanisms including regulation of cell signaling and gene expression. Clearly more research is required to more fully understand and elucidate the details on how they specifically target the different stages of skin carcinogenesis. Given the compelling evidence already available to date regarding phytochemicals found in fruits and vegetables as a prudent prevention and possibly intervention regimen for SCa, greater attention must be directed at changing menus and diets which tend to be low in vegetable and fruit content and are typically offered by the fast-food industry in many Western countries including the United States.

Summary Points

- Melanoma is neoplasm of melanocytes, which originate from neural crest cells, reside in the basal layer of the epidermis, and produce melanin
- Melanoma is the most common fatal skin cancer whose incidence rate has been steadly increasing in the past 30 years. A 3.0% increase per year has been seen since 1992 among white women aged 15–39 years while a 5.1% increase per year has been found in white adult men since 1985 as compared to a 4.1% increase per year in white adult women since 1975.
- Current treatment options involve surgery/local excision. For individuals with clinical evidence of lymph node enlargement or depth of invasion >4 mm, adjuvant therapy with IL-2, IFN-α should be considered. In stage IV (metastatic) disease—single-agent dacarbazine is the only chemical with small proven advantage
- Melanoma is notoriously resistant to all current modalities of cancer therapy, due to melanocytes with enhanced survival features
- Low frequency of p53 mutations in melanoma. In contrast, PI3K/AKT/PTEN pathway represents late but frequent event. Notably, PTEN overexpression can revert the invasive phenotype of human and mouse melanoma cell lines while blocking AKT by targeting PI3K inhibition of cell proliferation
- B-Raf is a Ras effector found to be mutated in 66% of human melanomas and also found in 82% of nevi—such may be an early step in progression
- Phytochemicals exert pleitropic effects: Induction of the cellular antioxidant network may provide chemoprotective effects in melanocytes; induction of apoptosis in melanoma cells may be important in establishing enhanced chemosensitivity

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Chapter 28
Soybean: Key Role in Skin Cancer

Sachin L. Badole and Sagar P. Mahamuni

Key Points

- Soybeans are a rich source of protein and phenolic compounds.
- Formulations containing soybean extract decrease oxidative damages of the skin.
- Genistein has potent antiphotocarcinogenic and antiphotoaging effects.
- Extracts of soybean seeds (EOS) reduce reactions to radiation and produced recovery in the skin.

Keywords Soyabean (*Glycine max* (L.) Merr.) • Genistein • PUVA-induced photodamage antiphotocarcinogenic effects • Antiphotoaging effects

Introduction

Glycine max (L.) Merr. (soybean) is a subtropical plant native to southeastern Asia. Soybean has been a dietary staple in Asian countries for at least 5,000 years. During the Chou dynasty in China fermentation techniques were discovered that allowed soybean to be prepared in more easily digestible forms such as tempeh, miso, and tamari soy sauce. Tofu was invented in second century China. Soybean was introduced to Europe in the 1700s and to the United States in the 1800s. Currently, Midwestern US farmers produce about half of the world's supply of soybeans. Soybeans are native to East Asia but only 45% of soybean production is located there. The other 55% of production is in the America. The United States produced 75 million tons of soybeans in 2000, of which more than one-third was exported. Other leading producers are Brazil, Argentina, Paraguay, China, and India [1, 2].

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Botanical Description

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Subfamily: Faboideae
Genus: *Glycine*
Species: *G. max*
Botanical name: *Glycine max* (L.) Merr.
Synonyms: *G. gracilis*, *G. soja*
Common name: Soybean, soya bean

The plant: It may grow not higher than 20 cm (7.8 in.), or grow up to 2 m (6.5 ft) high. The pods, stems, and leaves are covered with fine brown or gray hairs. *Leaves*: Trifoliolate, having three to four leaflets per leaf. Leaflets are 6–15 cm (2–6 in.) long and 2–7 cm (1–3 in.) broad. The leaves fall before the seeds are mature (Fig. 28.1a). *Flowers*: Big, inconspicuous, self-fertile flowers are borne in the axil of the leaf and are white, pink, or purple (Fig. 28.1b). *Fruit*: A hairy pod that grows in clusters of three to five, each pod is 3–8 cm long (1–3 in.) and usually contains two to four (rarely more) seeds (Fig. 28.1c). *Seeds*: 5–11 mm in diameter. Soybeans occur in various sizes and in many hull or seed coat colors, including black, brown, blue, yellow, green, and mottled. The hull of the mature bean is hard, water resistant, and protects the cotyledon and hypocotyl (or "germ") from damage. If the seed coat is cracked, the seed will not germinate. The scar, visible on the seed coat, is called the hilum (colors include black, brown, buff, gray, and yellow) and at one end of the hilum is the micropyle, or small opening in the seed coat which can allow the absorption of water for sprouting (Fig. 28.1d) [2].

Collection and Cultivation

Cultivation is successful in climates with hot summers, with optimum growing conditions in mean temperatures of 20–30°C; temperatures of below 20°C and over 40°C retard growth significantly. They can grow in a wide range of soils with optimum growth in moist alluvial soils with a good organic content. Soybeans, like most legumes, perform nitrogen fixation by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (syn. *Rhizobium japonicum*). However, for best results an inoculum of the correct strain of bacteria should be mixed with the soybean (or any legume) seed before planting. Modern crop cultivators generally reach a height of around 1 m (3 ft), and take 80–120 days from sowing to harvesting [1, 2].

Phytochemistry

Soybeans are composed of 43–48% protein, 18–21% oil, 4.9–6.8% sucrose, 0.8–1.2% raffinose, and 3.5–4.3% stachyose. Soybean seeds are a chief source for naturally occurring important isoflavones, genistein, diadzein, glycitein, and their glycosides and malonate conjugates are the main phenolic compounds [3]. Cyanidin-3-glucoside was the major anthocyanin of black soybean seed coats [4]. Delphinidin-3-glucoside, in the black-seeded variety, and pelargonidin-3-glucoside in the reddish-buff seed coats of T236 soybeans are other anthocyanins [5].

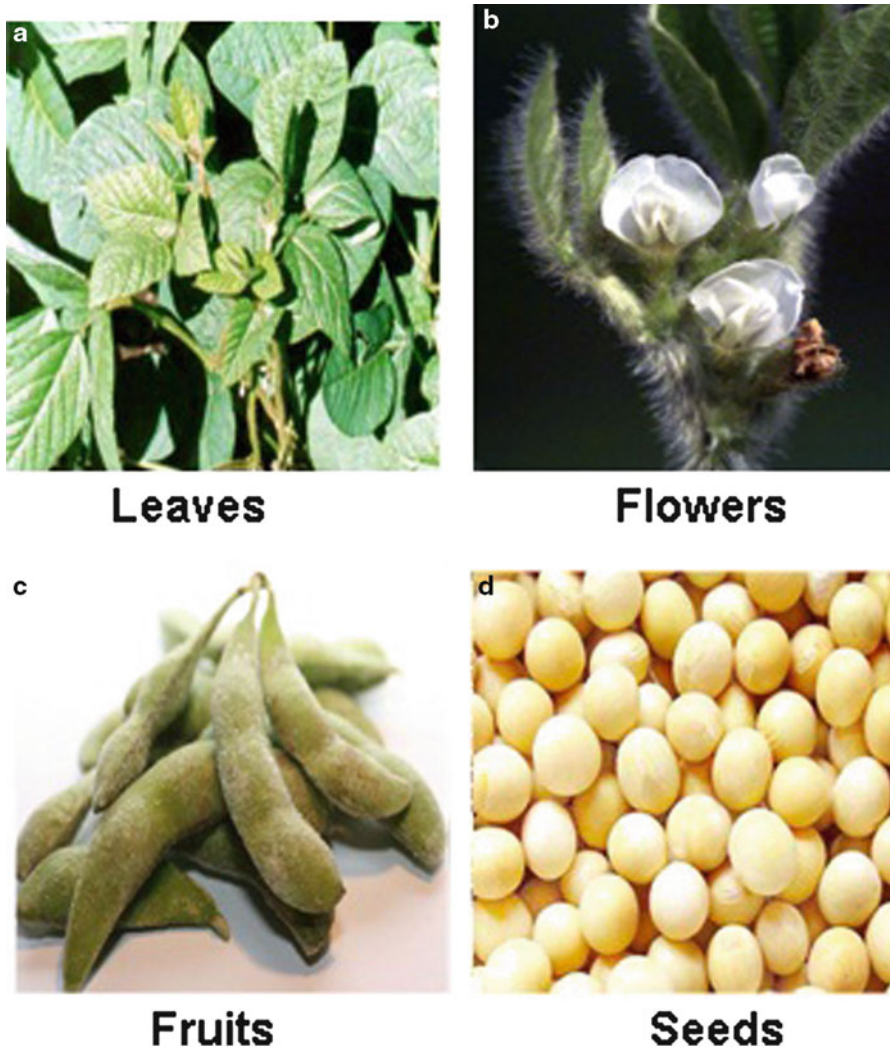

Fig. 28.1 Different parts of *Glycine max*: (**a**) Leaves, (**b**) flowers, (**c**) fruits, and (**d**) seeds

Application to Skin Cancer

Soyabean extracts from raw and roast soy hypocotyls showed inhibition of EBV-EA activation. The effect was stronger than the preparation from soybeans. Moreover, the treatment of mouse skin by the preparation of hypocotyls showed a delay of TPA-induced promotion of papillomas. As the hypocotyls include five times more isoflavones than soybeans, the inhibitory effect might come mainly from isoflavones. Isoflavones have a suppressive effect on proliferation of lymphocytes and many tumor cell lines in culture. Genistein induces G2-M arrest and other flavonoids (quercetin, luteolin, and flavone) induce G1 arrest. However, isoflavones are the major substances which have been thought to reduce the risk of cancer by hypocotyls [6]. Extraction from soybean and recombinant DNA production through the *lunasin* gene present two ways of producing lunasin in addition to chemical synthesis. Initial commercial soy products contain reasonable amounts of lunasin, ranging from 5.48 mg of lunasin/g of protein (defatted soy flour) to 16.52 mg of lunasin/g of protein (soy concentrate).

Synthetic lunasin is heat stable, surviving heat up to 100°C for 10 min. In vitro digestibility studies show that lunasin is digested by pancreatin but protected by chymotrypsin and trypsin inhibitors derived from soybean. Bioavailability studies of natural and recombinant forms of lunasin should help determine physiologically relevant doses of lunasin for cancer prevention. Lunasin is a unique 43-amino acid soybean peptide that contains at its carboxyl end the following: (a) nine Asp (D) residues, (b) an Arg-Gly-Asp (RGD) cell adhesion motif, and (c) a predicted helix with structural homology to a conserved region of chromatin-binding proteins. Transfection of mammalian cells with the *lunasin* gene arrests mitosis, leading to cell death. Exogenous application of the lunasin peptide inhibits chemical carcinogen-induced transformation of murine fibroblast cells to cancerous foci. Lunasin internalizes in the cell through the RGD cell adhesion motif, colocalizes with hypoacetylated chromatin, binds preferentially to deacetylated histone H_4 in vitro, and inhibits histone H_3 and H_4 acetylation in vivo in the presence of a histone deacetylase inhibitor. These results suggest a mechanism whereby lunasin selectively induces apoptosis, mostly in cells undergoing transformation, by preventing histone acetylation. Lunasin selectively induces apoptosis in E1A-transfected cells but not in nontransformed cells. In SENCAR mouse skin cancer model, dermal application of lunasin (250 g/week) reduces skin tumor incidence by 70%, decreases tumor yield/mouse, and delays the appearance of tumors by 2 weeks relative to the positive control. Lunasin is a new chemopreventive agent that functions possibly via a chromatin modification mechanism [7].

Genistein-containing soy materials in animal models of cancer significantly reduced the risk of cancer (incidence, latency, or tumor number). In addition, purified genistein delayed mammary tumor appearance in association with increased cell differentiation in mammary tissue in rats treated with 7,12-dimethylbenz[a]anthracene when administered neonatally, inhibited phorbol ester-induced H_2O_2 production in a model of skin cancer, and inhibited aberrant crypt formation in a model of colon cancer [8].

Long-term psoralen plus ultraviolet A radiation (PUVA) therapy is associated with an increased risk of squamous cell carcinoma and malignant melanoma. Genistein (4,5,7-trihydroxyisoflavone), a major isoflavone in soybeans and a specific inhibitor of protein tyrosine kinase, has been shown to inhibit UVB induced skin carcinogenesis in hairless mice. The protective effects of topical genistein on PUVA-induced photodamage are known. In two separate experiments, genistein in a dimethyl sulfoxide/acetone (1:9) solution was applied to SKH-1 female mice 1 h post 8-methoxy-psoralen dosing and 1 h prior to UVA irradiation. Application of genistein significantly decreased PUVA-induced skin thickening, and greatly diminished cutaneous erythema and ulceration in a dose-dependent manner. Histological examination showed that PUVA treatment of mouse skin induced dramatic inflammatory changes throughout the epidermis; topical genistein prevented these changes without noticeable adverse effects. Cells containing cleaved poly(ADP-ribose) polymerase (PARP) and active caspase-3 were significantly increased in PUVA-treated skin as compared with unexposed control skin. Topical genistein completely inhibited cleavage of PARP and caspase-3. Proliferating cell nuclear antigen (PCNA) positive cells were observed in suprabasal areas of the epidermis and were significantly decreased in PUVA treated skin compared with both control samples and samples treated with PUVA plus topical genistein. These results indicate that genistein protects the skin from PUVA-induced photodamage. Genistein may serve as a preventive agent against PUVA-induced photodamage and thus possibly PUVA-induced carcinogenesis. Clinical trials on the photoprotective ability of genistein are currently underway to determine the protective effects of genistein on PUVA-treated patients [9].

Antioxidant activity of the soybean extract (isoflavin beta) and of formulations added with this extract was evaluated using stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and deoxyribose as well as the lipid peroxidation inhibition assays. For all the assays the extract showed a dose-dependent activity, and IC_{50} of 21.03 lg/mL in lipid peroxidation inhibition, 161.8 lg/mL in DPPH, and 33.5 ng/mL in hydroxyl radical scavenging assay. The antioxidant activity of the extract added in the formulations could not be assessed using the deoxyribose assay. However, the lipid peroxidation inhibition and DPPH scavenging assays could be successfully applied for the antioxidant activity

evaluation of the formulations added with soybean extract to protect the skin against free radicals, which can be generated by the ultraviolet radiation exposure [10].

Soybean extract was dispersed in two different topical formulations, allowing for skin retention using modified Franz diffusion cells and *in vivo* activity of these formulations to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) increases in the skin of hairless mice was studied.

The physicochemical stability was evaluated by pH, globule size, and centrifugation test. Furthermore, functional stability was evaluated by antilipoperoxidative activity. The two topical formulations were stored at 4°C, 30°C/60% RH and 40°C/70% RH for 6 months. The evaluation of the antiperoxidative stability of soybean extract itself and incorporated in formulations did not demonstrate loss of activity by storage at 4°C/6 months. During 6 months of the study in different storage conditions the formulations 1 and 2 added or not with soybean extract were stable to physicochemical tests. The effect of antioxidant compounds detected by the inhibition of MDA formation was time dependent for formulation 2 as detected in the skin retention study. Pretreatment with formulation 1 or 2 significantly diminished TPA-induced H_2O_2 and MDA generation. Formulations containing soybean extract may be a topical source of antioxidant compounds that decrease oxidative damages of the skin [11].

Genistein potently inhibits the UVB-induced skin carcinogenesis and photodamage in animals. The possible mechanisms of the anticarcinogenic action include scavenging of reactive oxygen species, blocking of oxidative and photodynamic damage to DNA, inhibition of tyrosine protein kinase, downregulation of EGF-receptor phosphorylation and MAPK activation, and suppression of oncoprotein expression in UVB-irradiated cells and mouse skin. Genistein effectively blocked UVB-induced skin burns in humans as well as PUVA-induced photodamage and molecular alterations in hairless mouse skin. The antipromotional activities are primarily associated with the anti-inflammatory pathways, downregulation of TPK activities, and expression of protooncogenes associated with cell proliferation. Soybean isoflavone genistein has potent antiphotocarcinogenic and antiphotoaging effects and will have promising applications in the field of dermatology [12].

Extracts of soybean seeds (EOS), [*G. max* (L.) Merr.], reduced radiation dermatitis induced by radiation. It was reported that the reduction in skin injury using natural products from soybean seeds as protection against radiation. Radiation dermatitis in EOS-treated animals (0.25 g/cm^2 skin once daily) was less severe than that in the control group after 50 Gy of radiation. Within 29 days after radiation, the skin scores in the control group were higher than those in the EOS group. The histologic findings indicated that skin reactions after 50 Gy of radiation were mild in both groups. However, the skin reactions in the EOS group were less prominent than those in the control group. The EOS-treated rats had normal hair growth, but hair growth was suppressed in control rats. We conclude that EOS are able to reduce reactions to radiation and can achieve a better recovery in the skin [13].

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Chapter 29
Bioactive Polyacetylenes of Carrots in Cancer Prevention

Lars Porskjær Christensen

Key Points

- Epidemiological studies suggest that carrots play a central role in cancer prevention in particular in Europe and North America.
- Carrots contain bioactive polyacetylenes with cytotoxic and anti-inflammatory activity. In addition these polyacetylenes have shown to be bioavailable and to have anticancer effect in a preclinical rat study. Consequently, they may be considered as important nutraceuticals that contribute significantly to the cancer preventive effects of carrots.
- The role of bioactive polyacetylenes in carrots in cancer prevention is still to be confirmed in clinical and in further preclinical trials.
- Antioxidants such as carotenoids and flavonoids do not seem to play a central role in explaining the cancer preventive effect of carrots.
- Genotype, cultivation, and thermal processing have a significant impact on the content of polyacetylenes in carrots and may be important for improving the cancer preventive effects of this vegetable.

Keywords Carrots • Polyacetylenes • Cancer prevention • Anti-inflammatory activity • Cytotoxicity • Anticancer effect

Introduction

Many epidemiological studies have shown an inverse association of fruit and vegetable intake with cancer risk [1–5], and that the daily intake of fruit and vegetables should be around 400–600 g in order to decrease the risk of this disease [2, 6]. The cancer preventive effects of fruit and vegetables has for many years primarily been ascribed to their contents of vitamins, minerals, fibers and antioxidants, but still the compounds responsible for the cancer preventive effects of these foods are largely unknown. However, if we look at specific vegetables, it may be possible to give a more unambiguous answer to their cancer preventive effect. This is, for example, the case with carrots (*Daucus carota* L., Apiaceae).

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Epidemiological studies have shown that a high content of natural β(beta)-carotene in blood is correlated with a low incidence of several types of cancer, while intervention studies have shown that supplementation with β(beta)-carotene does not protect against development of this disease [7, 8]. However, in most European countries and North America more than 50% of the β(beta)-carotene intake is provided by carrots. In these regions of the world carrot consumption is better correlated with the intake of α(alpha)-carotene than with the intake of β(beta)-carotene [9]. Several studies have also found stronger negative correlations of cancer with the intake of α(alpha)-carotene rather than β(beta)-carotene [10–12]. However, a beneficial effect of any compound primarily found in carrots would give the same correlations. Based on these findings it is now widely accepted that carrots play a central role as a protecting vegetable against cancer. Until recently this was seen as a strong indication of a cancer-preventing effect of α(alpha)- or β(beta)-carotene or a combination of these [13]. Carotenoids and polyphenols (e.g., flavonoids) are well-known antioxidants in vitro and the cancer-preventing effects of carrots have often been explained by their relative high content of these antioxidants being able to reduce oxidative damage on cells from free radicals or other reactive molecules [5, 14].

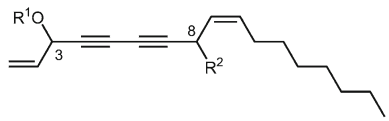
The antioxidant hypothesis is built on the assumption that antioxidants due to their reducing capacity are able to neutralize reactive oxygen and nitrogen species (ROS/RNS) that are generated constantly in the body. If the ROS/RNS outnumber the capability of the antioxidant defense system in the body this may lead to serious oxidative damages, due to reaction with vital compounds such as DNA, proteins, and lipids, which in the end can result in cancer and other diseases. However, it has never been proven that antioxidants obtained through our diet provide further protection against ROS/RNS in vivo than naturally occurring antioxidants such as superoxide dismutases and glutathione [15]. Intervention studies have also shown that supplementation with high levels of antioxidants does not protect against cancer [16]. In fact, it has been demonstrated that supplementation with high levels of antioxidants such as β(beta)-carotene may increase the risk of cancer [7, 8].

In most cases where antioxidants have shown effect against cancer, the mode of action of these compounds is probably not related to their antioxidant activity. So in order to explain the anticancer effect of carrots we have to focus on highly bioactive compounds with mechanism of actions different from antioxidants. Highly bioactive compounds are often toxic in high concentrations but may have beneficial effects in low concentrations and such bioactive compounds are indeed present in carrots and include sesquiterpenoids [17], phenylpropanoids [18], and polyacetylenes [19–21]. The polyacetylenes in carrots are of the falcarinol type (Fig. 29.1) and they have in recent years received considerable attention due to their anti-inflammatory activity, cytotoxicity, and potential anticancer effect [5, 19–26]. It is well known that there exist a close link between inflammation and cancer, and that inflammatory diseases can lead to cancer [27]. The cytotoxic and anti-inflammatory properties of falcarinol type polyacetylenes therefore seem to be a plausible explanation for the anticancer effect of carrots. In the human diet, carrots are the major dietary source of falcarinol type polyacetylenes, although they are also found in many other vegetables and medicinal plants of the Apiaceae family [19–21, 24]. Polyacetylenes of the falcarinol type could therefore turn out to be important nutraceuticals.

This chapter focuses on bioactive polyacetylenes in carrots in cancer prevention as well as factors such as genotype, cultivation, and thermal processing that may have a significant impact on the content of polyacetylenes in carrots and hence are important for improving the cancer preventive effects of this vegetable.

Polyacetylenes in Carrots

Polyacetylenes are fatty acid derivatives that are heat labile, sensitive to oxidation and may undergo photodecomposition if exposed to ultraviolet (UV) light [19, 28, 29]. To isolate, quantify, and characterize these natural products in carrots is therefore not an easy task. Sixteen polyacetylenes have been

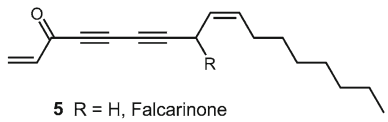


1 $R^1 = R^2 = H$, (3*R*)-Falcarinol (panaxynol)

2 $R^1 = H$, $R^2 = OH$, (3*R*,8*S*)-Falcarindiol

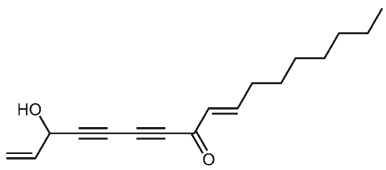
3 $R^1 = COCH_3$, $R^2 = OH$, (3*R*,8*S*)-Falcarindiol-3-acetate

4 $R^1 = H$, $R^2 = OCOCH_3$, Falcarindiol-8-acetate

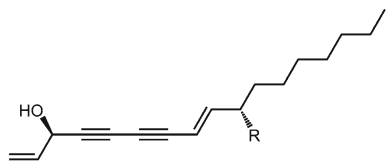


5 R = H, Falcarinone

6 R = OH, Falcarinolone

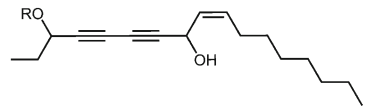


7 Isofalcarinolone



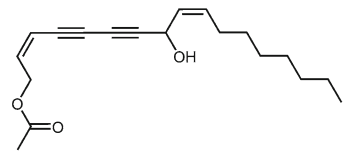
8 R = OH, Panaxydiol

9 R = OOH, Ginsenoyne K

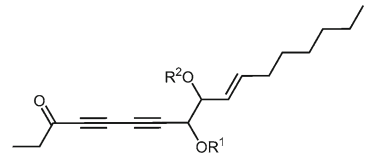


10 R = H, 1,2-Dihydrofalcarindiol

11 R = $COCH_3$,1,2-Dihydrofalcarindiol-3-acetate

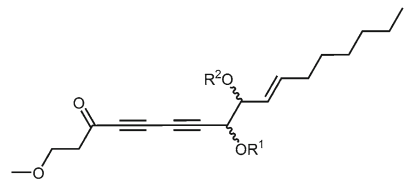


12 1-Acetoxy-(2*Z*,9*Z*)-heptadeca-2,9-diene-4,6-diyn-8-ol



13 $R^1 = COCH_3$, $R^2 = H$, (*E*)-Falcarindiolone-8-acetate

14 $R^1 = H$, $R^2 = COCH_3$, (*E*)-Falcarindiolone-9-acetate



15 $R^1 = COCH_3$, $R^2 = H$, (*E*)-1-Methoxy-falcarindiolone-8-acetate

16 $R^1 = H$, $R^2 = COCH_3$, (*E*)-1-Methoxy-falcarindiolone-9-acetate

Fig. 29.1 Chemical structures of polyacetylenes isolated from carrots

isolated from carrots, which are all aliphatic C_{17}-polyacetylenes of the falcarinol type (Fig. 29.1) [18–21, 24, 30–33].

The polyacetylenes in carrots have very characteristic UV spectra due to the presence of triple and double bonds and carbonyl groups in various conjugated configurations (Fig. 29.1). Consequently, qualitative and quantitative analysis of polyacetylenes in carrot extracts have mainly been performed by high-performance liquid chromatography (HPLC) combined by photodiode array or simple UV detection [19, 24, 26, 30, 34–42], although liquid chromatography–mass spectrometry (LC–MS) [19, 24, 34, 36, 43, 44] and gas chromatographic techniques [19, 45] are also suitable methods for analyzing these compounds. For extraction of polyacetylenes from carrots the most used method is a multiple extraction with ethyl acetate under stirring at room temperature in the dark in various time intervals resulting in an almost complete extraction of these compounds [36, 40, 41, 43]. For separation of carrot polyacetylenes by HPLC a reversed phase C_{18} column is often used in combination with a simple stepwise gradient consisting of aqueous acetonitrile containing increasing proportions of acetonitrile as the

mobile phase [19, 24, 34, 36, 40, 41]. The major polyacetylenes in carrots are falcarinol (**1**), falcarindiol (**2**), and falcarindiol-3-acetate (**3**), which normally appear in concentrations 6–60 mg kg^{-1} fresh weight (FW), 6–120 mg kg^{-1} FW, and 6–25 mg kg^{-1} FW, respectively [32, 35, 37, 40–42, 44, 45].

Bioactivity of Polyacetylenes in Carrots

Allergenicity and Alkylating Properties

Falcarinol is a potent contact allergen being responsible for allergic skin reactions from plants, including carrots [46–49]. The allergenic properties of falcarinol indicate that it is very reactive towards thiol and/or amino groups in proteins, thus capable of forming hapten-protein complexes (antigens) [46, 47]. The reactivity of falcarinol towards proteins is probably due to its hydrophobicity and its ability to form a stable carbocation (resonance stabilized) with the loss of water, thereby acting as a strong alkylating agent [19–22, 46]. No protein targets for falcarinol have been identified; however, it has recently been demonstrated that falcarinol covalently binds to the cannabinoid CB_1 receptor and induces pro-allergic effects in skin, thus confirming the alkylating properties of falcarinol [28]. Although, other polyacetylenes of the falcarinol type such as falcarindiol do not seem to be allergenic [47], they still have the possibility to generate relatively stable carbocations due to the presence of hydroxyl groups at C-3 and/or C-8 (Fig. 29.1). Consequently, they may also bind covalently to various biomolecules in almost the same way as falcarinol, thus explaining the bioactivities of falcarinol type polyacetylenes, including their potential anticancer effect.

Anti-inflammatory Activity

Recent data have shown that inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, invasion, and metastasis [27]. Inflammation leads for example to an induced expression of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. COX enzymes convert arachidonic acid to prostaglandins, which play a central role in many normal physiological processes and as inflammatory mediators. COX exists in two isoforms COX-1 and COX-2, and both COX enzymes are up-regulated in a variety of circumstances [50]. Normally COX-1 is constitutively expressed in a broad range of cells and tissues. In contrast, COX-2 is normally absent in most cells and tissues but it is highly induced in response to pro-inflammatory cytokines, hormones, and tumor promoters. Furthermore, COX-2 derived prostaglandin E2 is the major prostaglandin, which play an important role in cancer development as it can promote tumor growth activating signalling pathways controlling cell proliferation, migration, apoptosis, and/or angiogenesis [50]. Epidemiological studies and clinical trials indicate that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin can decrease the incidence of several types of cancers with up to 50%. The ability of NSAIDs to inhibit COX enzymes underlies their mechanism(s) of cancer prevention [27, 50]. Falcarinol and falcarindiol have shown to be strong inhibitors of LOXs (5-, 12- and 15-LOX) that like COX enzymes are involved in tumor-progression processes [51–54]. Furthermore, falcarindiol is an effective inhibitor of COX-1 and -2 [54–57], and in particular COX-1 where the lowest IC_{50}-value reported is 0.3 μ(micro)M [56]. Inhibitory activity of COX enzymes have also been demonstrated for falcarinol, falcarindiol-8-acetate (**4**), and panaxydiol (**8**), although the COX inhibitory activity of these polyacetylenes is considerably less compared to falcarindiol [53, 55, 57].

The transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κ(kappa)B) plays a key role for the inducible expression of genes mediating pro-inflammatory effects and is

therefore also an important target for the chemo-prevention for inflammation and cancer [27, 50]. A large variety of inflammatory signals lead to NF-κ(kappa)B activation, including lipopolysaccharide (LPS), nitric oxide (NO), and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α(alpha) [38]. It has been demonstrated that extracts of purple carrots, rich in polyphenols possess anti-inflammatory activity by decreasing LPS induced production of IL-6, TNF-α(alpha) and NO in macrophage cells in concentrations of 10 μg ml^{-1}. Bioassay-guided fractionation of the extracts revealed, however, that the anti-inflammatory activity were due to falcarinol, falcarindiol, and falcarindiol-3-acetate suggesting that they are responsible for this activity of carrots [38].

The anti-inflammatory activity of carrot polyacetylenes can be explained by their alkylating properties leading to an inactivation of for example COX and LOX enzymes and NF-κ(kappa)B. Thus, the anti-inflammatory activity of bioactive polyacetylenes may contribute to a deeper understanding of the anticancer effect of carrots.

Cytotoxicity

Falcarinol has been extensively studied for its cytotoxicity. Falcarinol has been shown to be highly cytotoxic to cancer cell lines such as leukemia (L-1210), human gastric adenocarcinoma (MK-1), mouse melanoma (B-16), human Caucasian colon adenocarcinoma (Caco-2), and mouse fibroblast-derived tumor cells (L-929) [58, 59]. The most toxic effect has been observed for MK-1 cells with an ED_{50} of 2.7 ng ml^{-1} [58]. In the same study falcarinol was found to be surprisingly less cytotoxic to normal cell cultures with ED_{50} values that were over 600 times higher compared to, for example, MK-1 cells. This is in contrast with a recent study by Purup et al. [22] who investigated the differential effects of falcarinol and falcarindiol on human intestinal (Int.) epithelial cells of cancer (Caco-2) and normal (FHs 74 Int.) origin, and found that the growth inhibitory effects of these polyacetylenes on normal and cancer cells were almost of the same magnitude (Fig. 29.2). The selective in vitro cytotoxicity of falcarinol against cancer cells compared to normal cells therefore appears to depend on the tested cell lines.

The cytotoxic activity of falcarindiol has also been demonstrated in numerous cancer cell lines [22, 24, 60–62], although it appears to be less bioactive than falcarinol (Fig. 29.2). However, falcarinol and falcarindiol are able to both inhibit and stimulate proliferation of Caco-2 cells depending on the concentration (Fig. 29.2). This biphasic effect (hormesis) on cell proliferation observed for these polyacetylenes has also been observed in other studies [23, 26, 63]. For example, in a study by Hansen et al. [26] it was shown that falcarinol could stimulate differentiation of primary mammalian cells in concentrations between 1 and 100 ng ml^{-1}. Toxic effects were found above >500 ng ml^{-1}, while β(beta)-carotene had no effect at even 100 μg ml^{-1} [26]. These results are in accordance with the hypothesis that toxic compounds have beneficial effects at certain lower concentrations [64]. Falcarinol and falcarindiol are therefore among the bioactive components that could explain the cancer preventive effects of carrots. This hypothesis is supported by recent studies on the anti-inflammatory compounds of carrots as described in the Sect. "Anti-inflammatory Activity." Finally, panaxydiol has shown medium level cytotoxicity against leukemia, lymphoma, and myeloma cell lines with IC_{50} values of approximately 30 μM [24, 65].

The molecular mechanism of falcarinol type polyacetylenes underlying their cytotoxic activity is most likely related to their alkylating properties as discussed in the Sect. "Allergenicity and Alkylating Properties". Further support for this hypothesis comes from a study by Purup et al. [22] who showed that falcarinol significantly inhibited cell proliferation in Caco-2 cells at 2.5 μg ml^{-1} ($P<0.01$), whereas its oxidized form, falcarinone (**5**), only inhibited proliferation in Caco-2 cells at 20 μg ml^{-1} ($P<0.001$). In addition, the dose of 20 μg ml^{-1} of falcarinone only caused around 50% reduction in cell proliferation compared to >90% reduction in cell proliferation for falcarinol at this concentration (Fig. 29.3).

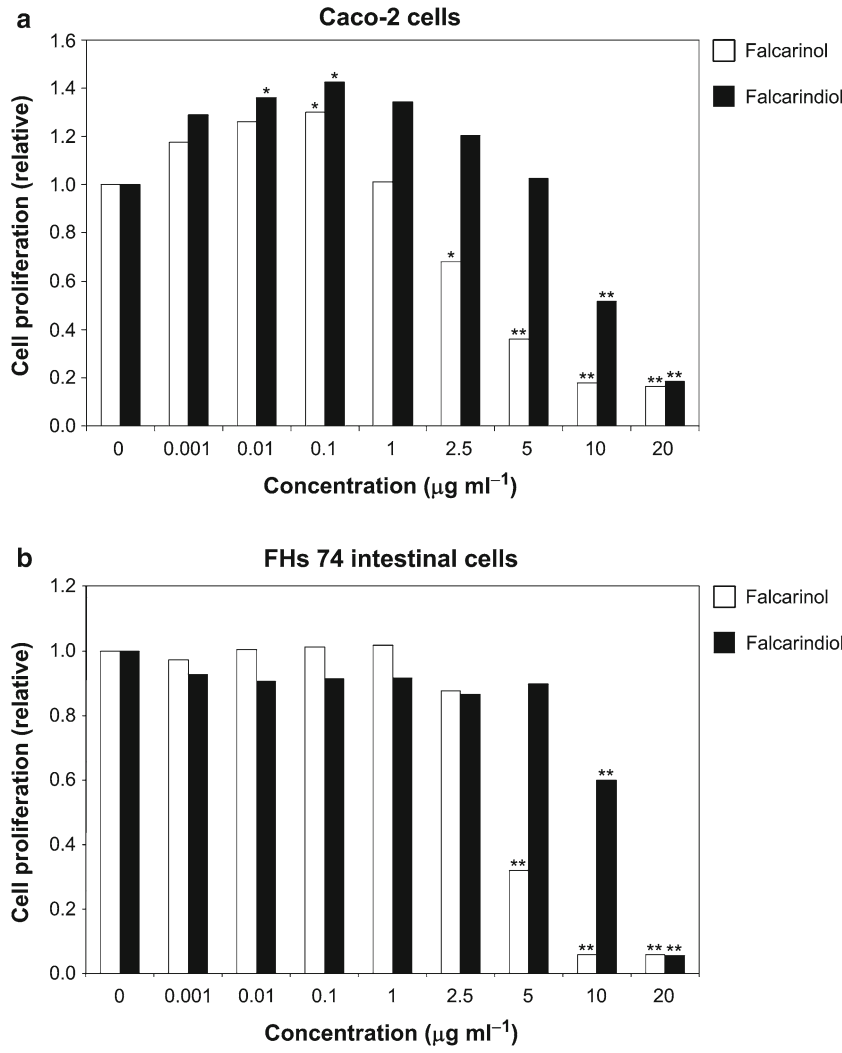


Fig. 29.2 Effect of increasing concentrations of falcarinol and falcarindiol on cell proliferation in human intestinal epithelial cells (**a**) of cancer origin (Caco-2 cells) and (**b**) normal origin (FHs 74 intestinal cells), cultured for 72 h in 0.625% fetal calf serum. Values are least-squares means obtained from cultures in quadruple samples from two experiments and presented as relative to proliferation obtained in medium without falcarinol or falcarindiol (basal medium). Standard error of the mean were 0.15 and 0.08 (relative values) for **a** and **b**, respectively. Values significantly different from proliferation obtained in basal medium are indicated: *, $P<0.05$; **, $P<0.001$

These results clearly demonstrate that falcarinone is a much less potent inhibitor of cell proliferation compared to falcarinol. Falcarinone lack a hydroxyl group at C-3; hence, this polyacetylene is not able to generate a reactive carbocation by the loss of water and to act as a strong alkylating agent in accordance with the above results. Other falcarinol type polyacetylenes such as falcarindiol and falcarindiol-3-acetate have the possibility to generate two reactive sites at C-3 and C-8 for nucleophilic attack. This reduces, however, the lipophilic character of these compounds and hence their reactivity. This is in accordance with the observed nonallergenic properties of falcarindiol [46, 47] and its less cytotoxic activity in comparison to falcarinol (Fig. 29.2). The proposed mode of action of falcarinol type polyacetylenes indicates synergistic, additive, and/or antagonistic effects between these bioactive

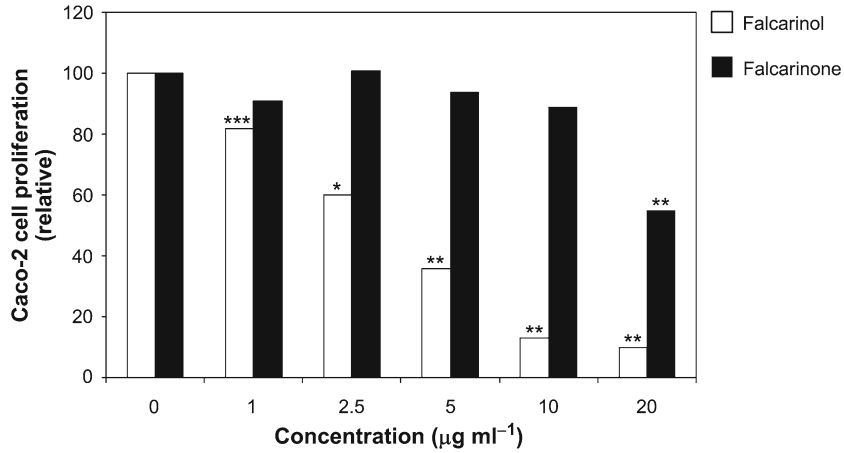


Fig. 29.3 Effect of increasing concentrations of falcarinol and falcarinone on cell proliferation in Caco-2 cells cultured for 72 h in 0.625% fetal calf serum. Values are least-squares means obtained from cultures in quadruple samples from two experiments and presented as relative to proliferation obtained in medium without falcarinol or falcarinone (basal medium). Standard error of the mean values was 6.4 (relative values). Values significantly different from proliferation obtained in basal medium are indicated: *, $P<0.01$; **, $P<0.001$. Values showing a tendency to be different from proliferation obtained in basal medium are indicated: ***, $P<0.1$

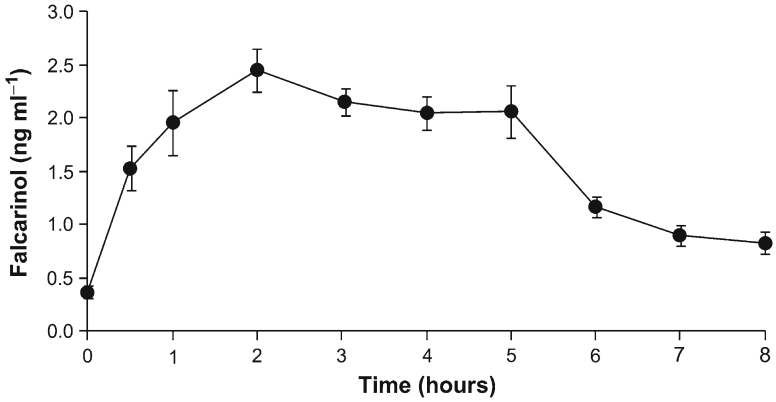


Fig. 29.4 Concentration of falcarinol in plasma of 14 volunteers as a function of time after ingestion of a breakfast meal consisting of 900 ml carrot juice containing 12.0 mg falcarinol. Mean ± standard error of mean

polyacetylenes, which may affect the anticancer activity of individual polyacetylenes as discussed in the Sect. "Synergistic Effects."

The suppressive effect of falcarinol and falcarindiol on cell proliferation of tumor cells [66, 67], is probably related to their ability to arrest the cell cycle progression at various phases of the cell cycles; hence, they may be able to induce apoptosis as demonstrated in cancer cells [23]. This support the hypothesis that the cytotoxicity and potential anticancer effect of falcarinol type polyacetylenes is due to their ability to interact with various biomolecules. Further support for the possible role of bioactive polyacetylenes in carrots in cancer prevention comes from bioavailability studies in humans. When falcarinol and falcarindiol were administered orally via carrot juice they were rapidly absorbed reaching maximum concentrations in serum of approximately 2.5 ng ml^{-1} at 2 h after administration (Fig. 29.4) [19, 21, 34]. This is within the concentration range where the in vitro data indicate a beneficial physiological effect, and a possible inhibitive effect on the proliferation of cancer cells [5, 19, 21–23].

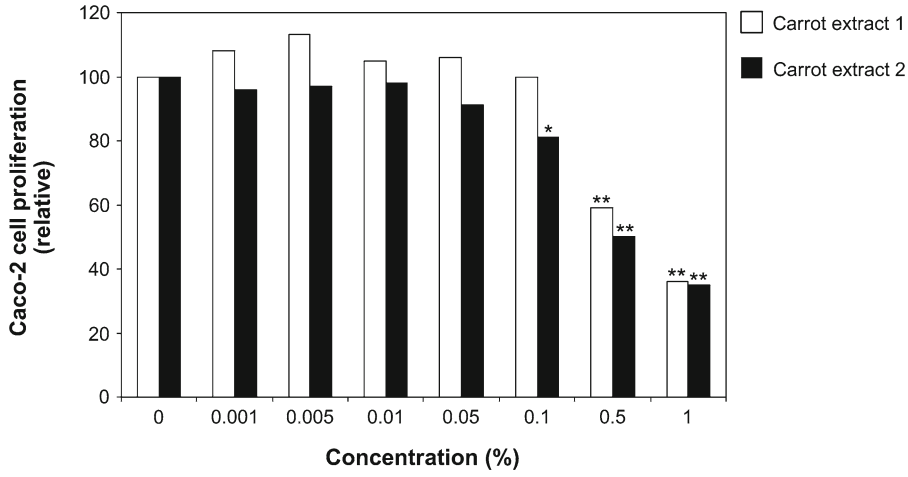


Table. Concentrations (μg ml^{-1}) of major polyacetylenes in ethyl acetate extracts from the carrot cultivars Bolero (extract no. 1) and Purple Haze (extract no. 2).

Carrot extract no.	Falcarinol	Falcarindiol	Falcarindiol-3-acetate
1	281	596	196
2	646	152	107

Fig. 29.5 Effect of increasing concentrations of carrot extracts on cell proliferation in Caco-2 cells cultured for 72 h in 0.625% fetal calf serum. Concentrations correspond to percentages of the stock solutions given in the *Table*. Values are least-squares means obtained from cultures in quadruple samples from two experiments and presented as relative to proliferation obtained in medium without extracts (basal medium). Standard error of the mean values was 2.8 (relative values). Values significantly different from proliferation obtained in basal medium are indicated: *, $P<0.05$; **, $P<0.001$. Carrot extract 1 contained approximately 50% less falcarinol compared to carrot extract 2, but approximately four and two times more falcarindiol and falcarindiol-3-acetate, respectively, than carrot extract 2 (*Table*)

Synergistic Effects

The possible synergistic interactions of polyacetylenes in combinations on the proliferation of human cancer cells have been studied in carrot extracts and on purified falcarinol type polyacetylenes. Purup et al. [22] tested the inhibitory effects of two ethyl acetate extracts from carrots (carrot extract 1 and 2) on the proliferation of Caco-2 cells (Fig. 29.5). Carrot extract 1 contained approximately 50% less falcarinol compared to carrot extract 2, but approximately four and two times more falcarindiol and falcarindiol-3-acetate, respectively, than carrot extract 2. Both extracts had significant inhibitory effects on cell proliferation in the highest concentration of 0.5 and 1% of extract in culture medium. However, carrot extract 2 tended ($P<0.10$) to be a more potent inhibitor of proliferation of Caco-2 cells than carrot extract 1, as significant inhibition was obtained also with 0.1% extract. This is in accordance with the fact that falcarinol is a more potent inhibitor of cell proliferation than falcarindiol (Fig. 29.2).

Comparing the effects of the carrot extracts with the effects of falcarinol or falcarindiol alone, Purup et al. [22] found that the mixture of polyacetylenes in the extracts was more potent in inhibiting cell proliferation. For example, the 1% carrot extract 1 prepared from a stock solution by diluting 100 times in cell culture medium contained falcarinol, falcarindiol, and falcarindiol-3-acetate in concentrations of 2.81 μg ml^{-1}, 5.96 μg ml^{-1}, and 1.96 μg ml^{-1}, respectively, and caused a reduction in cell proliferation to 36% (Fig. 29.5). To obtain a decrease in cell proliferation corresponding to 36%, it was shown in the same study that concentrations in falcarinol of 5 μg ml^{-1} or falcarindiol of 10–20 μg ml^{-1} were required (Fig. 29.2) [22]. However, the addition of carrot extract 2 to Caco-2 cells

Table 29.1 Effects of different ratios of falcarinol and falcarindiol on cell proliferation of Caco-2 cells in culture medium containing 0.625% fetal calf serum [22]

	Falcarinol (μg ml^{-1})			
	0	1	5	10
Falcarindiol (μg ml^{-1})	Caco-2 cells[a]			
0	1.0	1.01 ± 0.25	0.36 ± 0.25	0.18 ± 0.01
1	1.35 ± 0.36	0.71 ± 0.08[b]	0.58 ± 0.04	0.22 ± 0.08
5	1.03 ± 0.32	0.53 ± 0.06[b]	ND[c]	ND
10	0.52 ± 0.17	0.17 ± 0.03[b]	ND	ND

[a]Mean ± standard deviation is shown for each combination of falcarinol and falcarindiol. Data are presented relative to cell proliferation obtained in medium without polyacetylenes for two individual experiments
[b]Significant synergistic inhibitory effect on cell proliferation compared to single-compound assay ($P < 0.01$)
[c]ND = Not determined

did not decrease cell proliferation more than carrot extract 1 despite a concentration in falcarinol of 6.46 μg ml^{-1}. The above results indicate synergistic effects on Caco-2 cell proliferation between falcarinol, falcarindiol, and falcarindiol-3-acetate in carrot extracts. Synergistic effects between falcarinol type polyacetylenes were confirmed between purified falcarinol and falcarindiol [22]. Keeping one of the polyacetylenes constant at 1 μg ml^{-1}, Caco-2 cells were incubated with falcarinol and falcarindiol in different ratios. A synergistic response for the inhibitory effect of cell proliferation of Caco-2 cells was observed by adding falcarindiol in 1, 5, and 10 times the concentration of falcarinol (Table 29.1) [22].

The above results show that falcarinol type polyacetylenes in combinations may affect their cytotoxicity significantly. Synergistic interactions could therefore be an important factor in relation to the anticancer effect of carrot polyacetylenes, although this activity clearly depends on the concentration and the ratio of the compounds.

Anticancer Effect

Only a few studies have been conducted to investigate the anticancer effect of falcarinol type polyacetylenes in vivo. Preliminary evaluation of the cytotoxicity of falcarinol type polyacetylenes using the LOX melanoma mouse xenograft model demonstrated some potential for in vivo antitumor activity of falcarinol type polyacetylenes, including falcarinol and falcarindiol [61]. The most interesting proof for a potential anticancer effect of carrot polyacetylenes are, however, from a preclinical study on rats demonstrating inhibitory effects of carrots and falcarinol in an established rat model for colon cancer [25]. The rats were induced with colon cancer by injections of the carcinogen azoxymethane followed by feeding with freeze-dried carrots or purified falcarinol corresponding to a daily human consumption of 400–600 g FW of carrot [25]. Dietary treatments with carrot and falcarinol showed a significant ($P = 0.028$) tendency to reduce numbers of (pre)cancerous lesions (aberrant crypt foci) with increasing size of lesion from no difference relative to control for the smallest lesions to a one-third reduction for the fully developed tumors. The effect of the falcarinol diet was shown to have a larger effect on (pre)cancerous lesions compared to the carrot diet, although the difference was not significant. This is, however, contradictory to the synergistic effect observed between major carrot polyacetylenes (see Sect. "Synergistic Effects") and the results from the above in vivo study therefore suggest that other metabolites in carrots may interact with falcarinol and other polyacetylenes in carrots, thereby affecting their effectiveness in vivo [22].

The above results clearly indicate that the protective effect of carrots against cancer to a large extent can be explained by their content of falcarinol type polyacetylenes, rather than carotenoids or

polyphenols as previously has been suggested [10, 68]. By improving the content of polyacetylenes in raw and processed carrots it may be possible to increase our daily intake of carrot polyacetylenes and thereby optimize the cancer preventive effects of carrots.

Important Factors That Influence the Content of Polyacetylenes in Carrots

Genotype and Cultivation

Genotype has a huge impact on the content of polyacetylenes in carrots. In a study by Kidmose et al. [42] it was shown that the content of falcarinol, falcarindiol, and falcarindiol-3-acetate varied significantly between 4–16 mg kg^{-1} FW, 19–54 mg kg^{-1} FW, and 9–19 mg kg^{-1} FW, respectively, in six genotypes of organically grown carrots, which is also in accordance with other studies [36, 37, 40, 41]. The location where carrots are grown also has a significantly impact on the content of polyacetylenes in carrots (Table 29.2) [42], whereas the growth system appears to be of less importance with regard to the content of polyacetylenes [36].

Thermal Processing

Thermal processing is used to inactivate enzymes before frozen storage or as part of cooking meals and it has been shown that thermal processing has a huge impact on the content of polyacetylenes in carrots. In an unpublished study by Christensen [33], it was shown that the content of polyacetylenes in carrot pieces of different sizes was reduced by almost 50% after 6 min boiling (Fig. 29.6), in accordance with another study where boiling of carrot pieces for 15 min resulted in a reduction of polyacetylenes by almost 70% [26]. The content of falcarinol in boiled carrot pieces seem to correlate positively with the size of the pieces being significantly smaller in the smallest pieces, whereas such correlations does not exist for falcarindiol and falcarindiol-3-acetate (Fig. 29.6) [33]. Since falcarindiol and falcarindiol-3-acetate is primarily located in the peel of carrots, i.e., in pericyclic parenchyma tissue close to the periderm [40, 41, 69], the distance between the compound in the carrot and the water is independent of carrot size. The spatial distribution of falcarindiol and falcarindiol-3-acetate therefore explain the independence of carrot size on the content of these polyacetylenes in carrot pieces during boiling. In contrast falcarinol is primarily located in the phloem tissue close to the vascular

Table 29.2 Content of falcarinol, falcarindiol, and falcarindiol-3-acetate in carrot genotypes (in mg kg^{-1} fresh weight) grown at two locations in Denmark (A and B)

Genotype	Falcarinol		Falcarindiol		Falcarindiol-3-acetate	
Location	A	B	A	B	A	B
Bolero	8.7	4.4	39.0	19.3	13.8	8.9
Duke	15.0	12.5	44.3	36.4	14.1	13.5
Express	6.0	7.3	30.4	29.1	10.9	11.0
Fancy	7.3	7.2	53.6	27.9	18.7	16.3
Line 1	15.5	10.3	49.4	25.1	12.2	8.5
Cortez	5.5	5.4	30.1	22.8	12.6	9.7
LSD[a]	3.1	2.0	12.7	5.1	4.4	4.3

Data are means of two replications [42]
[a]LSD = Least significant difference at $P < 0.05$.

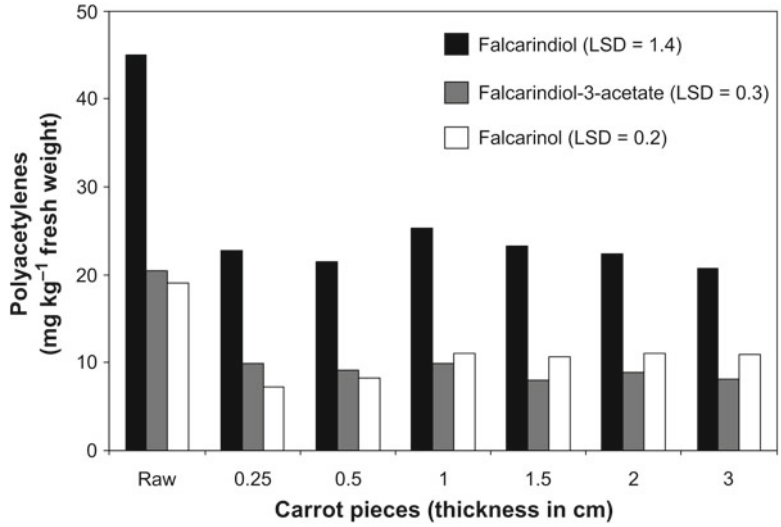


Fig. 29.6 The effect of boiling (6 min) on the content of major polyacetylenes in carrot pieces (cultivar Bolero) of different size. Values are given as the mean of two processing replications. *LSD* least significant difference at $P<0.05$

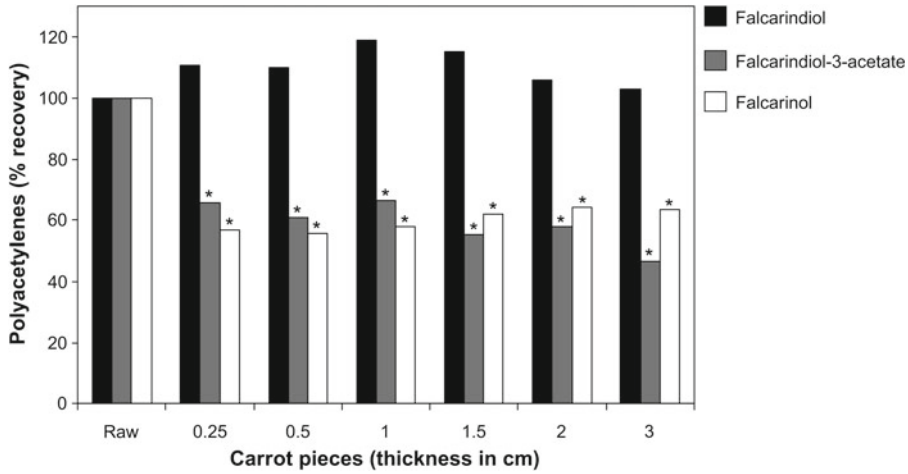


Fig. 29.7 Recovery (in %), i.e., the amount of polyacetylenes found in boiled carrots (cultivar Bolero) plus the boiled water compared to the amount in raw carrots. Columns marked with *asterisk* are significantly different ($P<0.05$) from the corresponding column representing the content in raw carrots. Values are given as the mean of two processing replications

cambium in the carrot root [40, 41, 69]. As the temperature in the phloem tissue during boiling depends on the size of the carrot pieces, the spatial distribution of falcarinol in the carrot root therefore explain the effect of boiling on this compound in the smallest carrot pieces. Steam blanching of carrot cubes or shreds at 90°C for a few minutes may result in losses of polyacetylenes up to 50% and indicates that polyacetylenes to some extent are degraded during high temperature heat treatment [26, 42].

To elucidate what happens with the polyacetylenes during boiling the total amount of each polyacetylene, i.e., the sum of polyacetylenes in carrot pieces plus the amount leached out in the boiled water, was determined, and the results are shown as percentage recovery in Fig. 29.7 [33].

Table 29.3 Content of falcarinol, falcarindiol, and falcarindiol-3-acetate in carrots (in mg kg^{-1} fresh weight) after 4 month storage at chilled (1°C) and frozen (−24°C) temperatures

Polyacetylene	Chilled storage	Frozen storage	LSD[a]
Falcarinol	16.2	11.0	4.2
Falcarindiol	117.2	86.9	31.7
Falcarindiol-3-acetate	25.5	17.1	6.4

Data are means of two genotypes (Bolero and Line 1) and two replications [42]
[a]LSD = Least significant difference at $P < 0.05$

Falcarindiol was not degraded during boiling, since the recoveries did not differ significantly from the content in the raw samples (Fig. 29.7). The tendency towards a recovery of falcarindiol greater than 100% can be explained by weight loss of the carrot pieces during boiling (10% weight loss after 6 min boiling [33]) or to a more efficient extraction of heat treated samples. Figure 29.7 also shows that the recovery of falcarindiol-3-acetate and falcarinol are significant lower compared to raw carrots. An explanation for this lower recovery of falcarindiol-3-acetate and falcarinol is probably due to a combination of degradation of the polyacetylenes and conversion to related polyacetylenes (Fig. 29.1) by oxidation and/or hydrolysis during heating.

The above results are not in accordance with those obtained by Rawson et al. [35] who found that during water immersion thermal processing of carrots at high temperatures (70–90°C) and long holding times (5–60 min) the contents of polyacetylenes increased with increasing temperatures. The differences observed in the levels of polyacetylenes of thermal processed carrots is probably due to the fact that the quantitative analysis of polyacetylenes in some studies are based on dry weight (DW) [35], whereas in other studies the quantitative analysis are performed on FW basis [26, 33, 42]. Thermal processing may result in increased extractability of compounds as heat induces solubilization of the intercellular cementing pectin, thus facilitating cell loosening [35]. In addition, a general increase in polyacetylene levels with longer holding times is probably attributed to the leaching of soluble solids from the root matrix, and retention of polyacetylenes, as these are largely insoluble in water [35]. As a result, the proportion of DW polyacetylene levels appears to increase, which is not the case if the quantitative measurements were performed on FW basis as the soluble solids to some extent would have been substituted by water.

Thermal processing reduces the content of bioactive polyacetylenes in carrots significantly. At the same time thermal processing changes the texture of carrots, which increases the accessibility of bioactive polyacetylenes and hence their bioavailability. Whether thermal processing has a positive or negative net effect on the cancer preventive effects of carrots is yet to be investigated.

Storage

It has been shown that the content of carrot polyacetylenes are significantly higher in unprocessed carrots stored for 4 month at chilled temperatures (1°C) compared to frozen temperatures (−24°C) (Table 29.3), which indicates that there is a net production of polyacetylenes during postharvest storage [42]. That the secondary metabolism in carrots is still active at chilled temperatures has been demonstrated by Kjeldsen et al. [70] who showed that storage of carrots at 1°C for 4 month resulted in a significant increase in the content of terpenoids compared to frozen stored carrots. Although storage only have a minor effect on the content of polyacetylenes compared to genotype and thermal processing, it appear to be beneficial to store carrots at low temperatures until processing to improve the content of bioactive polyacetylenes.

Summary

Epidemiological studies suggest that carrots play a central role in cancer prevention in particular in Europe and North America. Antioxidants such as carotenoids and flavonoids do not seem to play a central role in explaining the cancer preventive effect of carrots. However, carrots contain bioactive polyacetylenes of the falcarinol type with cytotoxic and anti-inflammatory activity. In addition these polyacetylenes have shown to be bioavailable and to have anticancer effect in preclinical trials; hence, they may be considered as important nutraceuticals that contribute significantly to the cancer preventive effects of carrots. Furthermore, the optimal concentration of polyacetylenes in carrots where the cancer preventive effect is obtained need to be determined. If this can be achieved the next step is to optimize the content of these compounds in carrots by developing new genotypes and/or processing techniques. In conclusion the role of bioactive polyacetylenes in carrots in skin and various specific cancers' prevention is strongly suggested but still to be confirmed in clinical and in further preclinical trials.

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Chapter 30
Chocolate: A Role in Skin Care and Cancer

Ronald Ross Watson and Amanda Berg

Key Points

- Dark chocolate with a high cocoa to milk content may play a role in protecting skin from melanoma development and enhancing overall skin health.
- High polyphenol flavonoids in bitter chocolate comparative to those inherent in antioxidative foods may improve susceptibility of cell damage due to UV radiation.
- Oral and topical application of high polyphenol cocoa extract may increase skin surfaces in elasticity, hydration, skin pigmenting, and firmness.
- Unrefined chocolate with 75% cacao may play a role in dermatological cosmetology by offering an organic alternative to commercial antiaging regimens in improving collagen synthesis.

Keywords Polyphenol • Flavonoid • Melanoma • Cocoa • Oxygen radical absorbance capacity • Deoxyribonucleic acid • Ultraviolet exposure • Laboratory erythema • Glycosaminoglycans • Collagen

Theobroma [1] the cacao tree has yielded an edible product since Europeans found it being used by Native Americans in the early 1500s. The tropical seeds have been roasted, husked, ground, liquefied, and defatted into victuals of the divine. Today it is processed into solid segments of marketable bars by adding sugar and chocolate powder to cocoa butter, the fatty content of the plant. Regulations established by the Food and Drug Administration take care to categorize the different typology of chocolate from white to milk to dark by the specifying the ratio of cocoa to milk solid it contains [1]. Dark chocolate specifically has exhibited its value in promotion of skin health and cancer prevention.

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Chemical Make-Up of Chocolate

As a living, breathing organism, cocoa contains several polyphenols. Polyphenols are aromatic ring-like structures and can be classified into three subgroups: phenolic acids, non-flavonoids, and flavonoids. Extract from cacao is especially rich in flavonoids, "a class of compounds that occur in a wide variety of vegetables, teas and red wines," [2]. Note this list of dietary foods. They are all nutritive plant by-products researched and believed to provide better heart and vascular health, promote cancer defenses, and potentially lower the prevalence of chronic disease like diabetes mellitus and neurodegenerative disorders.

This extra protective measure produced by the ingestion of chocolate could be primarily due to its exogenous antioxidant properties. According to the oxygen radical absorbance capacity (ORAC), a standard measuring indicator of antioxidant dynamics of a single food, dark chocolate rises to the top with a score of 13,120 in comparison with prunes at 5,770, spinach at 1,260, and broccoli at 890 [2]. The antioxidative metabolites aid in cancelling out free radicals and reactive oxygen species (ROS), agents that produce harmful ionic molecules, damaging a somatic system's homeostatic balance overtime [3].

Prevention from Skin Cancer

Does cocoa or its constituents prevent skin cancer? The epidermis is a large resilient organ aiming to protect and prevent damage to the internal features of our bodies. It proliferates regularly and several layers are useful in synthesizing the body's need for vitamin D from the sun. It also houses adiposities to cushion joints and water to provide evaporative cooling on a warm summer day. Beyond this it also serves as an armed barrier in the immune system to keep pathogens away from otherwise healthy tissue.

Cancer begins at the mitotic division of all cells. When skin cells regenerate without inhibitory mechanisms, overgrowth occurs. Exposures to cancer causing carcinogens and events create this blueprint "always on" mutation in the deoxyribonucleic acid (DNA) of cells. This excessive division of cells inherently becomes a tumor and begins to grow feeding off of an increased blood supply, much like an extra organ. Not all tumors present poor prognosis. Benign ones such as epidermal moles do not typically cause disease in human beings. However, malignant growths typically found in the three most prevalent sorts of cancer affecting the skin (melanoma, basal cell cancer, and squamous cell cancer) are not favorable. They are invasive in nature and can disturb nearby tissues with potential to still remain latent upon excision [4].

Cancer of the skin is the most prevalent kind at this moment. The National Cancer Institute estimates an incidence of 68,130 confirmed cases and 8,700 lives lost to epidermal malignancies in 2010 [5]. Melanoma, a highly metastatic variety, forms in the midst of melanocytes. These are the cells responsible for pigmentation and the crispy hue upon exposure to ultraviolet radiation. Basal cell skin cancer presents in the deepest layer of the epidermis in round basal cells. This type customarily affects fair skinned complexions. Squamous cell skin cancer, particularly representative in darker skin tones, pollutes squamous, thin, and flat-bodied cells. All three cancers are readily found on the face, ears, neck, and head, where most skin is exposed to harmful sunrays [4].

Cocoa beans' high polyphenol content may be beneficial in reducing the damaging effects of solar emission. Due to the higher antioxidant qualities of cocoa [6] than those found in super foods like pomegranates, green tea, and blueberries there is a strong suggestion of its intrinsic potential to fight carcinogenic principles of radiation. Studies have tested and confirm these characteristics. A study conducted by the School of Management and Science in London University of Arts validated the use of high-flavanol-containing chocolate as effective in boosting photo protection of skin [6]. It consisted

of a double blind experiment in which 28 subject were either orally administered 20 g of either high- or low-flavanol-containing chocolate daily. At the baseline and 12 week marks participants' forearms were subjected to UV light causing redness. This exposure simulates the sun burning process. Minimal UVB erythema dose readings were taken 24 h post UV contact. The findings: the group ingesting the high-flavanol chocolate possessed minimal UVB erythema dose readings that more than doubled over the time period. At baseline this value was 0 J/cm^2. After 12 weeks high-flavanol chocolate increased to 0.23 J/cm^2. In opposition, the placebo (low-flavanol chocolate) group readings remained unchanged at 0 J/cm^2 from baseline until the termination of 12 week interim [6]. Thus, chocolate rich in polyphenols required a higher UV exposure of its consumers to reach the same amount of skin damage.

Cocoa in Cosmetology

Cocoa extract, in topical and oral application, can provide superficial benefits to the soma. Experiments with the administration of high polyphenol extract have produced one or more desirable effects in skin such as increased elasticity, increased tonicity, increased firmness, reduced wrinkles (including wrinkle width and/or volume), reduced fine lines, increased hydration, decreased skin roughness, decreased scaling, improved skin structure and barrier and depigmenting of spots formed by aging [7]. Overall documented epidermal transfiguration has brought its effects to the marketplace. Modern spas have begun to lather outer derma in chocolate cream and follow up with a thermal blanket encasement for 20 minutes or longer. Upon removal of the emollient, calm, toned, rehydrated skin is revealed [9]. This process also induces fat melting, due to the physiological absorption of caffeine and polyphenols [8].

The effects of cocoa polyphenols on skin elasticity has been rated just as appropriate in creating a positive outcome compared to antiaging skin regimens. An experiment involving skin explants from a 68-year-old donor has demonstrated this. The procedure involved sole topical application of cocoa butter or a 1.5% cocoa polyphenol and cocoa butter blend. The third category was left untreated as the control. After 5 days of results the cocoa polyphenol treated tissue portrayed a mild increase in skin thickness and after 12 days an elevation of type I collagen density, as shown in Fig. 30.1. Type III and IV collagen is marked at a greater amount by day 5 also in the cocoa treated group. Glycosaminoglycans—components invaluable in connective tissue—are also slightly increased in the target group. None of these beneficial observations are found in the untreated or pure cocoa butter samples [2]. This expedient biochemical display has allowed for the conclusion that cocoa has earned a place beyond its notorious pathways of ingestion.

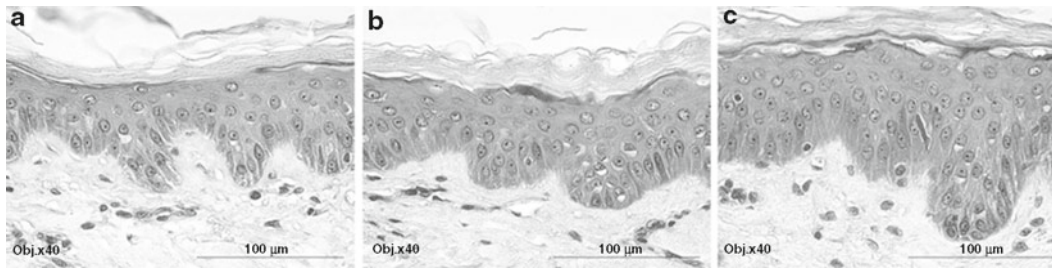

Fig. 30.1 (**a**) Untreated explant (**b**) Explant treated with cocoa butter (**c**) Explant treated with 0.75% cocoa butter polyphenol blend © Laboratoire BIO-EC, with permission

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Chapter 31
N-Acetylcysteine for Reduction of Oxidative Stress/ Damage and Prevention of Melanoma

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Key Points

- *N*-acetyl-L-cysteine is a potent antioxidant that is commercially available as an over-the-counter supplement that has demonstrated efficacy for several medical applications.
- The drug is FDA-approved for acetaminophen poisoning. Its metabolism and basis of its biological activity are well understood.
- *N*-acetyl-L-cysteine has been used in several experimental systems, ranging from skin cancer prevention to modification of psychological conditions.
- Here we review its biological basis and potential clinical applications, with emphasis on our work developing *N*-acetylcysteine as an agent for melanoma chemoprevention.

Keywords NAC • Oxidative stress • UV • Melanoma • Nevi

Abbreviations

8-OH-dG	8-Hydroxy-2′-deoxyguanine
Cys	Cysteine
GGTase	Gamma-glutamyl transferase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione *S*-transferase

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NAC	*N*-Acetylcysteine
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
NAPQI	*N*-Acetyl-*p*-quinonimine
Protein-SG	Protein-glutathione mixed disulfide
ROS	Reactive oxygen species
UV	Ultraviolet radiation
γ(gamma)-GCS	Gamma-glutamylcysteine synthase

N-Acetyl Cysteine Is a Prodrug That Supports the Biosynthesis of Glutathione

N-acetyl-L-cysteine (NAC) is a prodrug of L-cysteine, which is the rate-limiting amino acid required for the biosynthesis of glutathione (GSH) [1]. The tripeptide thiol GSH (γ(gamma)-glutamylcysteinylglycine) is the most abundant small-molecule antioxidant in the body with levels as high as 10 mM in some tissues [2]. NAC begins its transformation into GSH by rapid deacetylation in the liver, giving cysteine (Cys). Cys is imported into melanocytes (the cells from which melanomas arise) and other cells in either its reduced form by the transporter Slc1A4 [3] or in its oxidized form (cystine) by Slc7A11 [4]. GSH itself is present in the extracellular space, but cannot cross the cell membrane; Cys must first be salvaged by cleavage of the tripeptide by γ(gamma)-glutamyltranspeptidases (GGTases) [5]. Once inside the cell, the first peptide bond in GSH is formed between the γ(gamma)-carboxylate of glutamate and the amino group of Cys in a reaction catalyzed by γ(gamma)-glutamylcysteine synthase (γ(gamma)-GCS). Feedback inhibition of γ(gamma)-GCS results in the tight regulation of basal levels of GSH in most tissues. Glutathione synthase then catalyzes the formation of the peptide bond between the α-carboxylate of Cys and the amino group of glycine to give GSH (Fig. 31.1) [1].

The biological activity of NAC is generally attributed to its ability to reduce oxidative stress in tissues by relieving the depletion of GSH, which in turn arises from a variety of insults including ultraviolet radiation (UV). The vital cellular functions of GSH are as follows: (1) scavenging and metabolizing reactive oxygen species (ROS) and reactive nitrogen species, (2) detoxifying electrophiles arising from xenobiotics and/or their metabolites, as well as endogenous electrophilic species such as oxidized lipids, (3) providing a reservoir for Cys, and (4) modulating critical cellular processes such as DNA synthesis, microtubular-related processes and immune function (reviewed in reference 6). Here we focus on the first two processes which are important for the antioxidant activity

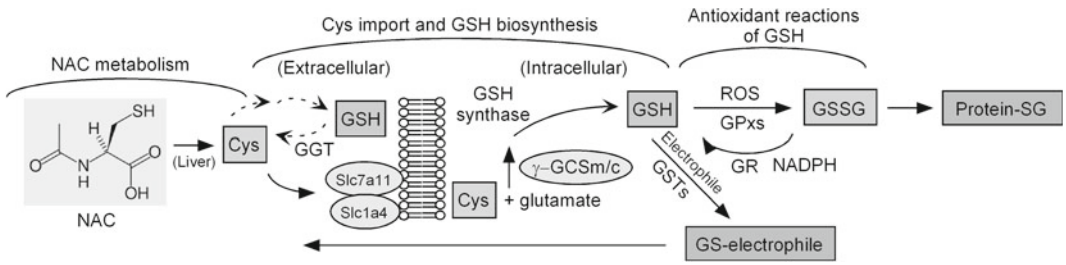

Fig. 31.1 The metabolism of NAC, the biosynthesis of GSH, and the antioxidant activities of GSH in the melanocyte are depicted schematically. *NAC metabolism*—NAC is deacetylated in the liver yielding Cys, which is imported cells by the transporters Slc1A4 and Slc7A11 (*Cys import*). Cys can also be salvaged by cleavage of GSH by GGTases. *GSH biosynthesis*—GSH is formed by the sequential actions of the γ(gamma)-GCS and GS. *Antioxidant reactions of GSH*—GSH is the reductant in the detoxification of hydrogen peroxide and peroxidized lipids in reactions catalyzed by GPxs, forming GSSG. Regeneration of GSH by reduction with NADPH is catalyzed by GR. Accumulated GSSG can oxidize protein thiols by formation of mixed protein-GSH disulfides (Protein-SG), thereby altering protein function. GSH is also consumed when electrophiles are detoxified by GSTs. Refer to list of abbreviations and text for further details

of GSH in the skin (Fig. 31.1). UV irradiation induces the formation of ROS [7], radicals, and oxidized lipids [8] in skin cells and tissues including human nevi [9]. Many toxic species are removed from the tissue in enzymatic reactions that consume GSH. GSH is the reductant in the detoxification of hydrogen peroxide and certain peroxidized lipids in reactions catalyzed by the glutathione peroxidases (GPxs) [10]. These reactions result in the formation the oxidized form of GSH, glutathione disulfide (GSSG). Regeneration of GSH by reduction with NADPH is catalyzed by glutathione reductase (GR) [1]. However, severe oxidative stress can overwhelm the cells' reductive capacity, and accumulated GSSG can oxidize critical protein thiols by formation of mixed protein-GSH disulfides, thereby altering protein function. This effect can be ameliorated by active export of GSSG, but the result is depletion of cellular GSH [11]. GSH levels are also decreased as a consequence of detoxification of electrophiles by the glutathione *S*-transferases (GSTs). These reactions involve the irreversible formation of covalent bonds between the thiol of GSH and the electrophile. While in almost all cases the resulting conjugate is much less toxic to the cell, it must be exported from the cell for processing and excretion; loss of GSH is the net result. The effects of the loss of this essential antioxidant on the cell can be catastrophic as illustrated below in our discussion of the molecular etiology of acetaminophen toxicity.

NAC for Acetaminophen Overdose

Elimination of acetaminophen from the body is facilitated by sulfation and glucuronidation [12]. If acetaminophen levels rise so quickly as to overwhelm these pathways, the drug is metabolized by the mixed-function oxidase cytochrome p450 CYP2E1 to the toxic electrophilic species *N*-acetyl-*p*-quinonimine (NAPQI). NAPQI can be safely eliminated from cells after formation of a covalent adduct to GSH in a reaction catalyzed by GSTs. However, in cases of overdose or in patients where GSH levels are already compromised, GSH becomes severely depleted, leaving unconjugated NAPQI free to react with proteins in the liver. This can result in failure of the organ if left untreated [13]. NAC is approved by the FDA for treatment of acetaminophen toxicity. Administration of NAC protects the liver by resupplying the tissue with Cys, which supports the synthesis of GSH and ultimately allows the safe elimination of NAPQI. For treatment of acetaminophen toxicity a total of 300 mg/kg NAC is administered in a loading dose of 150 mg/kg in 200 mL diluent over 60 min, followed by 50 mg/kg over 4 h and 100 mg/kg over 16 h [14].

NAC Sources

Acetadote® brand of NAC, available by prescription in the United States, is supplied by Cumberland Pharmaceuticals as a solution (10 and 20%) for i.v. administration (Table 31.1). We chose to use the aqueous solution (acetylcysteine) formulated for oral ingestion because it is packaged under nitrogen

Table 31.1 Sources and formulations of NAC

Prescription drug	Dose/formulation	Supplier	Cost
Acetylcysteine	10 and 20% for inhalation or oral ingestion	American Regent, Inc.; Mayne Pharma, Inc.	------
Acetadote®	10 and 20% for i.v. injection	Cumberland Pharmaceuticals	------
Dietary Supplements			
N-Acetylcysteine	1,000 mg tablet	Source Natural	120 for $20.79
N-Acetylcysteine	600 mg capsule	Now Foods	250 for $21.73
N-Acetylcysteine	600 mg capsule	Swanson Premium	100 for $6.49

which stabilizes the drug by eliminating contact with oxygen. Dietary supplements containing NAC are widely available in health food stores and on the Internet. FDA regulations concerning the manufacture of these products are different than those for prescription drugs, but all domestic and foreign companies that manufacture dietary supplements for distribution in the United States, must comply with the Dietary Supplement Current Good Manufacturing Practices (cGMPS) for quality control (http://www.fda.gov/Food/DietarySupplements/default.htm). The greatest concern with these products is loss of potency via oxidation, which can be accelerated if the drug is not stored in a cool and dry location.

Toxicology and Adverse Events

Single i.v. doses of NAC at 1,000 mg/kg in mice, 2,445 mg/kg in rats, 1,500 mg/kg in guinea pigs, 1,200 mg/kg in rabbits and 500 mg/kg in dogs were lethal. There are no well-controlled studies of NAC in pregnant women and the drug is classified as "Pregnancy Category B" [14].

In a post-marketing study involving 4,709 adults who received i.v. injections for acetaminophen overdose, the following adverse reactions were reported: urticaria or facial flushing (6.1%), pruritus (4.3%), respiratory symptoms (1.9%), edema (1.6%), hypotension (0.1%), and anaphylaxis (0.1%). In our clinical trial we have safely administered NAC orally to a total of 72 patients, although in our studies, the single oral dose of 1,200 mg [9], was far lower than the i.v. doses given for acetaminophen poisoning (300 mg/kg or 21,000 mg for a 70 kg person). To our knowledge, no adverse events have been reported by others following oral ingestion of the drug.

Oxidative Stress and Melanoma

There is correlative evidence to suggest that one link between UV radiation and melanoma is generation of oxidative damage [15, 16]. In the *Xiphophorus* fish model, the UV action spectrum for melanin-dependent oxidant production is identical to that for melanoma induction [17], and oxidative dysregulation in human melanoma cell lines correlates with aggressive behavior [18]. Melanocytes isolated from melanoma patients display increased sensitivity to peroxidizing agents that correlates with endogenous antioxidant imbalance [19], and elevated ROS have been found in melanocytes from dysplastic nevi relative to normal skin from the same individuals [20]. Interestingly, melanoma is quite rare in albino individuals who lack melanin [21], and, while melanocytes may be protected by endogenous melanin which can directly absorb photons and quench UV-generated ROS [22]. At higher UV doses oxidized melanin actually participates in the generation of ROS [17], and we recently found that melanocytes maintain higher levels of oxidative stress than keratinocytes or fibroblasts isolated from the same individuals [23], possibly due to ROS generated during melanin biosynthesis [24].

Multiple oxidizing species capable of damaging cellular structures and DNA are induced in the skin by UV; these include hydrogen peroxide, hydroxyl radical, superoxide, nitric oxide and oxidized lipids [25]. If high levels of these species persist, mutations in DNA can result directly from reactions with DNA causing the formation of modified bases such as 8-hydroxyguanine (8-OHdG). These DNA modifications can lead to mutations if not repaired prior to DNA replication [26]. Studies of early human melanoma lesions show loss of heterozygosity for DNA repair genes [27], and next-generation sequencing of a melanoma genome revealed a significant rate of the G→T transversion, which is a signature mutation arising from unrepaired oxidative DNA damage [28]. We previously showed that oral delivery of the antioxidant NAC can protect mouse skin against UV-induced generation of ROS and 8-OHdG, and depletion of GSH [7]. In addition, we found that administration of NAC just prior

to, and immediately following, UV exposure significantly delayed the onset of UV-induced melanoma in our animal model (discussed in detail below) [7]. This is direct evidence for a role of oxidative stress in UV-induced melanoma, and provides the rationale for targeting oxidative stress for melanoma chemoprevention. In summary, the production of melanin itself may increase oxidative stress in melanocytes, and oxidation of DNA has the potential to produce cancer-causing mutations in these cells.

Antioxidant Response and the Melanocyte

More that 90% of melanomas are thought to be sporadic, but of those that are familial, 20–40% arise in persons with germ-line mutations in the *Cdkn2a* locus [29]. The penetrance of mutations *Cdkn2a* varies according to geographic location, with an incidence rate of melanoma in carriers of 58% in Europe, 76% in the United States, and 91% in Australia, by 80 years of age. A recent meta-analysis of genetic modifiers in 96 families with germ-line *Cdkn2a* mutations showed that risk for melanoma was increased 4.6-fold for carriers that had two mutations in another gene important to melanocyte biology, *MC1R*. This increased risk is reflected in the median age of onset in *Cdkn2a* mutation carriers which is decreased from 47 years of age for *MC1R* wild-type individuals, to 37 years in *MC1R* mutants ($P<0.0001$) [30]. The melanocortin-1 receptor gene (*MC1R*) codes for a seven-pass transmembrane protein expressed on the cell surface of melanocytes [31]. Loss-of-function (LOF) mutations in *MC1R* commonly result in the red-hair phenotype (red hair, light eye color and the inability to tan) in humans. Functional MC1R protects the skin from the mutagenic effects of UV by promoting pigment (melanin) synthesis, and by upregulating the expression of DNA repair and antioxidant genes in melanocytes [32, 33]. In fact, the expression of antioxidant genes that have antioxidant response elements (AREs) in their promoters (including those controlling GSH biosynthesis, Fig. 31.1) is downstream of MC1R [34, 35]. Binding of the MC1R ligand stimulates transcription of *Nrf2*, which in turn encodes a subunit of the transcription factor complex that binds to AREs and activates transcription of antioxidant genes [32]. We therefore believe that impairment of both pigmentation *and* antioxidant response contribute to the fourfold increased risk for melanoma observed in individuals (without *Cdkn2a* mutations) carrying two LOF *MC1R* alleles [36]. This is consistent with the synergistic effect manifested by the increased melanoma risk observed in individuals harboring mutations in both *Cdkn2a* and *MC1R*, and highlights the importance of oxidative stress as a target for therapeutic intervention in the process of melanomagenisis.

How the Use of NAC Overcomes Pitfalls in Traditional Chemoprevention?

There are many obstacles associated with conventional chemoprevention approaches for cancer, which usually involve chronic drug administration. Besides maintaining and monitoring patient adherence over time, unintended toxicities may be associated with chronic ingestion of any agent, and it is generally not possible to assess clinical benefit until the end of the trial (i.e., did the intervention group develop less cancer?). Another consideration is that the chemopreventive agent may not be administered in conjunction with the specific oncogenic stimulus, which for many cancers is unknown. For melanoma, the long latency time and low (annual) risk of tumor development are such that large numbers of patients would need to be treated and monitored for many years to determine whether a given preventive agent is effective. Finally, a combination of these factors may yield unanticipated adverse (or paradoxical) results as observed in various prevention trials of antioxidants [37]. For example, patients who took β-carotene and retinol for 2 years exhibited an increased risk of lung cancer [38], and mixed antioxidant supplementation over 7 years was associated with increased risk of skin cancer

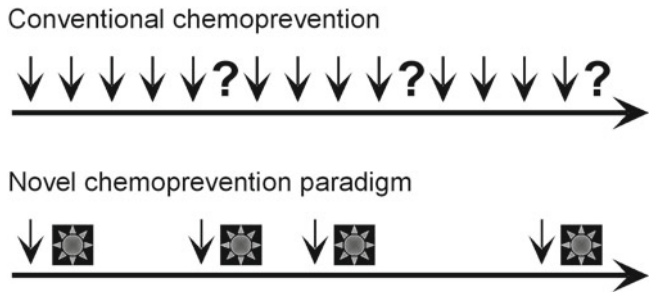

Fig. 31.2 Chemoprevention paradigms. (**a**) Conventional chemoprevention is characterized by chronic administration of an agent (*arrows*), usually not in conjunction with specific oncogenic stimulus (often unknown, *?*). (**b**) Our proposed novel use of NAC involves episodic administration (*arrows*) in anticipation of known oncogenic stimulus (UV exposure,). From the authors' previously published work [7]

in French women [39]. Here we propose a novel chemoprevention strategy for melanoma that bypasses most of these obstacles associated with conventional cancer chemoprevention. Patients could take NAC as a prophylactic "sunburn pill" in anticipation of sun exposure to protect their skin against UV-induced oxidative stress/damage. In sharp contrast to conventional chemoprevention protocols, in which an agent is administered chronically and (usually) independent of mutagenic insult that may be unknown or poorly defined (Fig. 31.2, conventional chemoprevention), this scenario would involve episodic drug administration, in conjunction with exposure to a presumed mutagenic insult (UV radiation), targeting a presumed mutation-initiating pathway (oxidative stress/damage) (Fig. 31.2, novel chemoprevention paradigm). Additional advantages of this approach over conventional strategies include avoidance of potential toxicities that could be associated with chronic ingestion of any agent, and the *presumed benefit* that would be afforded by reduction in potentially carcinogenic oxidative damage in the skin over the course of many UV exposures.

NAC Prevents UV-Induced Skin Cancers in Mouse Models

D'Agostini et al. [40] have shown that NAC is able to significantly modulate the formation of UV-induced skin tumors in the SKH-1 hairless mouse. In this study, mice were exposed daily to light from halogen quartz bulbs with emission covering a broad spectrum of visible light as well as UVA and UVB. NAC administration was initiated 3 days before the beginning of irradiation and throughout the remainder of the study in the drinking water at a dose calculated to deliver 1,000 mg/kg bodyweight daily. NAC affected both the tumor latency and multiplicity in this model. The earliest light-related skin lesions were detectable in animals treated with UV alone after 300 days and this was delayed until 390 days in irradiated mice treated with NAC. After 480 days the UV-treated mice had significantly more tumors compared to those treated with UV plus NAC (5.76 ± 1.06 versus 2.14 ± 0.57, $P < 0.001$). The nature of the light-induced skin lesions ranged from pre-neoplastic lesions such as epidermal hyperplasia, to benign tumors such as papillomas, evolving towards keratoacanthoma-like tumors, appendage/basal tumors, carcinomas in situ and squamocellular carcinomas. Of the animals treated with UV alone, 21% had squamocellular carcinomas, but none of the animals treated with NAC as well as UV developed these advanced tumors.

Our examination of the effects of NAC on melanoma began with a study of melanocytes in culture [7]. We found that hydrogen peroxide produced in UV-irradiated (960 J/m [2]) melan-a mouse melanocytes was reduced to the levels of untreated controls with the addition of 5 mM NAC to the culture

medium. We also found that free nonprotein thiols (principally GSH) were reduced by approximately 20% within 48 h after UV irradiation at the same dose. Addition of NAC (10 mM) to the medium of the irradiated cells boosted the level of free thiols to 130% of that in unirradiated controls. When we measured UV-induced DNA damage in melanocytes, we found that UV increased the number of cells staining positive for the oxidized base 8-OH-dG to fivefold more than that observed in control cells, and that 5 mM NAC reduced the number of positive cells to the same as that in unirradiated cells. However, immunochemical analysis of DNA isolated 48 h after treatment from UV-irradiated cells showed that NAC had little if any effect on UV-induced cyclobutane dimer formation. Thus, we concluded that the principle effect of NAC is on UV-induced oxidative stress (as measured by thiol depletion), and that oxidative damage to DNA was also relieved, but with no apparent effect on the formation/repair of direct photoproducts.

We next studied the effects of NAC in vivo using the HGF/survivin mouse model of melanoma [41]. The expression of the HGF transgene causes melanocytes in the skin of these mice to localize to the dermal/epidermal junction, in contrast to wild-type mice where the majority of melanocytes are found at the base of the hair follicle where they are protected from the mutagenic effects of UV [42]. HGF mice develop cutaneous melanomas when subjected to a single neonatal dose of UV radiation. Expression of the survivin transgene in the melanocytes of HGF mice decreases the latency of melanoma development and increases metastases both in the lymph nodes and in the lungs [41]. In our NAC study, the fact that tumor formation requires that the animals be irradiated shortly after birth, dictated that in order to deliver NAC orally, the drug must be supplied to the dams, then trans-placentally to their offspring and/or via the milk of nursing females. In order to show the efficacy of this delivery method, we first examined GSH levels and 8-OH-dG in the skin of neonatal HGF mice born to females given water containing NAC (equivalent to approximately 1.9 g/kg/day 2–3 days before until 2 weeks after delivery). Compared to the UV-irradiated skin of animals receiving no NAC from their mothers, the UV-irradiated skin of NAC treated animals contained significantly higher levels of free thiols and less positive staining for 8-OH-dG (Figure 3 in reference 7). Convinced that our delivery method was effective, we then compared the effects of NAC on UV-induced tumor formation. The time for 50% of animals to form tumors of at least 1 mm in diameter was increased from 13.8 weeks in control animals to 20.6 weeks in NAC-treated animals ($P = 0.0003$, Fig. 31.3). In addition, tumors were significantly smaller in treated animals at early time points, but this difference disappeared by the end of the study at 32 weeks. Tumors were collected for histological examination and metastases were determined by necropsy. There were no significant differences between cytologic atypia in tumors or in rates of metastasis to the lymph nodes and lungs. Thus, the predominant effect of orally delivered NAC before (and briefly following) UV irradiation was a delay in tumor formation.

Effects of NAC on UV-Irradiated Human Skin Tissues

Investigators at the University of Michigan studied the effects of topical NAC on the UV-induced signaling that leads to photoaging of human skin [43]. They found that in untreated skin, topical application of a 20% aqueous solution of NAC under occlusion, virtually eliminated the oxidized form of GSH (GSSG), and increased reduced GSH levels by 50%. They also showed that UV stimulates ERK/MAP kinases and promotes the accumulation of the protein factor cJun. The transcription of *cJun* is increased by oxidative stress [44], whereupon the resulting protein forms a heterodimeric complex with the constitutively expressed cFos [45]. This complex is known as the transcription factor AP-1. The accumulation of cJun is related to photoaging by virtue of the fact that the transcription of the matrix metalloproteinase (MMP) collagenase, an AP-1 target gene, is induced by UV. In their working model for the pathophysiology of photoaging, the Michigan group hypothesizes that damage to the extracellular matrix by UV-induced MMPs is imperfectly repaired after UV exposure, resulting in an

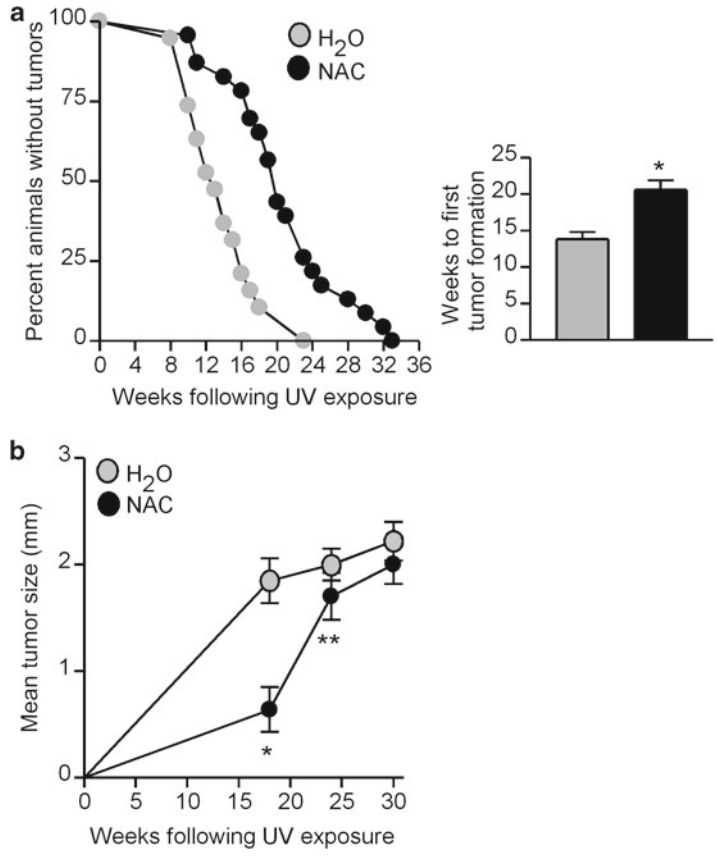

Fig. 31.3 Oral NAC delays onset of UV-induced melanoma. (**a**) Percent of animals without tumors. Pregnant female mice were provided with either water containing NAC ($n=19$) or water alone ($n=21$). Neonates from these females were irradiated with UV (3,900 J/m^2) 2 days after birth. Animals were monitored for 40 weeks for tumor formation. NAC-treated animals required significantly long to develop tumors ($P=0.0003$) (**b**) Tumor size. Tumors in NAC-treated animals were smaller at early timepoints but were similar in size by 30 weeks after irradiation. *$P<0.001$, **$P=0.14$. From the authors' previously published work [7]

invisible solar scar. With repeated intermittent UV exposures, the solar scars accumulate, eventually resulting in visible skin wrinkling or photoaging. When NAC was applied 24 h prior to irradiation of skin at two times the minimal erythemal dose (2 MED), the UV-induced accumulation of cJun and induction of collagenase transcription, was relieved. This is supportive of the prediction that by decreasing UV-induced MMP expression, NAC will prevent photoaging in human skin.

Oral NAC Protects Melanocytic Nevi Against UV-Induced Oxidative Stress

Melanocytes make up less than 10% of the cells in the human epidermis. Nevi (clonal neoplasms of melanocytes) have a significantly higher percentage of melanocytes and we therefore considered them an excellent model system for studying UV-induced oxidative stress in this cell type. In order to avoid any potential risks of exposing patients to UV radiation, we developed an ex vivo system for evaluating UV-induced oxidative stress in nevi. After removal from the patient, nevi were divided into roughly equal fragments and one was irradiated while the other was left as untreated control. The tissue was placed in a cell culture incubator and we found it to be viable for up to 72 h. Following treatment with UV at 400–4,000 J/m^2, we measured ROS levels that were significantly elevated 48 h after treatment in nevi treated with the highest dose. GSH levels in nevi treated with 4,000 J/m^2 UV were depressed by 25–30% at both 24 and 48 h after irradiation.

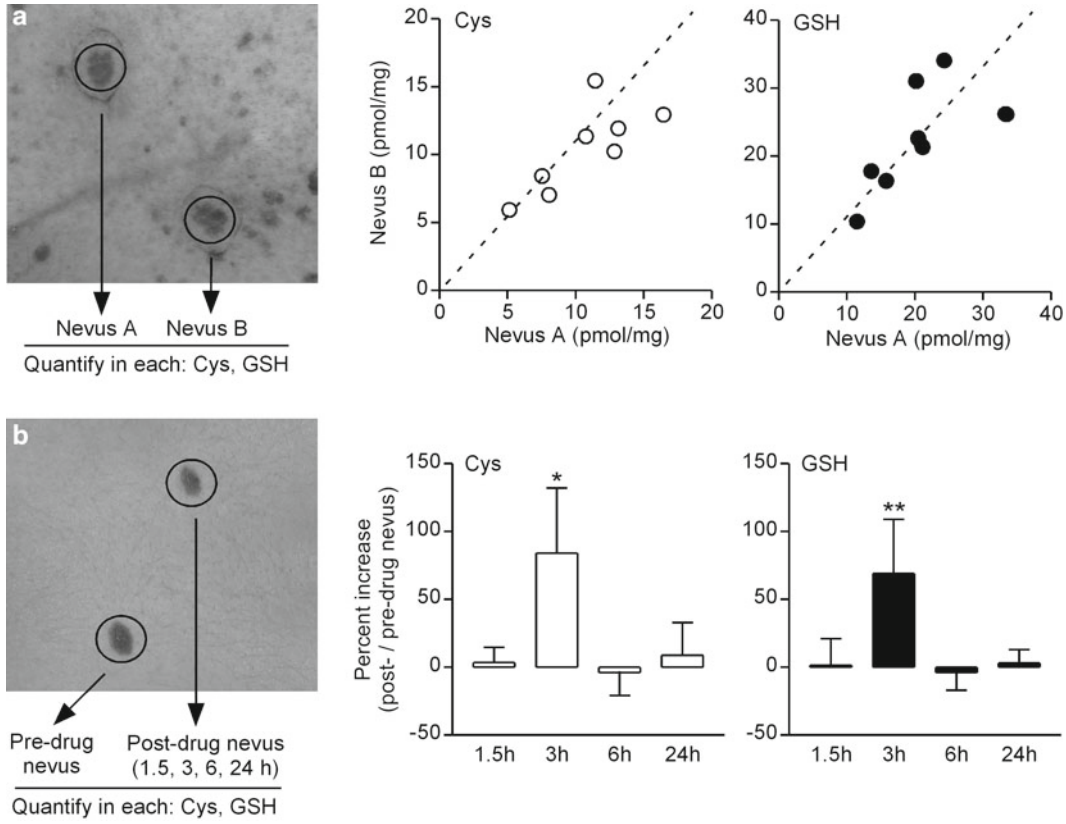

Fig. 31.4 Internevus correlation of oxidative biomarkers, and NAC-mediated oxidative modulation in nevi. (**a**) Two similar-appearing nevi were removed from individual patients ($n=8$), and Cys and GSH content was determined (*left*). For each patient, values for Cys (*left plot*, *open circles*) and GSH (*right plot*, *closed circles*) are expressed as a single data point reflecting each pair of nevi (nevus A, abscissa; nevus B, ordinate). *Dotted lines*, theoretical correlation where data points should fall for pairs of nevi with identical values. For Cys measurements, correlation coefficient (r) is 0.77 (95% confidence interval, 0.14–0.95; $P=0.03$). For GSH measurements, correlation coefficient (r) is 0.69 (95% confidence interval, −0.03 to 0.94; $P=0.06$). (**b**) Two similar-appearing nevi were removed from individual patients immediately before, and either 1.5, 3, 6, or 24 h ($n=5$–6 at each time point) following ingestion of 1,200 mg NAC (*left*). Cys and GSH content (normalized to nevus weight) were determined for each nevus, and data expressed as percent increase in postdrug versus predrug nevus (*right*). $*P=0.047$; $**P=0.016$ (Wilcoxon signed-rank tests). From the authors' previously published work [9]

Next we examined the safety and tolerability of oral NAC. We found that 600 mg NAC, administered in a single dose of a 20% solution diluted into tomato juice to mask the salty taste, was well tolerated by the first two patients. Therefore, the remaining patients (70) were given a 1,200 mg dose with the idea that the larger dose would have an increased chance to protect against oxidative stress. All patients were surveyed by telephone 24 h after drug ingestion in order to assess any side effects such as nausea or itching. In all cases the drug was well tolerated, confirming the safety of a single oral high dose of NAC.

We then conducted a pilot study of the delivery of NAC to nevi after oral administration. We felt that it was important, in determining parameters for suitable drug delivery to nevi, to use a "predrug" nevus as a reference to control for interpatient variability. However, we realized that this strategy would not work if there was significant variability in nevi from the same patient (Fig. 31.4a). In order to assess inter-nevus variability, we determined Cys and GSH levels in eight sets of two nevi of similar appearance, each harvested from the same patient. We found that while levels of these thiols varied

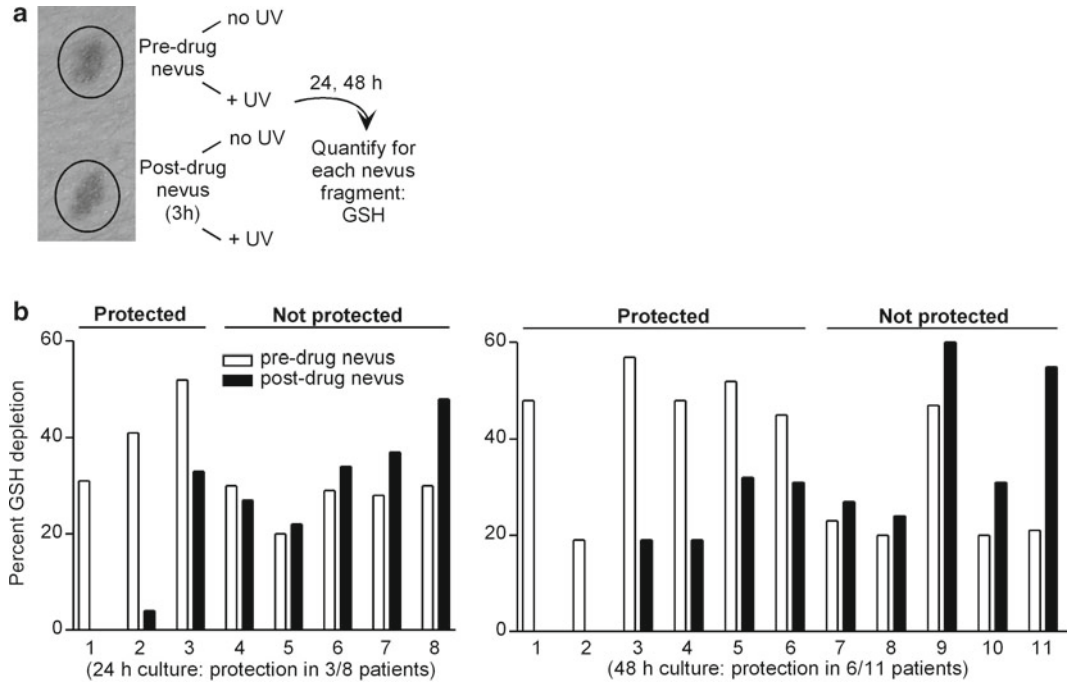

Fig. 31.5 NAC-mediated protection against UV-induced oxidative stress. (**a**) Two similar-appearing nevi were removed from 19 patients immediately before and 3 h following ingestion of 1,200 mg NAC. Fragments of each nevus were either untreated or UV irradiated (4,000 J/m2), then cultured for either 24 h (8 patients) or 48 h (11 patients). GSH content (normalized to nevus weight) was determined for each nevus fragment. (**b**) Data expressed as percent UV-induced GSH depletion (UV treated versus untreated) in fragments of predrug nevi (*open columns*) and postdrug nevi (*filled columns*) from each patient. For nevi cultured 24 h, there was protection (i.e., less GSH depletion) in three of eight patients (*left*); for nevi cultured 48 h, there was protection in 6 of 11 patients (*right*). From the authors' previously published work [9]

considerably between patients, there were good correlations between levels measured in nevi harvested from the same patient. In order to characterize the pharmacokinetics of Cys delivery to nevi, we removed a nevus just before drug ingestion, then either 1.5, 3, 6, or 24 h later (Fig. 31.4b). Using an HPLC-based assay to detect thiols in the nevus tissues, we found a significant elevation of both Cys and GSH at 3 h, which returned to baseline after 6 h.

Having determined the optimal timing for both drug delivery and UV-induced GSH depletion, we set about conducting a test of the effects of NAC administration on UV-induced oxidative stress in human nevi. The experimental design is shown in Fig. 31.5a. Patients with two suitable nevi were recruited from our high risk melanoma clinic. Before ingestion of the drug one nevus was removed, and 3 h after NAC administration, the second nevus was removed. Immediately after harvest, each nevus was divided in half and one portion was UV-irradiated. After 24–48 h, GSH levels were measured in each of the four nevus fragments from every patient. Our results showed that in 9 of the 19 participants treated in this study, UV-induced GSH depletion was relieved (Fig. 31.5b).

Our current research is aimed at determining why some of the patients' nevi were not protected by NAC. In new analyses of the data, we noted that tissues from patients responsive to NAC-mediated chemoprotection exhibited significantly higher levels of UV-induced GSH depletion in control (predrug) nevi than did nevi from patients that were not protected (Fig. 31.6a, Cassidy and Grossman, unpublished analysis). This may represent a threshold effect, whereby UV-induced production of oxidizing species in some patients may not have been sufficient to activate Nrf2-dependent antioxidant responses, which include transcriptional activation of Cys transporters and the GSH biosynthetic

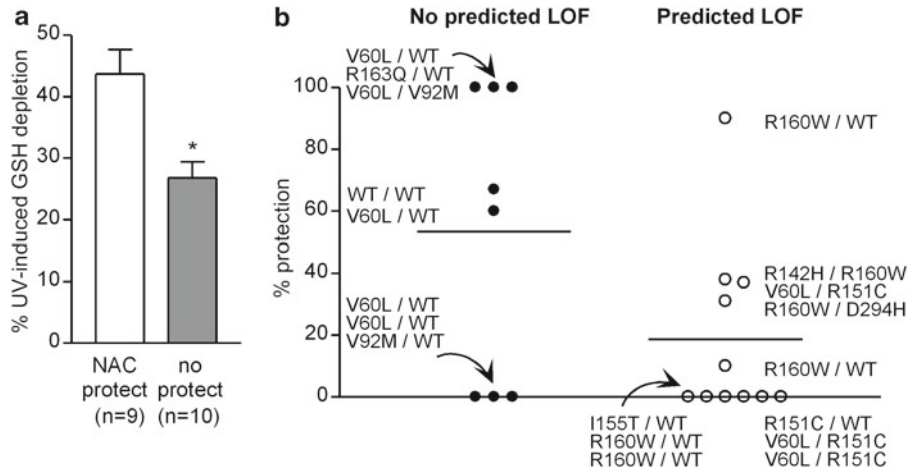

Fig. 31.6 Preliminary analysis of NAC-mediated protection and effect of *MC1R* mutation. (**a**) Magnitude of UV-induced GSH depletion in untreated nevi is greater in patients with nevi protected by NAC than those not protected. $*P = 0.003$ (2-tailed *t* test). (**b**) GSH levels were determined and percent protection is shown. Results are grouped based on indicated *MC1R* genotype (*left*, *filled circles*, no predicted loss of function (LOF); *right*, *open circles*, predicted LOF). *Solid line* represents mean percent protection

pathway [46]. This is a likely significant factor since our trial design included administration of an identical dose of UV to nevi from every participant, regardless of their skin type. Our future trial design will incorporate measurements of MED in normal skin and delivery of a "biologically equivalent" dose of UV as determined by this objective measurement of UV sensitivity. Using this design, persons with high MED get a higher UV dose than those with low MED with the intent to elicit an equivalent amount of oxidative stress in the nevi of each patient. This new strategy should result in a higher percentage of patients with significant GSH depletion in pre-drug nevus, which should then facilitate increased biosynthesis of GSH in the post-drug nevus and protection of the tissue against oxidative stress.

We also considered genetic factors that might make the antioxidant response of some nevi less robust than others. To explore this concept, we retrospectively sequenced *MC1R* (which as discussed above is known to affect oxidative stress in melanocytes) in the patients from our completed study. Interestingly, we found that while five of eight patients with wild-type *MC1R* or non-LOF polymorphisms experienced protection from UV-induced depletion of GSH, 7 of 11 patients with one or more LOF *MC1R* mutations were *not* protected (Fig. 31.6b, Cassidy and Grossman, unpublished). Given the dependence of Nrf2-mediated antioxidant gene transcription in melanocytes on MC1R activation [34], it is plausible that loss of MC1R function in these patients in the context of GSH depletion by UV, may compromise the downstream antioxidant response, leading to an inability to upregulate GSH synthesis. Thus, *MC1R* genotype and sensitivity of nevi to UV-induced oxidative stress may be important biomarkers of chemoprevention efficacy in our system, and incorporating these factors into our trial design are the focus of our continued efforts to develop personalized melanoma chemoprevention strategies.

Other Potential Applications

There are numerous potential therapeutic applications for NAC in humans, in addition to those relating to skin cancer detailed above. Most of these additional applications exploit the antioxidant activity of NAC, which may be beneficial in ameliorating the effects of acute or chronic inflammation and

oxidative damage in various organs. In patients with pulmonary fibrosis, NAC (600 mg three times daily) improved lung function in patients already taking immunosuppressive drugs [47]. In patients undergoing cardiac angioplasty, combined i.v. and oral dosing of NAC before and after the procedure prevented contrast dye-induced nephropathy [48]. Aberrant ROS production also may play a role in the autoimmune disease systemic lupus erythematosis (SLE), and NAC can decrease auto-antibody production in SLE-prone mice [49]. Oral NAC (600 mg three times daily for 2 weeks) improved endothelial dysfunction in SLE patients [50], and a clinical trial to test whether oral NAC can decrease auto-antibody production in these patients is underway (ClinicalTrials.gov, NCT00775476). Finally, in cystic fibrosis, NAC scavenges myeloperoxidase activity in patient sputum [51] and stimulates chloride efflux from airway epithelial cells [52], and a recent phase II trial in patients receiving either 700 mg or 2,800 mg orally showed that extracellular GSH increased in sputum although there was no alteration in clinical or inflammatory parameters [53]. In patients with history of preterm labor and bacterial vaginosis, oral NAC (600 mg daily) significantly increased gestational age at delivery compared to placebo [54].

NAC attenuates decline in muscle Na+/K+-pump activity [55], and oral NAC (1,800 mg) reduces respiratory muscle fatigue during heavy exercise [56]. NAC reduces keratinocyte proliferation, and there is a case report of a patient with lamellar ichthyosis improving following 5 weeks of topical application of NAC [57]. NAC penetrates the blood–brain barrier, and given the known role of its metabolite Cys as a modulator of the glutamatergic system and potentiator of dopamine release, NAC may potentially influence reward-reinforcement pathways in the brain [58]. Thus, NAC may be therapeutically useful in psychiatric disorders allegedly related to oxidative stress (e.g., schizophrenia, bipolar disorder) as well as psychiatric syndromes characterized by impulsive/compulsive symptoms (e.g., trichotillomania, pathological nail biting, gambling, drug addiction) [59]. In adult patients with trichotillomania (compulsive hair pulling), 1,200 mg oral NAC daily was found to reduce hair pulling in over half the patients [60]. One study in marijuana users found that 2,400 mg NAC per day decreased drug use [61], and another in cocaine addicts found that 2,400 mg NAC reduced cravings for cocaine [62]. On the other hand, NAC at doses up to 2,400 mg per day does not appear to be useful for tobacco cessation [63]. A placebo-controlled trial found that 1,000 mg per day NAC plus existing medication over 6 months improved global function and akathisia in schizophrenia patients [64]. A 6-month trial of 2,000 mg NAC per day in patients with bipolar disorder showed reduction in depressive symptoms [65]. Finally, NAC has been tested against placebo in Alzheimer's disease following 3 and 6 months of treatment, and comparison of interval change favored NAC treatment on nearly every outcome measure, although significant differences were obtained only for a subset of cognitive tasks [66].

NAC has an excellent record of safety and efficacy in treating a wide range of conditions in which oxidative stress plays a critical role, and many more clinical trials are currently in progress (ClinicalTrials.gov). In this chapter, we have presented data from promising clinical and animal studies illustrating the potential utility of NAC in protecting against the consequences of UV-induced oxidative stress in the skin including photoaging as well as skin cancers. We believe that the insights that we and others have gained into molecular mechanisms of UV-induced oxidative stress and the protective effects of NAC, provide robust intermediate biomarkers of efficacy, and that larger scale phase III trials using photodamage and cancer incidence as endpoints could be justified in the near future.

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Chapter 32
An Indian Spice: Turmeric, in Relation to Skin Health and Cancer

Shree Acharya and Ronald Ross Watson

Key Points

- The effects of turmeric's circumin component is being critically studied in animals as well as humans.
- Turmeric's potential skin health benefits vary in scope.
- Much more research needed on spice to prove how effective it actually is in cancer prevention and treatment.
- Turmeric a preventative spice that exemplifies curative properties.
- Turmeric is flavorful as well as very defensive against cancer.

Keywords Turmeric • Cancer • Curcumin • Indian foods

Herbs and spices not only invigorate the senses but also bring forth a natural, yet aromatic addition to any food item. Turmeric (*Curcuma longa*), having been used many centuries prior to discoveries of its potential health benefits, is a yellow, warm, and pleasantly bitter spice used in flavoring up many exquisite Indian dishes. Pertaining to skin health, turmeric has long been deemed by native users to have beneficial effects, as well as therapeutic properties, and has obtained a name to fame in many recent studies. Medicinal practices with the herb have been passed on through many generations in some Asian cultures, namely, Chinese and Indian.

This specific spice that has been studied with much fervor, namely, turmeric, will be the main focus of this analysis. Though the results are at the very early stages of development, it has been seen that turmeric may help to prevent cancer from mounting. The several types of cancer that have responded positively to turmeric tests are breast, prostate, skin, and colon cancer, in which curcumin plays a major role [1]. One astonishing component as well as active ingredient in the herb turmeric is known as curcumin, which has been classified as a "powerful antioxidant." In cases of cancer, antioxidants have been shown to reduce the amount of free radicals present in the human body, which can "damage

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cell membranes, tamper with DNA, and even cause cell death" [1]. Hence, curcumin is very beneficial in proper and non-degenerative cell health, and is an astonishing component of turmeric that is being extensively studied.

Turmeric is composed of curcuminoids, fatty acids, and even some essential oils [2]. In order to determine the benefits of the bioactive curcuminoids, which consists of curcumin types I, II, and III, recent studies have been generated to show that "whole turmeric or the extracted curcuminoids appear to be active in many disease processes with specific reference to chronic ailments such as cardiovascular, degenerative, infective and inflammatory disorders as well as cancers" [2]. One of the major effects of turmeric comes through in its ability to act as an anti-mutagen. After supplementing dosages of turmeric to rats, urine samples were collected and tested for the presence of mutagens. Turmeric's anti-mutagenic properties were assessed against the ubiquitous pollutant benzo(a)pyrene [B(a)P] in rats [2]. Hence, the samples were compared to the amount of mutagens in normal urine. "Mutagens in urine were significantly reduced by dietary exposure to turmeric" [2]. The amount of turmeric in the dietary samples given to the mice was 0.5%, which showed a high preventative characteristic within 4 weeks of feeding [2]. Due to these findings, there is a positive established linkage between the health benefits of turmeric and it's effect on the decrease of mutagens in rats.

In regard to tumor inhibition and prevention, another experiment was done where rats were fed varying doses of turmeric in order to determine the effects of turmeric and curcumin on DNA protection. A P32 post label assay showed that "the percentage of reduction ranged from 61 to 70% for 0.1 to 3% turmeric respectively while curcumin (0.03%) resulted in 92% reduction" [2]. This data exemplifies the premier effect of curcumin in reducing harmful effects on DNA function and structure. With curcumin in the system, breaks in DNA were repaired to a greater effect in peripheral cells [2]. Due to the anti-mutagenic and protective properties of turmeric and curcumin, the likelihood of cancer seems to become less. This is due to less mutational risks as well as more effective repair methods, after which the DNA can function normally.

Tracking the progress of certain Phase-1 clinical trials, notably in prevention techniques of skin cancer, it has been shown that curcumin "inhibits carcinogenesis of murine skin…" [3]. In order to place a valid stance at this conclusion, patients were given increasing doses of curcumin, of course, only progressing if the amount (in mg) developed no signs of toxicity to the patients. By analysis of tissue samples, there was a "histologic improvement of precancerous lesions… in two out of six patients with Bowen's disease" [3] (also known as squamous cell carcinoma). Though specifics such as creating an effective dose for patients has not be established, this clinical trial has presented more ground, as well as a respectable guide for prospective studies. Experimentation mainly shows the positive biological "effect of curcumin on the chemoprevention" of many types of cancer, primarily skin [3]. In relation and support to this experiment, there have been further studies in the effects of curcumin in the down-regulation of cyclin-D1 in squamous cell carcinoma [4]. "Cyclin-D1 is a proto-oncogene that is overexpressed in many cancers," and by using curcumin, a down-regulation of cyclin-D1 is shown [4]. This basically entails that curcumin helps to suppress levels of proliferation caused by cyclin-D1, and instead, promotes proteolysis to inhibit cancer cell formation. These results were found in "selective cell lines" and hence, it can be said that curcumin plays a role in acting as an anti-proliferative agent in those specific cell lines [4].

In terms of a large-scale disease on which curcumin has been tested, melanoma is a great example. In an article in "Molecular Cancer Therapeutics," it is stated that curcumin promotes a pro-apoptotic effect of cells involved in melanoma [5]. As the dose of curcumin in the human cell line was increased, there was an increase in apoptosis of melanoma cells, due to inhibition of the many pathways in abnormal cell regulation. The only adverse effect of curcumin doses in human cells, which this article explicitly states, is the fact that curcumin can lower the "responsiveness of immune effector cells… that possess antitumor properties" [5]. In this way, though there are definitely some positive effects of

the active ingredient curcumin, this article states that it could also inhibit major types of cells in the human body that are already programmed to fight skin cancer (in this case).

Based off of these collected evidences, it can be said that the evolution of tumor inhibition in the skin due to circumin-containing turmeric, which has observed regulatory effects on carcinomas, can be prospectively depleted with the use of curcumin containing medicines. In terms of the people who have taken this potent herb as a medicine, there has been a wide range of results. The higher the dose becomes, the more likely there could be an introduction of toxicity in the body. In a recent study, it was demonstrated that the toxicity of curcumin does not come into affect if dosage is under 8,000 mg/day when taken by mouth, in a study done over 3 months [3]. As long as factors such as toxicity and dosage amounts are controlled, there is a plausibility that curcumin may be an answer to down-regulating cancerous tumors.

Considering the larger picture, turmeric is not solely a component of the various amounts of spices that are prevalent in having preventative, but also exhibits medicinal effects. Other Indian spices that are in the process of being studied for their antioxidant effects, including turmeric, are those of ginger, cloves, and even various plant seeds similar to cumin. There are phytochemicals in these "commonly consumed plant foods" which are usually not toxic, and can also help prevent chronic diseases [6]. Frequently called the "functional foods" due to their therapeutic properties, these plant components are being evaluated and tested in order to determine their effective potentials in disease prevention. So far, there has been a positive correlation in the consumption of these functional, phytochemical-containing foods, and the "low prevalence of non-communicable disease… from epidemiological observations" [6]. These phytochemicals have many properties in foods, including a synergistic or even antagonistic effect in the human body [6]. Due to this variation in their coherent behavior, phytochemical antioxidants are being very carefully studied in order to verify whether their medicinal forms would be more beneficial rather than harmful [6].

Pertaining to skin health, curcumin has been shown to lower the levels of enzymes in the body that cause inflammation [1]. According to the NIH's National Center for Complementary and Alternative Medicine, turmeric has been successful in treatment of "eczema and wound healing" when applied directly to the skin [7]. Though there is not yet much evidence to prove that turmeric is always an effective anti-inflammatory powder, or even one that reduces infection, many studies are focusing their research on the potential benefits of application of this potent powder on the skin. Turmeric has also shown to improve overall skin health. For example, it has been shown to be beneficial therapy that can alleviate acne, restore resiliency of the skin, as well as provide an anti-aging effect on the skin [8]. It can also act as a protective barrier towards "sun exposure, and contact with environmental irritants including pollution, smoke, and ozone" [8]. Hence, there have been certain alleged situations in which turmeric plays the restorative roles mentioned above, though they are not based off of a wide scale population analysis. Therefore, treatment with turmeric is not yet a major component in the field of medicine [8].

To summarize, turmeric's curcumin component has been the primary point of focus for this chapter due to the rigorous studies that are being done in animals as well as humans, in order to see if there actually are preventative or curative effects of the herbal component in regard to skin tumors and carcinomas in general. Also, turmeric's potential skin health benefits vary in scope, as seen by observations, yet this method of treatment is not very prevalent in medical culture today. This is due to the fact that there has yet to be much more research done on the spice in order to prove how effective it actually is. It can be seen from this discussion, that turmeric is definitely a preventative spice that exemplifies curative properties, but must be more efficiently and vigorously studied in order to be fully accepted before it is prescribed as a general medication. Budding results have the possibility to show that turmeric is most certainly a flavorful, as well as a very defensive "spice for life."

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Chapter 33
Green Tea (*Camellia sinesis*): Key Role in Skin Cancer

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Key Points

- Green tea preparation is a very common beverage and leaves contain polyphenols, alkaloids, saponins, and amino acids.
- Green tea extract, polyphenolic fraction from green tea, and epigallocatechin gallate (EGCG) have been found effective against a variety of skin tumors induced by chemicals and radiations.
- EGCG when administered orally, topically, and intraperitoneally is found effective against skin a variety of cancers.
- EGCG is effective against photocarcinogenesis and skin papilloma.

Keywords Green tea • EGCG • Skin cancer

Introduction

Green tea (*Camellia sinesis* Linn. Kutze belonging to the family Theaceae) is a heavily branched shrub cultivated and usually picked as young shoots. It can reaches up to 30 or more feet if not properly being pruned but is preferentially restricted up to a height of 2–3 ft by pruning for proper cultivation. Archeological data suggests that tea leaves steeped in boiling water were consumed as many as 5,000 years ago. Botanical evidence indicates that India and China were among the first countries to cultivate tea. The herb is commonly harvested in various parts of the subtropical regions of China, India, Indonesia, Europe, Kenya, and Zimbabwe. Tea plants are considered as indigenous to Asia and China, but also commercially grown in Africa, Indonesia, Malaysia, and Sri Lanka. The young buds and leaves are usually consumed for various purposes. Although, there are three main varieties of tea: green, black, and oolong; the difference between the teas is in their processing.

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Climate, soil, and harvesting techniques play the most significant role in developing the different varieties of teas. Green tea is produced from the same tea plant as black tea; however, the former is made from unfermented leaves and dried quickly. The quick drying procedure retains the goodness of tea which makes green tea as a popular dietary supplement. According to the original method, leaves are firstly placed on matting, which are in turn placed on boiling water so that the exposition to steam inactivates the enzymes (polyphenol oxidases), thus preventing polyphenol oxidation and preserving the chlorophyll. Subsequently, the tea is spread under the sun, pressed, and dried [1–4].

Botanical Description

Kingdom: Plantae—plants
Subkingdom: Tracheobionta—vascular plants
Superdivison: Spermatophyta—seed plants
Divson: Magnoliophyta—flowering plants
Class: Magnoliophyta—dicotyledons
Subclass: Dilleniidae
Order: Theales
Family: Theaceae—tea plant
Genus: *Camellia* L.—camellia
Botanical name: *Camellia sinesis* Linn Kutze
Synonyms: *Thea sinesis*

Flowers: It has attractive white flowers with distinctive rose-like blooms. Flowers pedicellate, usually one to two in the axiles of leaves of young shot, sometimes more than two (Fig. 33.1a). *Leaves*: Evergreen, dark-green in color, hairy, oblong-ovate, petiolate, simple, and elliptical. Generally, 5–10 cm long and 2–4 cm wide while blunt at apex and first two thirds of margin shortly serrate (serrated edges) (Fig. 33.1b). *Seeds*: Sub-orbicular or hemispherical 10–14 mm long, dull brown to reddish brown, smooth [1–3].

Collection and Cultivation

Tea is a subtropical species and is grown on a plantation scale in many parts of the world, where the climate is moist, warm and where winter is not too cold. It flourishes at optimum temperature range between 29.5 and 13.0°C. A well-distributed rainfall in the range of 125–750 cm is considered good for tea cultivation. Tea can be grown on a soil rich in organic matter or well drained and friable loam. It grows well not only on hill slopes but also on low lying flat lands. It is cultivated from almost sea level to about 2,460 m. It is grown at an altitude above 1,050 m in an area such as Darjeeling, where the growth pauses in winter or as in Sri Lanka where seasonal slowing down of growth occurs [1].

Phytochemistry

Green tea phytoconstituents are mainly categorized into tea polyphenols, alkaloids, amino acids, saponins, vitamins, and fragrance elements. Fresh tea leaf majorly contains polyphenols particularly known as catechins which attributes up to 30% of the dry leaf weight. Among different types, following

Fig. 33.1 Different parts and forms of tea. (**a**) tea flowers, (**b**) fresh tea leaves, (**c**) marketed green tea

types were present in tea (+)-catechin, (−)-epicatechin (3.0–9.0%), (+)-gallocatechin, (−)-epigallocatechin (1.0–12.0%), (−)-epicatechin gallate (8.0–18.0%), and epigallocatechin gallate (30–53%) [5]. Abundance of polyphenol varies as per the area cultivation in addition to age of the leaves. Other polyphenols co constituents are flavonol glycosides and depsides such as chlorogenic acid, coumaryl quinic acid, and theogallin (3-galloylquinic acid) which is exceptionally belongs to tea [6]. Green tea also contains alkaloids among which caffeine is reportedly present at an approximate 3% along with small quantity of the other methylxanthines such as theobromine and theophylline. Tea leaves contain amino acids, which are reported to contribute not only for fresh taste but also for sweetness. This greatly affects refreshing taste of tea leaves, fragrance, and tea water color. Amino acid content mainly stays in tender fresh tea leaves and it becomes less as tea leaves grows old. Fine green tea has higher amount of amino acids than other sorts of teas which might be due to its natural and unfermented manufacturing method, which keeps the freshness of tender tea leaves. The amino acid L-theanine (5-*N*-ethylglutamine) is also unique to tea. Tea accumulates aluminum and manganese. Carbohydrate content is high in tea, but only 1–4% of its content is solvable. Rough and old tea leaves contain higher sugar as compared to fresh and smooth leaves. The carbohydrate of solvable sugar contributes to sweet taste in tea water. Green tea composition is very similar to that of the fresh leaf except for a few enzymatically catalyzed changes which occur extremely rapidly following plucking [2]. Other phytoconstituents are essential vitamins (C and the group B), although the most relevant one is folic acid since vitamin C gets completely degraded as a consequence of the processes applied to the plants. The leaves are also reported to contain essential oil [1].

Application to Skin Cancer

Topical application or oral feeding of green tea in drinking water exhibited protection against complete carcinogenesis in SENCAR and BALB/c mice and skin tumor initiation induced by polycyclic aromatic hydrocarbon [7]. Green tea has ability to inhibit UV-B radiation-induced photocarcinogenesis in SKH-1 hairless mice [8].

Green tea polyphenols (GTP) has ability to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced epidermal ornithine decarboxylase (ODC) activity when applied topically in dose dependent manner. GTP also exhibit time dependent effect as when GTP was applied 30 min prior to topical application of TPA it shown maximum effect. In addition induction of epidermal ODC activity caused by several structurally different skin tumor promoters was also inhibited by GTP in mice. Among various types of catechins, the major catechin of green tea, epigallocatechin-3-gallate (EGCG), has inhibitory effects against TPA induced epidermal ODC activity when compared with several other naturally occurring polyphenols [9].

GTP is also effective in TPA-induced and 7,12-dimethylbenz(a) anthracene-(DMBA) initiated skin tumor in SENCAR mice. Study highlighted the significant and dose-dependent protective effect of GTP when applied topically in varying dose (1–24 mg) 30 min prior to TPA induction. Accordingly, animals pretreated (30 min prior) with GTP exhibited lowering of tumor body burden parameters viz. average volume per tumor, total number of tumors per group, tumor volume per mouse and number of tumors per animal as compared to non treatment group. In addition, GTP has been found effective quantitatively against the TPA-induced epidermal cyclooxygenase, lipoxygenase, edema, and hyperplasia (these four are typically considered as markers of skin tumor promotion), which is also considered as underlying mechanism of anti-skin tumor promoting effects of GTP [10].

Green tea extract in varying dose (1.25% or 2.5%) as the sole source of drinking water was found effective as inhibitor of the development of skin tumors induced by various tumor promoting agents. It was also found to inhibit tumor induced by various agents such as ultraviolet B light (UV-B), 12-*O*-tetradecanoylphorbol-13-acetate, 7,12-dimethylbenz(a)anthracene (DMBA) in single or combination in female SKH-1 mice. In addition, it found to inhibit tumor induced by topical application of DMBA followed by 12-*O*-tetra-decanoylphorbol-13-acetate and inhibited by administration of 1.25% green tea extract as the sole source of drinking water. Moreover, comparative study of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on UV-B-induced skin carcinogenesis in DMBA-initiated SKH-1 mice shown that the decaffeinated green tea had a marked inhibitory effect but exhibit slightly less effect than the regular teas [11]. Another study also supported the same hypothesis and reported that caffeine contributes vital role as inhibitor of carcinogenesis induced by UV-B [12].

The continuous administration of green tea in the drinking water or with i.p. injections of a green tea polyphenol fraction or (−)-epigallocatechin gallate is found effective in UV light-induced or chemically induced skin papillomas in female CD-1 mice. Tumor inducers composition mainly includes the 200 nmol of 7,12-dimethylbenz[a]anthracene (DMBA), 5 nmol of TPA and 180 mJ/cm^2 of UV-B, which were administered at fixed time interval and for fixed duration. Findings of present study revealed that green tea (p.o.), green tea polyphenol fraction (i.p.), or EGCG (i.p.) inhibited the growth and/or caused the regression of experimentally induced skin papillomas [13].

In addition to above studies shielding effect of topical application of polyphenolic fraction from green tea against spontaneous as well as benzoyl peroxide (BPO) and 4-nitroquinoline-*N*-oxide (4-NQO)-enhanced malignant conversion of chemically induced skin papillomas in SENCAR mice. Inhibition of conversion of benign tumors to malignant cancer is vital and considered responsible for chemopreventive effect. In this study topical application of 7,12-dimethylbenz(a)anthracene followed by twice a week application of 12-*O*-tetradecanoylphorbol-13-acetate were used as a tumor-initiating and tumor-promoting agent, respectively, to induce papillomas in SENCAR mice. These findings not only suggest chemopreventive effects (against both tumor initiation and promotion stages of multistage

carcinogenesis) but also shielding effect specifically against tumor progression induced by BPO and 4-NQO of green tea polyphenols [14].

EGCG when applied topically exhibited protective effect against UVB induced contact hypersensitivity and tolerance induction by contact sensitizer 2,4-dinitrofluorobenzene (both are reported to cause photocarcinogenesis). Mechanism underlined for this action might be due to ability of EGCG to reduce the number of CD11b+ monocyte/macrophage and neutrophills infiltration in inflammatory skin lesions which are thought to contribute for immunosuppressive state contributed by UV-B. In addition, EGCG decreases number of (IL)-10 in skin as well as in draining lymphatic node along with decrease in (IL)-12 which is considered as contributory factor for contact sensitivity. Furthermore, the mechanism of protection against photocarcinogenesis was supported by another study, which explains the effects of topical application of EGCG on C3H/HeN mice after UVB exposure. Results indicated that EGCG not only inhibited leukocytes infiltration (specially CD11b1 cell type) and myeloperoxidase activity but also reduces the quantity of antigen-presenting cells. EGCG also has ability to encounter the oxidative stress induced by UVB by decreasing production of H_2O_2 and (nitric oxide) NO at UV-B-irradiated sites such as epidermis and dermis [15]. Which suggests ability of EGCG to inhibit UVB induced immunosuppression and photocarcinogenesis.

Green tea when administered orally decreases number of tumors per mouse, size of the parametrial fat pads and thickness of dermal fat (directly under tumors and layer away from tumors) in UV-B pretreated high-risk SKH-1 mice. Green tea has particularly better potential to decrease the thickness of the dermal fat layer under large tumors than small tumors [16].

Topical application of EGCG (6.5 μmol/day for 5 days for 18 weeks) has been proved to inhibit UV-B induced skin tumors in SKH-1 hairless mice. Topical treatment resulted in decrease in number of both malignant and nonmalignant skin tumors. Immunohistochemical studies highlighted increased apoptosis as quantified by the no effect on apoptosis in nontumor areas, caspase 3-positive cells in nonmalignant skin tumors by 72% and in squamous cell carcinomas by 56%. In addition, effect of topical application of EGCG has also been reported as small inhibitory effect on proliferation in nonmalignant tumors and similar, but non significant, inhibitory effect on proliferation in malignant tumors [17].

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Chapter 34
Camellia sinensis (Tea) in the Prevention of UV-Induced Carcinogenesis: A Mechanistic Overview

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Key Points

- Ultraviolet radiation (UVR)-induced cancers include melanomas and two types of malignant keratinocytes, the basal-cell carcinomas (BCC) and squamous-cell carcinomas (SCC).
- Sun-protection products reduce the risk of erythema and DNA damage but are observed to be not that effective in preventing UVR-induced ill effects and immune suppression. One approach to protect humans from the deleterious effects of UV irradiation is to use chemopreventive agents.
- The polyphenols present in tea (*Camellia sinensis*) have been reported to possess health benefits, including protection from UV carcinogenesis and other ill effects.
- Tea and its polyphenols (1) act as free radical scavengers and antioxidants, (2) inhibit enzymes like inducible nitric oxide synthase, lipoxygenases, cyclooxygenases, and xanthine oxidase involved in the inflammatory reactions, (3) modulate the signal transduction pathways involved in cell proliferation, transformation, inflammation, apoptosis, metastasis, and invasion, (4) inhibit transcription factors like activator protein-1 and nuclear factor kB, (5) inhibit mutagenesis or enhance DNA

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repair, (6) modulate apoptosis, (7) modulate the immune system by inducing immunoregulatory cytokine interleukin IL12, and inhibit UV-induced immunosuppression and stimulate the cytotoxic T cells in a tumor microenvironment, (8) affect fat layers of the skin, and (9) inhibit angiogenesis and metastasis. This review addresses the scientific observations validating the protective effects of tea and its phytochemicals in preventing UV-induced carcinogenesis.

Keywords Skin cancer • Chemoprevention • Photoageing • Botanical antioxidants • Ultraviolet radiation • Immune modulators • *Camellia sinensis* • Green tea • Black tea and oolong tea

Introduction

Globally, skin cancers, comprising of basal cell carcinoma, squamous cell carcinoma, and melanoma, are the leading form of cancer [1]. They are a major health problem in many countries and chronic exposure to the solar radiations that contains ultraviolet radiation (UVR) is considered to be the most important cause for skin cancers. Additionally, the rapid depletion of the ozone from stratosphere and increase in outdoor activities has lead to an enhancement in the exposure of skin to environmental ultraviolet radiation and along with it the incidence of skin cancer. One approach to protect humans from the deleterious effects of UV irradiation is to use chemopreventive agents, which by definition is a means of cancer control where the disease can be entirely prevented, slowed, or reversed by topical or oral administration of naturally occurring or synthetic compounds or their mixtures. Ideally chemopreventive compounds must be nontoxic, antimutagenic, anticarcinogenic, and have the ability to exert inhibitory effects on diverse cellular events associated with multistage carcinogenesis [1]. The use of dietary agents possessing myriad beneficial effects is considered to be ideal as they are mostly nontoxic at the consumed levels and are acceptable to every individual. Studies carried out in the past three decades have conclusively shown that *C. sinensis* (family Theaceae), commonly known as tea, has been scientifically investigated and shown to be effective in preventing skin cancer.

C. sinensis in the Prevention of UV-Induced Skin Cancer

Tea, a plant native to China and Southeast Asia has been cultivated and consumed by humans for thousands of years [2]. Historical evidence suggest that the tea plant was native of China, Burma, Thailand, Laos, and Vietnam but today are also cultivated in Sri Lanka, India, and Japan. Globally, tea is the second most widely consumed beverage after water. Teas from the genus Camellia are generally divided into three main categories based on their processing method. Green tea (unfermented), oolong tea (partially fermented), and black tea (fully fermented) are manufactured from the same tea plant, *C. sinensis* [2–4].

For green tea manufacture, leaves are immediately heated or rapidly dried to inactivate polyphenol oxidase and native microflora which catalyzes the aerobic oxidation of tea catechins. This process generally protects tea catechins from oxidization, as long as processing steps are carried out in a timely manner. For black tea, tea leaves are crushed and allowed to wither to induce oxidization and fermentation prior to drying. The characteristic color, reduced bitterness and astringency, and general flavor are derived from this process giving black tea a marked distinction from green tea. Of the total commercial tea production worldwide, about 80% is consumed in the form of black tea, 18% in the form of green tea and 2% as oolong tea. Black tea is consumed principally in Europe, North America, and North Africa, green tea throughout Asia, and oolong tea in China and Taiwan [2–4].

Phytochemistry

Tea is one of the most investigated plants and detailed information on the phytochemical constituents is available. The active compounds of green tea are the catechins [(−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG)] (−), proanthocyanidins, flavonols [kaempferol, quercetin, and myricetin in the form of glycosides] (−), gallic acids, and theanine, while that of black tea are thearubigins and theaflavins (Fig. 34.1). The relatively less commonly used oolong tea is reported to contain monomeric catechins, theaflavins, and thearubigins [2]. Tea leaves also contain about 2–5% of the alkaloids caffeine and small quantities of

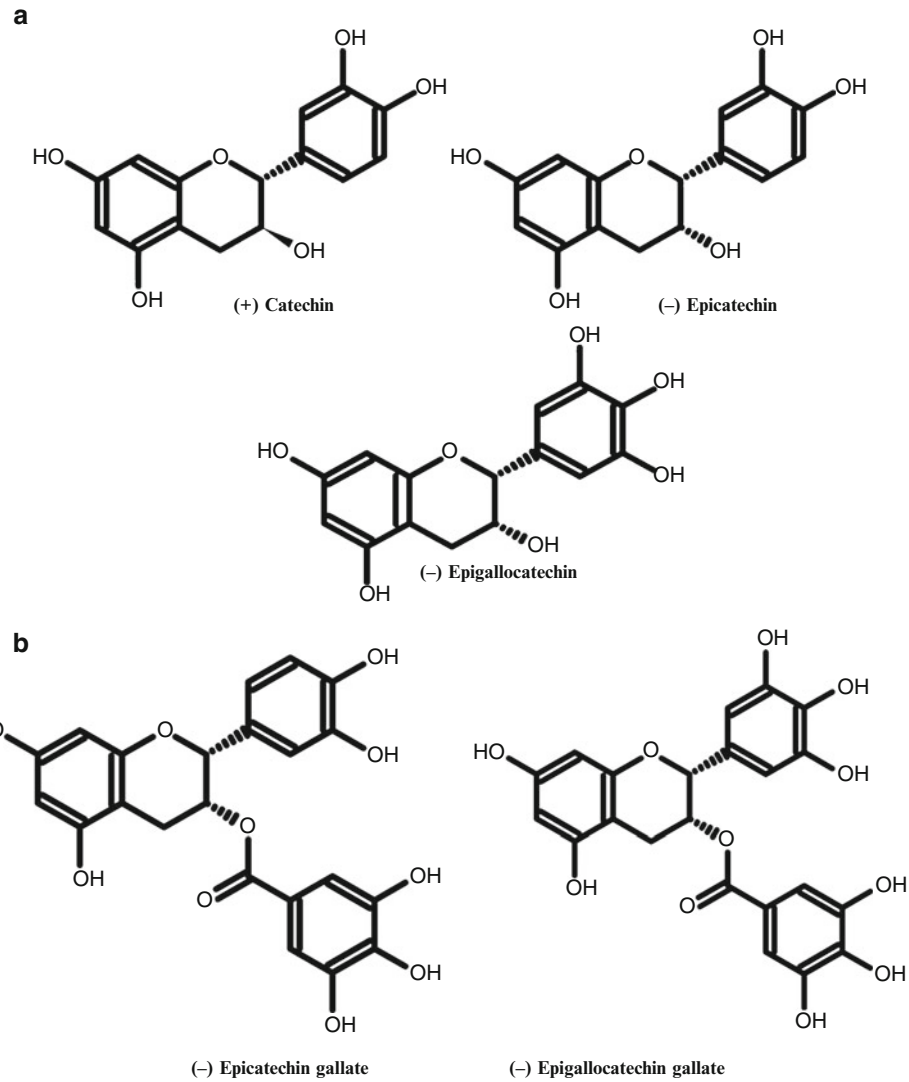

Fig. 34.1 (**a**–**c**) Polyphenols in green tea, (**d**–**f**) polyphenols in black tea

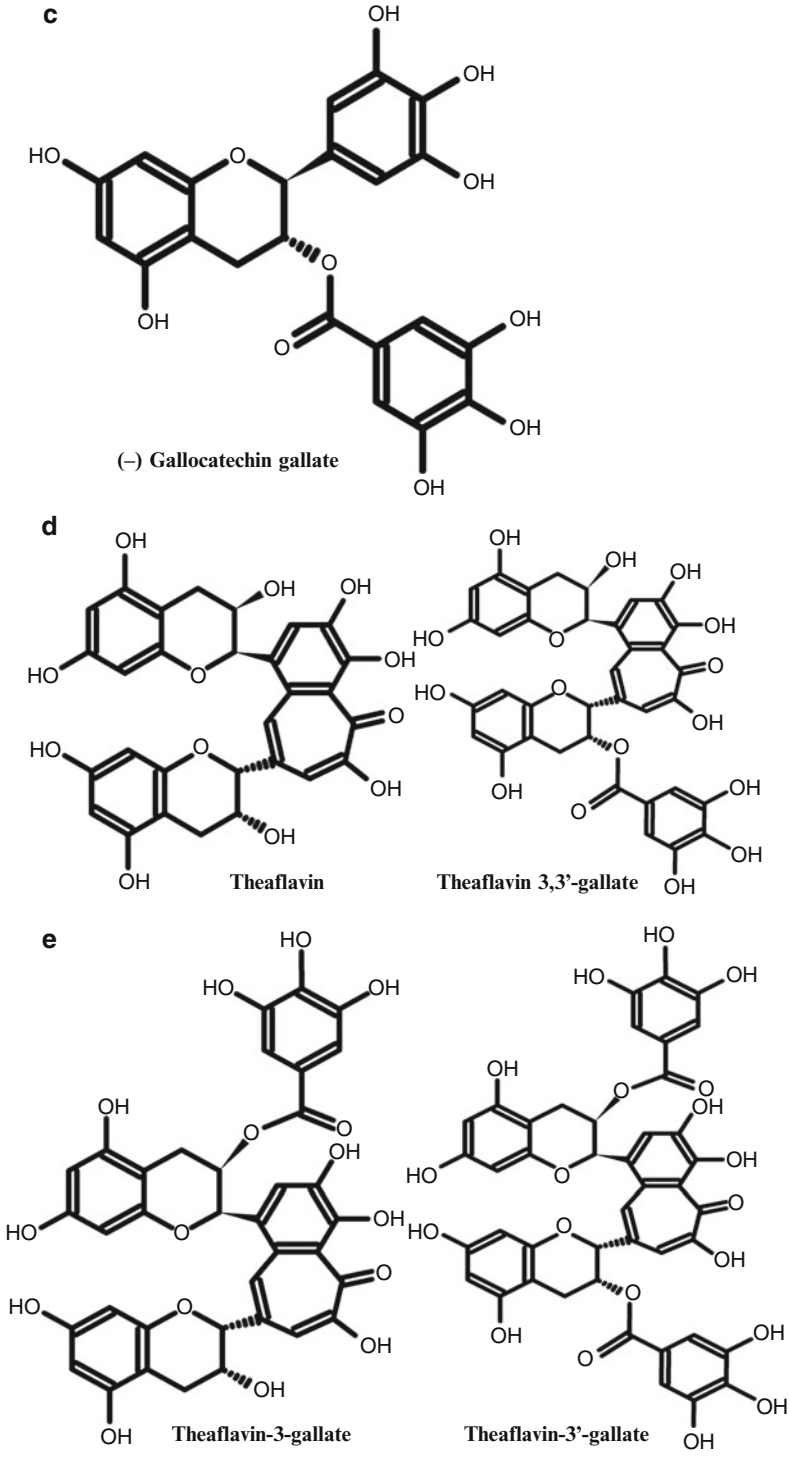

Fig. 34.2 (continued)

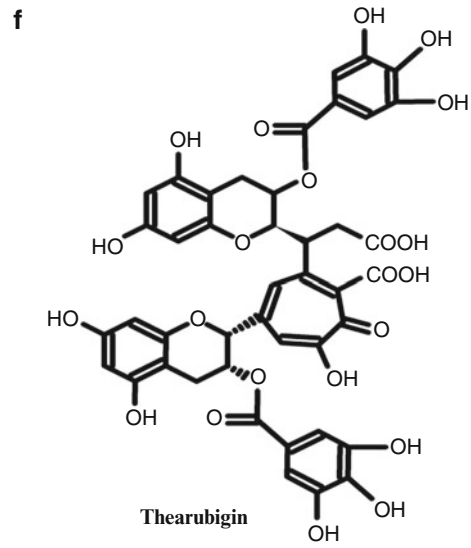

Fig. 34.2 (continued)

theobromine and theophylline. Other related compounds in this class include isotheaflavins, neotheaflavins, theaflavic acids and epitheaflavic acids, theafulvins, and theacitrins [2].

Tea Prevents UV Carcinogenesis in Experimental Systems

Tea is arguably the highly investigated natural product and has been studied for its anticancer and chemopreventive effects [3]. Animal studies have shown that green tea polyphenols protected mice against ultraviolet B radiation (UVB)-induced photocarcinogenesis [4]. Additionally, the individual fractions and the principle photochemical EGCG were also effective in inhibiting the growth of established skin papillomas in mice [5]. When compared to the placebo treated irradiated controls, irrespective of route of administration (oral or topical, intraperitoneal), the green tea polyphenols fraction and EGCG were effective in reducing the number of tumors [6]. Subsequent studies also showed that the polyphenolic fraction isolated from green tea protected against the UVB radiation-induced effects in the skin of SKH-1 hairless mice [7]. Gensler et al. [8] have also observed that the topical administration of pure EGCG prevents photocarcinogenesis.

With regard to black tea studies have shown that administering black tea reduced the UVA+B light-induced skin papillomas and that the chemopreventive effect was better than that by the green tea [9]. Studies have also shown that black tea, green tea, decaffeinated green and black teas also inhibited the formation and size of malignant and nonmalignant tumors [10–12]. Studies have also shown that black tea, green tea, decaffeinated black tea, and decaffeinated green tea were effective against DMBA-initiated and the light-induced skin carcinogenesis in SKH-1 mice [12]. Subsequent studies by many investigators have further confirmed the chemopreventive effects of the green tea, black tea, and their phytochemicals against the UV-induced skin carcinogenesis.

Corroborating the preclinical observations Hakim et al. [13, 14] in their population based study have observed an inverse association between tea consumption and the occurrence of squamous cell carcinoma of the skin in 450 older adults in Arizona, USA. A 6-month clinical trial in 118 patients with recalcitrant atopic dermatitis (a non-tumor lesion) has also showed that more than half the subjects had moderate to marked improvement after consuming oolong tea [15]. Together all these observations indicate the usefulness of tea in preventing UV-induced skin damage and photocarcinogenesis. In the following sections the various mechanisms responsible for the chemopreventive effects are addressed.

Mechanism Involved in Preventing Photocarcinogenesis

Free Radical Scavenging and Antioxidant Properties

Exposure to UVR causes excess generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) thereby leading to oxidative stress and nitrosative stress. Among ROS and RNS, the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide (NO), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2) are the most cytotoxic and damage the cell [16]. Scientific studies have shown that the composite extracts as well as the various organic fraction of both green and black teas have been reported to effective against hydrogen peroxide (H_2O_2) in vitro [17]. EGCG, the principal constituent decreased the release of intracellular hydrogen peroxide in HaCaT cells [18] and normal human epidermal keratinocytes [19]. EGCG treatment prevented UVB-induced production of H_2O_2 and nitric oxide (NO) by decreasing the inducible nitric oxide synthase-expressing cells in both epidermis and dermis at UVB irradiated site in mouse [19]. Additionally, both EGCG and theaflavins reduced nitric oxide production by suppressing inducible nitric oxide synthase [20]. Recently, Jagdeo and Brody [21] have also reported that the green tea polyphenols alone, and in combination with caffeine, inhibited the upregulation of H_2O_2-generated free radicals and 4-hydroxy-2-nonenal (HNE) in human skin fibroblasts WS-1 cells in vitro. The authors observed that when compared to the green tea polyphenols, caffeine alone had limited antioxidant properties.

The antioxidant enzymes SOD, GPx and catalase cooperate or work in a synergistic method to protect cell against oxidative stress. The SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide which then gets acted upon by the GPx and catalase to give the water. By this process the toxic free radicals are converted in to the nontoxic water molecule. When an appropriate balance exists between these three enzymes, the oxidative stress is reduced and the cells are protected from the cytotoxic and mutagenic effects of the ROS [16]. Cell culture studies with keratinocytes have shown that the treatment with tea polyphenols restored the glutathione peroxidase (GPx) levels, decreased UVA-induced lactate dehydrogenase and lipid peroxidation production [22]. Agarwal et al. [7] for the first time observed that the oral feeding of green tea polyphenols to mice resulted in significant protection against UVB radiation-mediated depletion of the antioxidant defense system in the epidermis. Additionally, the topical application of green tea polyphenols as well as EGCG prevented single or multiple UVB irradiation-induced depletion of antioxidant enzymes (glutathione peroxidase, catalase) and the glutathione levels in mice [23]. Together all these observations clearly indicate the usefulness of tea and its polyphenols to be effective antioxidants and to mediate their protective effects at least in part through this mechanism. Pretreatment with EGCG is also shown to halt the UV-induced decrease in GSH level and to afforded protection to the antioxidant enzyme GPx in human volunteers [24].

Prevention of Mutagenesis

The process of UV-induced carcinogenesis is extended and involves a complex series of events of which the induction of DNA damage and mutation is the primary event. The composite extracts as well as the various organic fraction of both green and black teas have been reported to effective in preventing UV-induced generation of 8-hydroxy 2′-deoxyguanosine (8-OHdG) in vitro [17]. The tea tannin components are also shown to be effective in inhibiting the UV-induced sister chromatid exchanges (SCEs) and chromosome aberrations in the normal human cells but not in XP cells, suggesting tea tannins were effective only in the normal cells [25]. To further substantiate these observations, studies by Tobi et al. [18] have shown that the treatment of HaCaT cells with EGCG reduced UVA-induced DNA single strand breaks and inhibited the mutagenic effects. Studies with cultured HaCaT cells have also shown that EGCG reduced UVA-induced DNA single strand breaks and inhibited the mutagenic effects Tobi et al. [18]. Studies with the living skin equivalents have shown that when compared to the radiation alone cohort's treatment with EGCG protected against UVB-induced damage and breakdown, and to reduce the levels of 8-hydroxy-deoxyguanosine (8-OHdG) [26].

Animal studies have shown that the application of EGCG to mouse skin also inhibited the malignant transformation of papillomas to carcinomas and decreased UVB-induced global DNA hypomethylation pattern, a molecular hallmark of human cancer [27] and to block UV-induced DNA hypermethylation and histone modifications in the skin required for the silencing of tumor suppressor genes like Cip1/p21, p16 (INK4a) [28].

With regard to humans, studies have shown that the topical application of green tea polyphenols prior to single exposure of UVB inhibited both UVB-induced erythema response as well as CPD formation in both epidermis and dermis [24, 29]. Studies with human volunteers have also shown that consumption of green tea lowered the UVA-induced damage [30]. Studies with human volunteers have also shown that the topical application of GTP (approximately 1 mg/cm^2 of skin area) is shown to inhibit UVB-induced erythema response as well as CPD [29]. Together, all these observations clearly indicate that green tea polyphenols affords protection against UV-induced mutagenesis, thereby preventing a critical step in initiation of skin cancer.

Anti-inflammatory Effects of Tea

Exposure of skin to UV causes inflammatory response which then manifests as erythema, edema and hyperplastic epithelial changes [31, 32]. Long-term oral feeding of green tea polyphenols in drinking water protected SKH-1 hairless mice against UV-induced cutaneous edema, erythema. Green tea polyphenols halted the depletion of antioxidant defense enzymes in the epidermis and also decreased the prostaglandin metabolism by inhibiting cyclooxygenase activity [33]. Topical application of green tea polyphenols before UV exposure decreased UV-induced hyperplastic response, myloperoxidase activity, and number of infiltrating inflammatory leukocytes, and protected against UV-induced inhibition of contact hypersensitivity response in mice [34]. The polyphenols extracted from green teas were shown to possess anti-inflammatory effect on mouse skin and inhibited epidermal lipid peroxidation [35]. Application of EGCG before exposure to UVB also reduced the bifold-skin thickness, inflammation, erythema, production of prostaglandin metabolites, infiltration of leukocytes and myloperoxidase activity [34, 35].

Animal studies with HWY/Slc hairless rats has shown that the regular intake of EGCG (1,500 ppm EGCG for 8 weeks) strengthens the skin's tolerance by increasing minimal erythema dose and thus prevents UV-induced perturbation of epidermal barrier function and skin damage [36]. UVB irradiation induces thinning of the epidermis and studies with living skin equivalents have shown that

treatment with EGCG prevented this [26]. EGCG treatment reduced UVA-induced skin damage (roughness and sagginess) and prevented decrease in the levels of dermal collagen in hairless mouse skin [37]. EGCG treatment is also shown to block UV-induced increase in collagen secretion and collagenase mRNA level in fibroblast culture [37]. These results suggest that EGCG is a potent candidate for systemic photoprotection.

Clinical studies have also shown that the application of green tea polyphenols before UV irradiation from a solar simulator on the untanned backs of normal volunteers reduced erythema in a dose-dependent manner [38]. This study showed that EGCG and ECG polyphenolic fractions were most efficient at inhibiting erythema, whereas EGC and EC had minimal effect. Histologic studies showed that green tea extracts reduced the number of sunburn cells and protected epidermal Langerhans cells from UV damage [38]. Topical application of EGCG prior to UVB radiation in human volunteers decreased the UV-induced production of hydrogen peroxide, epidermal lipid peroxidation and the infiltration of the inflammatory CD11b (+) leukocytes [24, 39]. The topical application of EGCG before UVB (4 MED) exposure significantly blocked UVB-induced infiltration of leukocytes and reduced myeloperoxidase activity, decreased UVB-induced erythema, and caused fewer dead cells in the epidermis of human volunteers [39].

Recently, Heinrich et al. [40] have carried out a 12-week, double-blind, placebo-controlled study with 60 female volunteers on either a beverage with green tea polyphenols (providing 1,402 mg total catechins/day) or a control beverage and measured the skin photoprotection, structure, and function at baseline (week 0), week 6, and week 12 following exposure of the skin areas to 1.25 minimal erythemal dose of radiation from a solar simulator. The authors observed that in the cohorts receiving green tea, the UV-induced erythema decreased by 16 and 25% after 6 and 12 week, respectively and the anatomical features like elasticity, roughness, scaling, density and water homeostasis was better than the place treated cohorts. Intake of the green tea polyphenol beverage for 12 week increased blood flow and oxygen delivery to the skin. In a separate, randomized, double-blind, single-dose (0.5, 1.0, and 2.0 g) study the authors also observed that intake of green tea polyphenols increased the blood flow with a peak at 30 min post ingestion [40].

Modulation of Enzymes

Tea and their components have demonstrated to affect multiple enzymes associated with carcinogenesis. Agarwal et al. [7] for the first time observed that the oral feeding of green tea polyphenols to mice resulted in significant protection against UVB radiation-mediated induction of epidermal ornithine decarboxylase (ODC) and cyclooxygenase (COX) enzyme activities, which play an important role in cutaneous inflammation, cell proliferation and tumor promotion. Recent studies also demonstrate that EGCG reduced dihydrofolate reductase activity, which would affect nucleic acid and protein synthesis [20]. Studies with human skin cells have shown that EGCG modulates UVA-induced activation of haemoxygenase, collagenase, and cyclooxygenase gene expression [41]. All these reports suggest that the tea polyphenols act on multiple targets and achieve control of the carcinogenesis process.

Modulation of Signal Transduction Molecules

Signal transduction pathways mediated by STAT3, Akt, ERK, JNK, and MAPK are recognized as potential molecular targets for cancer treatment and prevention [42]. Studies with cultured normal human epidermal keratinocytes (NHEK) have shown that pretreatment with EGCG inhibited

UVB-induced hydrogen peroxide production and to inhibit UVB-induced phosphorylation of ERK1/2, JNK, and p38 proteins [19]. EGCG is also shown to promote keratinocyte survival and to inhibit UV-induced apoptosis by phosphorylating Ser112 and Ser136 of Bad protein through Erk and Akt pathways [43]. Treatment of 30.7b Ras 12, a Ras-transformed mouse epidermal cells with EGCG and theaflavins decreased the level of phosphorylated Erk1/2 and Mek1/2, and the association between Raf-1 and Mek1 [44]. EGCG is also shown to directly inhibit the kinase activity of Erk1/2 by competing with Elk-1 in both normal [43] and Ras-transformed cells [45]. Studies with living skin equivalents have also shown that EGCG treatment protects against UVB irradiation by suppressing JNK and p38 MAPK activation [26]. EGCG and theaflavins inhibited STAT1 (Ser727), ERKs, JNKs, PDK1, and p90RSK2 phosphorylation [46]. Animal studies have also shown that the application of EGCG to mouse skin in a hydrophilic-based cream inhibited UVB-induced phosphorylation of ERK1/2, JNK, and p38 proteins of MAPK family in a time-dependent manner [23].

Modulation of Key Transcription Factors

Activation of signaling pathways converge upon a group of proteins known as the transcription factors. These proteins bind to the specific consensus sequences (*cis* elements) in the promoter regions of the effector genes and transactivate or repress the gene expression. Studies have shown that the transcription factors/activators like AR, Sp1, STATs, E2F, Egr1, c-Myc, HIF-1α, NF-kB, AP-1, ETS2, GLI, and p53 in the process of carcinogenesis and intervention [47]. Cell culture studies with JB6 cells have shown that EGCG and theaflavins inhibited AP-1dependent transcriptional activity through the inhibition of a c-Jun NH_2-terminal kinase-dependent [48] and to inhibit the UVB-induced phosphatidylinositol 3-kinase activation [49]. Additionally, the UVB-induced AP-1 activation was suppressed by both EGCG and theaflavins, and was also accompanied by inhibition of both ERK and c-Jun N-terminal kinase [50].

EGCG is shown to inhibit UVB-induced increase in the transcriptional activation of the c-fos gene [51, 52], while theaflavins to inhibit UVB-induced increase in the transcriptional activation of the c Jun gene [50]. EGCG is also shown to suppress activation of AP-1 in cultured human keratinocytes [53]. Studies with normal human epidermal keratinocytes have also shown that EGCG inhibits UVB-induced activation of NFkB [54, 55] and to attenuate the UV-induced apoptosis; Fas ligand activation and expression of IL-6 suggesting multiple signal transduction pathways are affected [55]. Additionally, studies have also reported that EGCG protects against the deleterious effects of both UVB and UVA radiation by inhibiting the activities of nuclear transcription factors NF-kB and AP-1 binding activities [37].

Studies with human volunteers have also shown that topical application of green tea extract at low, cosmetically usable concentrations reduces UVB-mediated epithelial damage without tachyphylaxis over 5 weeks [56]. Molecular studies showed that treatment with green tea extract did not affect UV-induced erythema and thymidine dimer formation but reduced UV-induced p53 expression and the number of apoptotic keratinocytes (sunburn cells and TUNEL-positive cells) [56]. Together all these observations indicate green tea extract to be suitable as an everyday photochemopreventive agent [56].

Modulation of Apoptosis

Apoptosis, a form of programmed cell death, plays a fundamental role in the maintenance of tissues and organ systems by providing a controlled cell deletion to balanced cell proliferation. Studies have

confirmed that many dietary chemopreventive agents can preferentially inhibit the growth of mutated, preneoplastic and tumor cells by targeting one or more signaling intermediates thereby leading to induction of apoptosis [42]. Tea and tea polyphenols are reported to induce apoptosis of cultured neoplastic cells by generating H_2O_2 [45], upregulating proapoptotic Bax, decreasing antiapoptotic Bcl-2 and Bcl-xl proteins, induce loss in mitochondrial transmembrane potential, stimulate release of cytochrome *c*, increase Apaf formation, activate caspases and breaking of poly (ADP-ribose) polymerase (PARP) proteins [47]. EGCG causes cell cycle arrest and induces apoptotic activity in neoplastic cells by the p53-dependent pathway and that the ablation of either p21 or Bax prevents p53-dependent apoptosis [57].

Cell culture studies with keratinocyte cell line have shown that EGCG treatment decreased UVB-induced cell cytotoxicity and apoptosis, inhibited the mRNA expressions of apoptosis-regulatory gene (p53 and p21) and c-fos gene [58]. Studies have also shown that the topical application of the EGCG to aged human skin stimulated the proliferation of epidermal keratinocytes (thereby increasing the epidermal thickness) and to concomitantly inhibit UV-induced apoptosis of epidermal keratinocytes. Mechanistic studies showed that EGCG promotes keratinocyte survival and inhibits the UV-induced apoptosis via two mechanisms: by phosphorylating Ser112 and Ser136 of Bad protein through Erk and Akt pathways, respectively, and by increasing the Bcl-2-to-Bax ratio [43].

EGCG causes a dose-dependent decrease in the viability and growth of the melanoma cell lines (A-375 amelanotic malignant melanoma and Hs-294T metastatic melanoma), while at similar EGCG concentrations; the normal human epidermal melanocytes (NHEM) were not affected [59]. EGCG decreased the cell proliferation, colony formation ability and to induce apoptosis by the downmodulation of anti-apoptotic protein Bcl2, upregulation of proapoptotic Bax and activation of caspases-3, -7 and -9 [59].

With regard to photocarcinogenesis, the oral pretreatment of SKH-1 mice with green tea enhanced the ultraviolet (UV)-induced increases in the number of p53-positive cells, p21-positive cells, and apoptotic sunburn cells in the epidermis [60]. Topical applications of caffeine or EGCG inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice [61]. Generally, the growth rate of pre-neoplastic or neoplastic cells exceeds that of normal cells due to dysregulation of their cell-growth and cell-death machineries and these studies clearly suggest that the tea polyphenols remove the genetically damaged, pre-initiated or neoplastic cells from the body and protect against neoplasia.

Affect on Fat Layers of the Skin

In an interesting observation, Lu et al. [61] and Conney et al. [12] have reported that that oral administration of green tea, black tea and caffeine decreases the size of the parametrial fat pad and the thickness of the dermal fat layer both away from the tumor and directly under it. Studies with obese mice have shown the levels of oxidative stress are greater in the adipose tissue than in other tissues and are accompanied by augmented expression of NADPH oxidase and decreased levels of antioxidant enzymes [62]. Further, the macrophages, which are an important source of inflammatory responses, infiltrate and accumulate in the obese adipose tissues [63]. Katiyar and Meeran [64] have observed that chronic exposure to UVB resulted in greater oxidative stress in the skin of obese mice in terms of higher levels of H_2O_2 and NO production, photo-oxidative damage of lipids and proteins, and greater depletion of antioxidant defense enzymes, like glutathione, glutathione peroxidase, and catalase. Further the authors also observed that when compared with the wild-type mice, the obese mice exhibited higher levels of phosphorylation of ERK1/2, JNK, and p38 proteins of the MAPK family, greater activation of NF-κB/p65, and higher levels of circulating proinflammatory cytokines, including TNF-α, IL-1β and IL-6 on UVB irradiation. When the results of Lu et al. [60] and Conney et al. [12]

are considered with the observations of Katiyar and Meeran, [64] it is logical to assume that by reducing fat in the skin of mice, tea prevents carcinogenesis by a unique mechanism.

Influence on the Dermal Immune System

Studies have shown that some of the deleterious effects like exacerbation of infectious diseases, premature aging of the skin and induction of skin cancer are mediated at least in part by UV radiation mediated immune suppression [34, 65]. In one of the earliest studies, Katiyar et al. [34] have shown green tea polyphenols to be protective against UVB-radiation-induced edema responses, local and systemic suppression of contact hypersensitivity in C3H/HeN mice. In this study, immunosuppression was assessed by contact sensitization with 2, 4-dinitrofluorobenzene applied to UVB-irradiated skin (local suppression) or to a distant site (systemic suppression), while double skin fold swelling was used as the experimental end point to measure of UVB-induced inflammation. Among the four major epicatechin derivatives present in green tea polyphenols, the major constituent EGCG was observed to be the best in affording protection against UVB-caused contact hypersensitivity and inflammatory responses [34].

EGCG treatment is also shown to prevent UVB-induced depletion in the number of antigen-presenting cells in both epidermis and dermis at UVB irradiated site [39]. Detail studies by Katiyar et al., [33] have shown that the prevention of UVB-induced immunosuppression in mice by the topical treatment of EGCG might be associated with alterations in IL-10 and IL-12 production. EGCG treatment before UVB exposure reduced number of CD11b+ monocytes/macrophages and neutrophils infiltrating into skin inflammatory lesions, which are considered to be responsible for creating the UV-induced immunosuppressive state [33, 39, 66]. In addition, EGCG was also found to decrease UVB-induced production of immunomodulatory cytokine IL-10 in skin as well as in draining lymph nodes (DLN), whereas production of IL-12, which is considered to be a mediator and adjuvant for induction of contact sensitivity, was found to be markedly increased in draining lymph nodes when compared with mice exposed to UVB alone [66].

Green Tea Inhibits UV-Induced Immunosuppression and Photocarcinogenesis Through IL-12-Dependent DNA Repair

Reports suggest that IL-12 has the ability to remove or repair UV-induced CPDs by inducing the nucleotide excision repair (NER) pathways, thereby contributing towards prevention of UV-radiation-induced immunosuppression [67, 68]. Using fibroblasts from patients suffering from xeroderma pigmentosum (XPA gene is an essential component of the NER and cells with a mutated XPA gene completely lack NER function) and from normal healthy persons, Meeran et al. [69, 70] have shown that EGCG does not prevent the immediate formation of CPDs after UVB exposure thereby excluding the UVB-radiation-filtering effect. However, when the cells were analyzed 24 h after UVB irradiation, the numbers of CPD+ cells were significantly reduced in NER-proficient cells but not in NER-deficient cells from XPA patients, suggesting that EGCG might accelerate the repair of UVB-induced CPDs through NER mechanism. In a separate study, the authors have also shown that the numbers of CPD+ cells were significantly lower in EGCG-treated WT mice than in WT mice exposed to UVB (without EGCG) [69, 70]. In the IL-12 KO mice EGCG did not reduce the dimers, when compared with the concurrent irradiated controls (IL-12 KO without EGCG) clearly suggesting that EGCG-induced IL-12 may contribute to the repair of UV-damaged DNA and that the differences in DNA repair between WT and IL-12 KO may be due to the absence of IL-12 [69, 70].

UV-induced DNA damage is an important molecular trigger for the migration of antigen presenting cells (i.e., Langerhans cells in the epidermis) from the skin to the draining lymph nodes. DNA damage in antigen presenting cells impairs their capacity to present antigen, which in turn results in lack of sensitization [71]. CPD-containing antigen presenting cells have been found in the draining lymph nodes of UV-exposed mice and these antigen-presenting cells were identified to be of epidermal origin and to exhibit an impaired Ag presentation capacity [71]. Treatment with EGCG resulted in a significant reduction in the numbers of CPD positive cells in the draining lymph nodes of UV-exposed WT mice compared to UV-exposed WT mice that did not receive EGCG. In contrast, there was no significant difference in the number of CPD positive cells in the draining lymph nodes between EGCG-treated and non-EGCG-treated UV exposed IL-12 KO mice suggesting EGCG-induced IL-12-mediated repair of CPD dimers in cells [69, 70].

Topical treatment with EGCG inhibited photocarcinogenesis in terms of tumor incidence, tumor multiplicity, and tumor growth or tumor size in WT (C3H/HeN) mice, but not in IL-12 KO mice, indicating that the prevention of UVB-induced skin cancer by EGCG requires IL-12. EGCG removed or rapidly repaired UVB-induced CPDs in WT mice compared to IL-12 KO mice [69, 70]. Furthermore, the treatment of EGCG-treated IL-12 KO mice with recombinant IL-12 removes or repairs UVB-induced CPD positive cells. This information further supports the finding that EGCG promotes the removal or the repair of damaged DNA in UVB-exposed skin through a mechanism that requires IL-12 activity [69, 70]. Treatment with EGCG also caused reduction in the number of sunburn cells in WT mice, while in IL-12 KO mice it was unproductive implying that this difference in the repair kinetics of sunburn cells in IL-12 KO and WT mice may be attributed due to the induction of IL-12 in WT mice by EGCG. This mechanistic information strongly support and explain the chemopreventive activity of green tea polyphenols against photocarcinogenesis.

Green tea polyphenols were effective in preventing the UV-induced immunosuppression only in the NER-proficient mice indicating it mediates the protective effect at least in part through the NER pathway. Cell culture studies showed that green tea polyphenols repaired UV-induced CPDs in xeroderma pigmentosum complementation group A (XPA)-proficient cells of a healthy person but not in XPA-deficient cells obtained from XPA patients, indicating that a NER mechanism is involved in DNA repair [72]. In vitro studies with KB cells and normal human keratinocytes have also shown that treatment with green tea polyphenols reduced the in UVB-induced apoptosis, and that this effect was completely reversed upon addition of an anti-IL-12-antibody, indicating that the reduction of UV-induced cell death by green tea polyphenols is mediated via IL-12 [73]. Green tea polyphenols also induced secretion of IL-12 in keratinocytes [73].

Stimulation of Cytotoxic T Cells in Skin Tumors

The CD8+ T cells, which are effector cells in the cytotoxic response of the host to UV-induced skin tumor cells, are of significance in protecting against UV-induced carcinogenesis [74]. Mantena et al. [75] have shown that the administration of green tea polyphenols in drinking water inhibits photocarcinogenesis in mice, and that this activity was mediated, at least in part, by recruitment of the cytotoxic T cells in a tumor microenvironment. IL-12 has been shown to stimulate production of IFN-γ and to stimulate development of cytotoxic T cells (e.g., CD8+ T cells), which are tumoricidal and causes inhibition or regression of tumors. In this study the authors observed that the oral administration of green tea polyphenols enhanced the number of CD8+ T cells in tumors when compared with the non-green tea polyphenols-treated UVB-exposed mice. The ability of the oral administration of green tea polyphenols to enhance the infiltration or recruitment of higher numbers of CD8+ T cells in the tumor microenvironment may act to enhance the immunosurveillance mediated by these cells, thereby reducing the incidence of tumors [74, 75].

Meeran et al. [69, 70] also observed similar observations when mice were topically treated with EGCG and subjected to photocarcinogenesis. However, the degree of the chemopreventive effect of EGCG applied topically was greater than that of green tea polyphenols given in drinking water. Presumably, this difference may be due to the higher concentration of EGCG with topical application compared with the concentration of green tea polyphenols with oral administration. The chemopreventive effect of orally administered green tea polyphenols in mice was also substantial and as it is easily obtained from green tea beverage, could be used for the prevention of UV-induced skin cancer and other harmful effects of UV radiation at cost-effective terms.

Inhibition of Angiogenesis and Metastasis in Skin Tumors by Tea

Angiogenesis and metastasis are key process in the cancer progression and their prevention is vital. Studies with cultured human melanoma cell line A375 have shown that theaflavin downregulates MMP-2 by affecting multiple regulatory molecules like FAK, EGFR and ERK [76]. Studies with artificial skin composed of cultured keratinocytes on a collagen matrix populated with fibroblasts has shown that treatment with EGCG prior to UVA irradiation decreases the level of MMPs production and to increase TIMP-1 expression level. EGCG also suppresses the activities of the gelatinases, to augment the expressions of the TIMP-1 and to inhibit extracellular matrix degradation induced by UV [77].

Studies have shown that the green tea polyphenols prevent ultraviolet light-induced oxidative damage and expression of matrix metalloproteinases in mouse skin [78]. To further substantiate these observations Garbisa et al. [79] have also reported that green tea inhibited the tumor invasion potential of tumor cells. Tea polyphenols reduced angiogenesis, in part by decreasing vascular endothelial growth factor production and receptor phosphorylation [20]. Studies have also shown that the oral administration of green tea polyphenols inhibited the expression and activity of both MMP-2 and MMP-9 in tumors and to concomitantly increase the expression of TIMP1 [74]. Green tea polyphenols inhibited the expression of vascular endothelial cell antigens, such as CD31 and VEGF, in UVB-induced tumors. The increased expressions of these proteins play an important role in tumor growth, invasion and metastasis due to the promotion of a new vasculature that supports tumor growth [74]. Thus, the administration of green tea polyphenols in drinking water has significant antiangiogenic effects and has the potential to reduce the growth or to cause the regression of tumors through this mechanism [74]. Similar effects were also observed when SKH-1 hairless mice were topically treated with EGCG [75]. Again, the antiangiogenic effect of EGCG, when applied topically, was greater than that observed with green tea polyphenols given in drinking water [75].

EGCG is shown to inhibit tumour invasion and angiogenesis by inhibiting MMP-2 and MMP-9 [80]. EGCG modulates the expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in fibroblasts irradiated with ultraviolet A [81]. Further, EGCG also inhibits urokinase (uPA), an important proteolytic enzyme in a dose dependent manner at nontoxic concentrations [82]. Cumulatively all these observations clearly indicates another major pathway by which green tea polyphenols can inhibit tumor growth by inhibiting the vascular endothelial growth factor (VEGF) and the matrix metalloproteinase (MMP)-2 and MMP-9, with concomitant increase in TIMPs [83, 84].

Reverting the Hypermethylation and Histone Modifications

Studies in the recent past have emphasized that the altered epigenetic modification patterns play a cardinal role in tumorigenesis. The epigenetic control of transcription is regulated by enzymes that

mediate covalent modifications at gene-regulatory regions and histone proteins around which chromosomal DNA is wound. Some of the enzymes mediating the chromatin epigenetic reactions (like DNA methyltransferase and histone deacetylase) are deregulated in cancer and are considered to be ideal targets for developing anticancer/chemopreventive agents [85]. Studies have shown that the topical application of EGCG (approximately 1 mg/cm^2 skin area in hydrophilic cream) protected against UV-induced skin carcinogenesis and that this was mediated at least in part by the inhibition of UVB-induced global DNA hypomethylation [27]. Recently, Nandakumar et al. [86] have reported that treatment of A431 cells with EGCG decreased the levels of 5-methylcytosine, activity of DNA methyltransferase (DNMT), messenger RNA (mRNA), and protein levels of DNMT1, DNMT3a, and DNMT3b. EGCG also decreased the levels of histone deacetylase activity and increased levels of acetylated lysine 9 and 14 on histone H3 (H3-Lys 9 and 14) and acetylated lysine 5, 12, and 16 on histone H4 but decreased levels of methylated H3-Lys 9. Additionally, EGCG treatment resulted in re-expression of the mRNA and proteins of silenced tumor suppressor genes, p16INK4a and Cip1/p21. Together these observations indicate that (−)-epicatechins from green tea have the ability to block UV-induced DNA hypermethylation and histone modifications in the skin required for the silencing of tumor suppressor genes [e.g., Cip1/p21, p16(INK4a)] [28], thereby contributing towards the chemopreventive effects.

Conclusions

Mounting evidence show that tea and their compounds due to their antioxidant properties, inhibitory effects on signal transduction pathways, cell proliferation, angiogenesis, capacity for apoptosis induction, immune protective effects are promising chemopreventive agents against ultraviolet-induced skin cancers (Fig. 34.2). The future challenge will be to combine these strategies as well as to devise new ones that are effective in preventing the harmful effects of UV radiation and thereby translating them for effective human application alone or in combination with other protective agents.

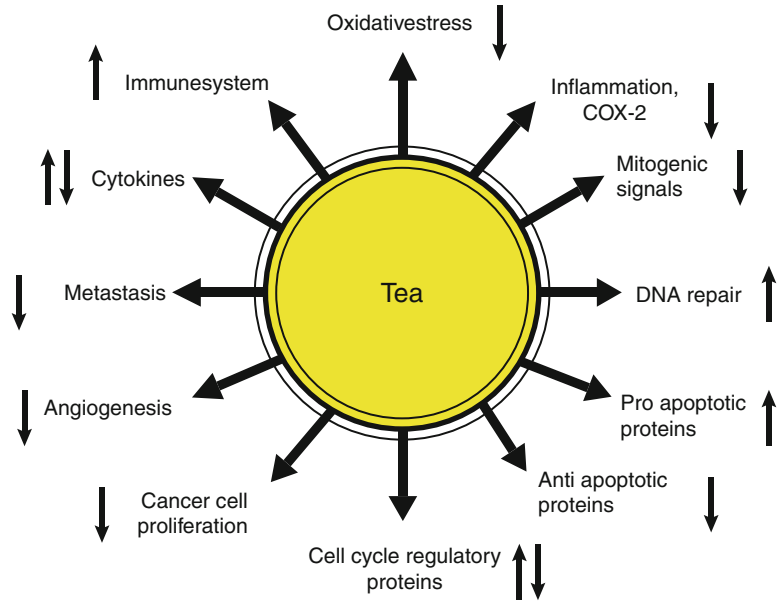

Fig. 34.2 Mechanisms by which tea and its constituents mediate the cancer preventive effects against UV-carcinogenesis (*arrow up* increase, *arrow down* decrease)

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Chapter 35
Melanoma and Leptin

Arash Sabetisoofyani

Key Points

- Anthropometrical measures, such as height, weight, and body mass index (BMI), have been associated with an increased risk of several malignancies, including melanoma [4–6].
- Obesity increases the expression of angiogenic factors, such as leptin, that may contribute to tumor growth.
- Leptin, a hormone secreted by adipose tissue, controls food intake and energy balance by providing signals to the hypothalamus [7].
- Serum levels of leptin are positively related with body composition and insulin levels, female sex, and alcohol consumption and inversely related to cigarette smoking [8–10].
- High intake of specific agents, such as antioxidants and retinoid-rich food, has been linked to a protective effect against melanoma development.

Keywords Melanoma • Obesity • Leptin • Angiogenic factor • Antioxidant

Introduction

Obesity has increased dramatically in the United States in the past three decades; approximately32 % of adults are obese with a BMI greater than 30 kg/m^2 [11]. Epidemiological studies have shown that there is a strong, positive correlation between BMI and the incidence of several types of cancer, such as colon, breast, ovarian, prostate, renal cell carcinoma, and melanoma. Calle et al. concluded that obesity may account for 50 % of cancer deaths in women aged 50 years or older [12]. However, there have been few studies that have demonstrated a direct cause and effect relationship between obesity and cancer. Moreover, the epidemiological evidence suggests that the response to obesity is not the same for all tumors. Consequently, the mechanisms by which obesity may influence carcinogenesis are poorly understood. Adipose tissue secretes several cytokines (i.e., adipokines) that are believed to promote inflammation, cell proliferation, and angiogenesis. Leptin, an adipokine that is produced in proportion to the mass of adipose tissue and which circulates in the blood, has been suggested to link

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obesity with tumor growth [13–15]. Leptin stimulates cell proliferation in several tumor cell lines, enhances endothelial cell migration in vitro, and has been suggested to be an angiogenic factor [16, 17]. Thus, leptin has several effects that could contribute to tumor growth. However, most of the studies supporting leptin's role in promoting cell proliferation and angiogenesis have been conducted in vitro and have involved the use of large, pharmacological concentrations of leptin to demonstrate the effects.

Structure

Leptin is an adipocyte-derived hormone that acts as a major regulator for food intake and energy homeostasis. Leptin deficiency or resistance can result in profound obesity, diabetes, and infertility in humans. Since its discovery, our understanding of leptin's biological functions has expanded from antiobesity to broad effects on reproduction, hematopoiesis, angiogenesis, blood pressure, bone mass, lymphoid organ homeostasis, and T lymphocyte systems. Leptin receptor belongs to the class I cytokine receptor superfamily. At least five isoforms of leptin receptor exist, primarily because of alternate splicing. The longest form is capable of full signal transduction. The short forms may serve as leptin-binding proteins and play a role in leptin transporting across the blood–brain barrier.

Background

The few published animal studies attempting to determine whether leptin and obesity promote tumor growth have produced mixed results. Some studies support the hypothesis that the absence of leptin signaling attenuates mammary tumor growth in mice [18–20]. For example, Gonzalez et al. demonstrated that mouse mammary tumor growth was dramatically reduced when mice were treated with a leptin receptor antagonist [21], suggesting that leptin signaling may be necessary for certain types of breast tumor growth. In apparent contradiction to these studies, obese Zucker rats, which have a mutation in the leptin receptor, developed more mammary tumors at an earlier age than lean Zucker rats after exposure to the carcinogen [22]. Until recently, there had been no studies that have examined the relationship between obesity and the incidence of melanoma. One mechanism by which obesity has been suggested to contribute to enhanced tumor growth is by promoting angiogenesis. The mechanisms that mediate the effects of food restriction on tumor growth are unclear but likely involve numerous metabolic pathways, including those involved in angiogenesis. The mechanisms for leptin's effects on tumor growth are still uncertain, although a role for leptin in promoting angiogenesis has been previously suggested. There is indirect evidence from clinical studies that leptin may enhance tumor growth by stimulating angiogenesis. For example, leptin expression correlated well with VEGF expression in human gastric cancers and both factors were associated with poor patient prognosis [23]. Results from a recent study in mice showed that mammary tumor VEGF and tumor growth were significantly reduced when mice were treated with the leptin receptor antagonist. Leptin has been identified in several types of human cancers and may also be linked to poor prognosis. In two studies, leptin and leptin receptor expression were significantly increased in primary and metastatic breast cancer relative to noncancerous tissues in women [24, 25]. In a clinical study of colorectal cancer, leptin expression was positively correlated with tumor grade [26]. Serum leptin and leptin receptor expression in renal cell carcinomas was well correlated with progression-free survival, venous invasion, and lymph node metastasis [27]. Leptin has also been shown to be expressed in uterine and endometrial cancers [28]. There is very little previous information on the relationship between leptin and melanoma. One epidemiological study reported that high serum leptin was positively correlated with melanoma risk [29].

Conclusion

Epidemiological studies suggest that hyperglycemia and hyperinsulinemia are associated with increased risk for development of cancer. However, further studies are needed for testing the hypothesis that hyperinsulinemia increases tumor growth in this model of melanoma. The mechanisms by which obesity promotes melanoma growth likely involve increased angiogenesis, since tumors from the obese mice have more VEGF. Leptin deficiency attenuates but does not abolish melanoma tumor growth. A key factor in obesity-induced angiogenesis that may be involved in promoting melanoma growth is inflammation. Obesity is often described as a low-level inflammatory state and the literature is replete with studies supporting a central role for macrophages in tumor angiogenesis. During weight gain, adipocytes increase in size, preadipocytes differentiate into mature adipocytes, and there is a sudden increase in the metabolic demand of the tissue. Adipocytes respond by secreting inflammatory cytokines that recruit macrophages. Macrophages, in turn, initiate angiogenesis by secreting matrix metalloproteinases to break down the extracellular matrix and basement membranes of nearby blood vessels. Together with adipocytes, macrophages secrete angiogenic factors that promote the proliferation and migration of endothelial cells. Once the new blood vessel is formed, macrophages secrete different cytokines that remodel the stroma to support their growth. The similarities between angiogenesis in obesity and cancer suggest that macrophages are central to both scenarios. Future studies should examine whether inhibition of inflammation in obesity reduces tumor growth.

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Part VII
Plants and Plant Components and Non-cancerous Skin Diseases

Chapter 36
Curcuma longa: Use for Skin Disease Care

Anand A. Zanwar, Sachin L. Badole, and Farid Menaa

Key Points

- *Curcuma longa* is commonly known as turmeric and rhizome powder of *C. longa* is used in various food preparations for preservation and freshness and imparts a characteristic flavor and color.
- It is the main ingredient in different types of skin cosmetics as it gives good complexion to the skin and so it is applied to face as a depilatory and facial tonic.
- *C. longa* is useful in the treatment of both aging and age-related chronic degenerative diseases.
- *C. longa* plays an important role in cancerous skin lesions, psoriasis, and wound healing.
- Most of the formulations containing *C. longa* are found to be safe and nontoxic.

Keywords *Curcuma longa* • Curcumin • Psoriasis • Skin aging • Turmeric • Wound healing

Introduction

Curcuma longa is commonly known as turmeric. *C. longa* is cultivated in warm, rainy regions of the world such as India, China, Indonesia, Jamaica, and Peru. *C. longa* is commonly cultivated in all parts of India, especially in West Bengal, Tamil Nadu, and Maharashtra. *C. longa* is given special importance to humans as rhizome powder (Fig. 36.1: photograph of rhizomes and powder of *C. longa*) and is used in various food preparations for preservation and freshness and imparts a characteristic flavor and color. Turmeric, which belongs to a group of aromatic spices, had been originally used as a food additive in curries to improve the storage condition, palatability, and preservation of food. It has been given special importance due to the strong antiseptic properties. Turmeric is commonly used for all

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Fig. 36.1 Photograph of rhizomes and powder of *Curcuma longa*

kinds of poisonous affections, ulcers, and wounds. It is the main ingredient in different types of skin cosmetics as it gives good complexion to the skin and so it is applied to face as a depilatory and facial tonic. "*Haridra Khand*," a compound containing powdered turmeric, sugar, and many other ingredients, is a well-known preparation for cold, cough, and flu and for skin diseases [1, 2].

The roots of *C. longa* are generally extracted by solvent-solvent extraction method. Normally, extracted by solvent extraction. Clinically it has proved as a bactericidal and now its use is more than being merely cosmetic. The importance of *C. longa* in medicine is increased due to the potent antioxidant properties of naturally occurring phenolic compounds in *C. longa* [3].

C. longa is used in the treatment of diseases due to morbid *vata*, *pitta*, and *kapha*, diabetes, eye diseases, ulcers, edema, anemia, anorexia, leprosy, and scrofula. Apart from this it is used as blood purifier by destroying the pathogenic organisms. Different formulations containing *C. longa* are commonly used in the treatment of ringworm, obstinate itching, eczema, and other parasitic skin diseases and turmeric is applied to facilitate the process of scabbing in chicken pox and small pox. Turmeric and alum powder in the proportion of 1:20 is blown into the ear in chronic otorrhea [4].

A preparation containing *Cynodon dactylon*, *Lawsonia alba*, *Acacia catchulonga*, *C. longa*, and *Terminalia chebula*, when mixed in specified amount with alcohol and water it form a paste. After boiling, filtering, cooling and adding camphor to this paste, it can be used in case of laceration and cut wounds [5].

Scientific Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Subclass: Zingiberidae
Order: Zingiberales
Family: Zingiberaceae
Genus: *Curcuma*
Species: *C. longa*
Scientific name: *Curcuma longa*

Common Names

English: Turmeric
Sanskrit: Haridra, varavarnini
Hindi: Haldi, halda
Bengali: Haldi
Malayalam: Manjal, pachamanjal, varattumanjal
Tamil: Mancal
Kannada: Haldi, arasina
Telugu: Pasapu
Marathi: Haldi

Botanical Description

Plant: It is a perennial herb, 60–90 cm in height, with a short stem and tufts of erect leaves.

Leaves: Leaves are simple, very large, petiole as long as the blade, oblong-lanceolate, tapering to the base up to 45 cm long.

Flower: Flowers are pale yellow, arranged in spikes concealed by the sheathing petioles and flowering bracts are pale green.

Rhizomes: Rhizomes are oblong, ovate, pyriform, cylindric, orange colored, and often short-branched (Fig. 36.1: Photograph of rhizomes of *C. longa*).

Traditional Uses of *C. longa*

Traditionally, *C. longa* has been used as a main ingredient in different types of foodstuff, cosmetic products, and medicine. Major part used is roots. It is also used as a spice for preparation of curry and also provides flavor. It is used a coloring agent in cheese, butter, and other foods. In folk medicine, turmeric and natural *C. longa*oids have been applied as therapeutic preparations over the centuries in different parts of the world. It is a major component in many ayurvedic preparations in India. In ayurvedic medicine, *C. longa* is a well-documented treatment for various respiratory conditions and for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough, and sinusitis. In traditional Chinese medicine, it is used to cure diseases associated with abdominal pain. In ancient Hindu medicine, it was used to treat sprains and swelling. It has traditionally been used for the treatment of anti-inflammatory and many of its therapeutic effects have been confirmed by modern scientific research, which include antioxidant, anti-inflammatory, anti-carcinogenic, and antimicrobial, hepatoprotective, thrombosuppressive, cardiovascular, hypoglycemic, and antiarthritic. Apart from this *C. longa* have been used in cardiac and diabetic complications also. The most compelling and key rationale for the continuing traditional therapeutic use of *C. longa* is its extremely good safety profile [6]. In case of urticaria a bit is taken orally daily for some days and grass along with *C. longa* is crushed and the paste is applied on the skin. The roots are crushed and applied directly on the infected skin after scratching it for ringworm. The extracted juice is mixed with milk and ghee and the mixture is orally taken for scabies. Equal amounts of the grass juice and *C. longa* juice are mixed and orally taken. Dried fruit peel of *Musa* spp. is crushed along with *C. longa* and the mixture is applied on

the infected skin used in leprosy. The juice of *Ocimum sanctum* is mixed with *C. longa* and orally taken for urticaria, urticaria, ringworm, scabies, dry skin/cracks, wrinkled skin, prickly heat, measles, cosmetics, etc. [7].

Role of *C. longa* in Skin Disease

C. longa in Cancerous Skin Lesions

Topical application of *C. longa* (ethanol extract of turmeric/ointment) to external cancerous skin lesions provided remarkable symptomatic relief that was in many cases relatively durable (lasting several months) and in all cases (except for a single adverse reaction in one subject) extremely safe. The positive effects included less itching in almost all cases and reduced lesion odor in 90%, dry lesions in 70%, and smaller lesion size and pain mitigation in 10% as reported by Kuttan et al. [8].

C. longa Beneficially Effects in Psoriasis

Psoriasis is the case of pro-inflammatory and potentially arthritis inducing skin disease wherein *C. longa* have been shown to have beneficial effects antipsoriatic effects of curcumin's evaluated by measuring its action on phosphorylase kinase activity. Increased levels are considered as a surrogate marker in psoriatic disease. In study reported by Heng et al., 2000, following four groups were made: (1) active untreated psoriasis; (2) resolving psoriasis treated with calcipotriol, a vitamin D3 analogue and an indirect inhibitor of phosphorylase kinase; (3) resolving psoriasis treated with *C. longa*; and (4) normal non-psoriatic subjects. It was observed that phosphorylase kinase activity was highest in the patients with psoriatic subjects, lower in the calcipotriol-treated subjects, even lower in the *C. longa*-treated group, and lowest in normal subjects. Interestingly, the decreased phosphorylase kinase activity in calcipotriol- and *C. longa*-treated patients was associated with corresponding decreases in the expression of keratinocyte transferring receptor, severity of parakeratosis, and density of epidermal CD8+ T cells. In the patients with active psoriasis it was shown that topical treatment with *C. longa* results in resolution of the psoriatic activity as assessed by clinical, histological, and immunological criteria [9].

The anti-psoriatic effects of *C. longa* may be due to the presence of antioxidants. The antioxidants may exert a positive effect on psoriasis-linked inflammation. Also the hydro-alcoholic extract of *C. longa*, in addition to exposure to white light (of photon flux density of 93 mol m^{-2} s^{-1}), may be a useful alternative to the standard psoriasis treatment with psoralen and UVA. *C. longa* and related products containing polyphenolic compounds show specific action in wound healing because of high antioxidant activity against free radical-induced autoxidation of unsaturated fatty acids. Also these antioxidants to protect the organism against the pathogenic results of the oxygen free radicals play a key role in both aging and the main age-related chronic degenerative diseases [10].

C. longa Beneficially Effects in Wound Healing

In this study the effect of *C. longa* on wound healing in mice exposed to whole-body-γ-radiation was observed by creating wound on the dorsum (below the rib cage) of mice whole-body irradiated to 2,

4, 6, or 8 Gy. The wound contraction was continuously monitored periodically and video imaging recording of the wound was carried out. The collagen, hexosamine, DNA, nitric oxide, and histological profiles were evaluated at various postirradiation days in mice treated and not treated with *C. longa* before exposure to 0 or 6 Gy. It was observed that the whole-body exposure resulted in a dose-dependent delay in wound contraction and prolongation of wound healing time. Irradiation caused a significant reduction in collagen, hexosamine, DNA, and nitric oxide synthesis. On the other hand pretreatment with *C. longa* significantly enhanced the rate of wound contraction; decreased mean wound healing time; increased synthesis of collagen, hexosamine, DNA, and nitric oxide; and improved fibroblast and vascular densities. This study demonstrates that *C. longa* pretreatment accelerated the repair of excision wound in mice whole-body exposed to γ-radiation [11].

In another study curcumin was binded with collagen. An attempt was made to slowly deliverer the antioxidants like curcumin from collagen matrix called curcumin incorporated collagen matrix (CICM). This CICM treated was compared with control- and collagen-treated rats in wound healing model. It was evident from biochemical parameters and histological analysis that there was increased wound reduction, enhanced cell proliferation, and efficient free radical scavenging in CICM group compared to untreated group. Moreover the in vitro antioxidant activity (2,20-azobisisobutyronitrile assay) of CICM suggested that CICM quenches free radicals more efficiently. Indicating topical application of CICM to support dermal wound healing [12].

C. longa Beneficially Effects in Aging

Tricutan gel is a formulation containing different types of herbal extracts along with *C. longa* which is traditionally used for treatment of skin conditions, together with dimethylaminoethanol. In randomized, placebo-controlled, double-blind, split-face study in 28 women, 34–67 years old, the effectiveness of Tricutan in improving skin firmness and elasticity in photoaged facial skin was screened. In this study treatment with Tricutan and placebo ($n=25$) was given for 4 weeks. By using the speed of propagation of ultrasound shear waves in the skin as end point, the skin firmness and elasticity were determined. The Tricutan treatment resulted in significant reduction in propagation speed suggesting increased firmness. In the self-evaluation in the women, the effect of treatment with Tricutan was found to be significant compared to placebo. In the clinical evaluation, Tricutan showed better treatment result compared to placebo. Based on this preliminary observation Tricutan can be used in the treatment of aging skin [13].

Toxicity Profile of *C. longa*

High dose of turmeric and ethanolic turmeric extract (5%) from *C. longa* in subchronic toxicity study showed a significant reduction in body weight gain and alterations in absolute and/or relative liver weights and hepatotoxicity (focal necrosis or focal necrosis with regeneration) in mice and rats. In case of acute toxicity study of mice, 0.2 or 1% for 14 days also showed hepatotoxicity [14].

Curcumin loaded polymeric nanoparticles of Eudragit S100 were subjected for acute-toxicity study, subacute-toxicity study (28 days), and various genotoxicity studies like in vivo micronucleus assay, in vivo chromosomal aberration assay, and in vivo comet assay. Up to dose level of 2,000 mg/kg the formulation was found to be safe and nontoxic in the acute-toxicity study. Similarly Eudragit S100 in subacute-toxicity study proved to be safe for prolonged administration at therapeutic dose of 100 mg/kg and at twice the therapeutic dose. Cellular safety of the Eudragit S100

was assessed at the therapeutic and at dose equivalent to thrice the therapeutic dose by genotoxicity study. Indicating safety of Eudragit S100 for oral administration for a short as well as a prolonged duration [15]. Mild irritative contact dermatitis was reported in few subjects in clinical study [14].

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Chapter 37
Western Diet-Mediated mTORC1-Signaling in Acne, Psoriasis, Atopic Dermatitis, and Related Diseases of Civilization: Therapeutic Role of Plant-Derived Natural mTORC1 Inhibitors

Bodo C. Melnik

Key Points

- Nutrient signaling of Western diet plays a fundamental role in the pathogenesis of epidemic skin diseases.
- Western style nutrition is characterized by high calorie uptake, high glycemic load, high fat consumption, and increasing intake of leucine-rich dairy- and meat proteins.
- Metabolic signals of Western diet are sensed by the nutrient-sensitive kinase, mammalian target of rapamycin complex 1 (mTORC1).
- mTORC1 integrates signals of cellular energy, growth factors (insulin, IGF-1) and protein-derived signals, predominately leucine.
- Exaggerated mTORC1 activity stimulated by Western diet plays a pivotal role in the pathogenesis of acne, psoriasis, atopic dermatitis, obesity, type 2 diabetes, cancer, and neurodegenerative disorders.
- Plant-derived polyphenols and isoflavonoids are inhibitors of mTORC1.
- Restriction of insulinotropic food as well as leucine-rich animal- and dairy proteins attenuates mTORC1 activity and has beneficial effects on mTORC1-promoted Western diseases.
- Patients should be encouraged to increase their intake of fruit and vegetables containing natural mTORC1 inhibitors and less leucine than animal-derived proteins.

Keywords Acne • Atopic dermatitis • Psoriasis • mTORC1 (mammalian target of rapamycin complex 1) • Nutrient signaling • Plant-derived mTORC1 inhibitors

Abbreviations

2DG	2-Desoxyglucose
4E-BP	Eukaryotic initiation factor (eIF) 4E-binding protein
AD	Atopic dermatitis
Akt	Akt kinase (protein kinase B)
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acid

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BCAT2	Branched-chain aminotransferase-2
DHT	Dihydrotestosterone
DIM	3,3′-Diindolylmethane
EGCG	Epigallocatechin-3-gallate
ERK	Mitogen-activated protein kinase
FoxO	Forkhead box class O transcription factor
GDP	Guanosine diphosphate
GH	Growth hormone
GHR	Growth hormone receptor
GIP	Glucose-dependent insulinotropic polypeptide
GR	Glucocorticoid receptor
GTP	Guanosine triphosphate
IgE	Immunoglobulin E
IGF	Insulin-like growth factor
IGF1R	IGF-1 receptor
IKK	Inhibitor of kappa light chain gene enhancer in B cells
IL	Interleukin
IRS	Insulin receptor substrate
KLF	Krüppel-like factor
LDL	Low density lipoprotein
LKB	Liver kinase B
mTOR	Mammalian target of rapamycin
NALA	*N*-Acetylleucine amide
NF-κB	Nuclear factor kappa B
PCOS	Polycystic ovary syndrome
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositol-3 kinase
PTEN	Phosphatase and tensin homolog on chromosome 10
Rag	Ras-related GTP-binding protein
Raptor	Regulatory associated protein of mTOR
REDD1	Regulated in development and DNA damage responses
Rheb	Ras homolog enriched in brain
Rictor	Rapamycin-insensitive companion of mTOR
RSK	Ribosomal S6 kinase
S6K	p70 S6 Kinase
SREBP	Sterol regulatory element binding protein
TCR	T cell receptor
TNF	Tumor necrosis factor
TOR	Target of rapamycin
TSC	Tuberous sclerosis complex
TSC1	Hamartin
TSC2	Tuberin

Introduction

It is the purpose of this chapter to highlight the role of endocrine signaling of Western diet, a fundamental environmental factor involved in the pathogenesis of acne, psoriasis, atopic diseases, and other epidemic diseases of civilization. Western style nutrition is characterized by high calorie uptake, high

glycemic load, high fat intake, and increased dairy protein and meat consumption. Metabolic signals of Western diet are sensed by a nutrient-sensitive kinase, the *mammalian target of rapamycin complex 1* (mTORC1), which integrates signals of cellular energy, growth factors like insulin and insulin-like growth factor-1 (IGF-1) and protein-derived signals, predominately provided by the essential branched-chain amino acid leucine. This chapter will focus on the role of excessive protein consumption resulting in increased leucine signaling, which, combined with high insulinotropic signaling of Western diet, promotes increased mTORC1 activity. Exaggerated mTORC1 activity will be shown to have fundamental impacts on the pathogenesis of all epidemic Western diseases like acne, psoriasis, atopic dermatitis, obesity, type 2 diabetes, cancer, and neurodegenerative disorders. It will be demonstrated that all plant-derived polyphenols and isoflavonoids, which exert protective and therapeutic effects in various age-related diseases, are in fact direct or indirect inhibitors of mTORC1. These new insights offer a rational approach for the prevention and treatment of common skin diseases and most other Western diet-related diseases of civilization by restriction of insulinotropic food, as well as leucine-rich animal and dairy proteins and offer a rational basis for the natural attenuation of mTORC1 activity by increasing the consumption of more fruit and vegetables containing several natural mTORC1 inhibitors.

mTORC1: The Convergence Point of Nutrient-Derived Signaling

Recent discoveries in the field of molecular biology have established the key role of the nutrient-sensitive mammalian target of rapamycin complex 1 (mTORC1) protein kinase in cell regulation and cell function. mTORC1 signaling stimulates gene transcription, translation, ribosome biogenesis, protein synthesis, cell growth, cell proliferation, lipid synthesis but suppresses the mechanisms of autophagy [1–6]. mTOR is a multi-domain protein of approximately 300 kDa exhibiting a protein kinase domain at its C-terminus related to phosphoinositol-3-kinases (PI3K). In mammalian cells two functionally different mTOR complexes exist: mTORC1 and mTORC2. Among other functional proteins, mTORC1 contains the important partner protein *Raptor*, which interacts with substrates for mTORC1-mediated phosphorylation. mTORC1 controls the G1/S transition and G2/M progression of the cell cycle [3]. In contrast to mTORC2, which contains the partner protein *Rictor*, only mTORC1 plays a special role in sensing cellular nutrients, amino acids, and energy (ATP) levels important for cell growth and proliferation. Liver kinase B1 (LKB1) and AMP-activated protein kinase (AMPK) are critical regulators of mTORC1 [7]. Most functions of mTORC1 are inhibited by rapamycin, a triene macrolide antibiotic synthesized by *Streptomyces hygroscopicus* [6].

mTORC1 has to be regarded as a pivotal convergence point in cell signaling, because it integrates many intra- and extracellular signals such as growth factors (insulin, IGF-1), energy-sensing signals (glucose, the AMP/ATP-ratio regulating AMPK), and most importantly the availability of sufficient amounts of amino acids, especially the branched-chain essential amino acid (BCAA) leucine for mTORC1 activation [8] (Fig. 37.1).

Recent advances in molecular biology have elucidated two parallel mechanisms of mTORC1 activation: (1) the upstream activation of the small GTPase Rheb (Ras homolog enriched in brain) by growth factor signals and high cellular energy levels, and (2) the amino acid-dependent translocation of inactive mTORC1 to active Rheb localized at late endosome or lysosome compartments [9–11] (Fig. 37.1). The activity of Rheb is tightly regulated by the tuberous sclerosis proteins TSC1 (hamartin) and TSC2 (tuberin), which form a functional heterodimeric complex. Intriguingly, loss-of-function mutations of either the *TSC1* or *TSC2* gene cause the hamartoma syndrome *tuberous sclerosis*. TSC1 stabilizes TSC2 that possesses a GTPase-activating protein, which hydrolyses GTP to GDP. The TSC1/TSC2 complex provides this function to Rheb leading to inactivation of Rheb. Insulin and IGF-1, which both activate the kinase Akt (protein kinase B) as well as other growth-related kinases

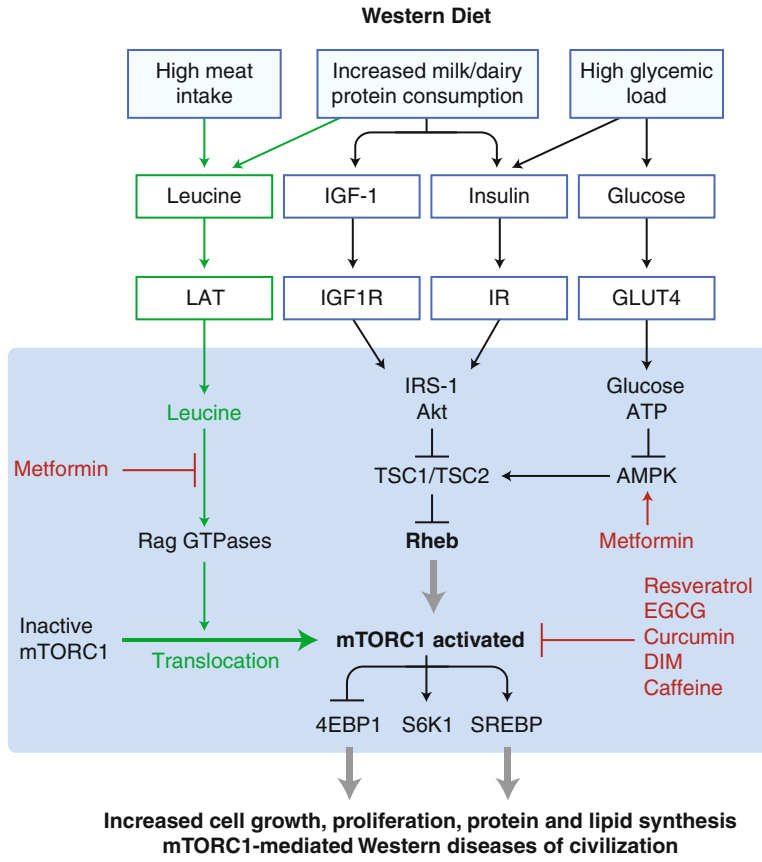

Fig. 37.1 mTORC1 signaling of Western diet: Dairy proteins increase leucine- and insulin/IGF-1-signaling, which both independently activate mTORC1. Leucine activates mTORC1 by translocation of inactive mTORC1 to Rheb-enriched membrane compartments. High glucose intake increases insulin signaling and elevates cellular ATP levels resulting in AMPK suppression, thus inactivated TSC2 upregulates Rheb, the final mTORC1 activator. mTORC1 activates protein synthesis via S6K1 and 4EBP1 and upregulates lipid syntheses via SREBP. Metformin inhibits mTORC1 activity by inhibiting leucine signaling and by stimulating AMPK activity. Plant-derived natural mTORC1 inhibitors (resveratrol, EGCG, curcumin, DIM and caffeine) downregulate exacerbated mTORC1 signaling by Western diet. (Abbreviations, see text)

such as ERK and RSK phosphorylate TSC2, thereby inhibiting the function of the TSC1/TSC2 complex. This inhibition leads to activation of Rheb and finally activation of mTORC1 [12–14] (Fig. 37.1).

Besides the important input of growth factor signaling on mTORC1 activation, AMPK, an essential energy sensor, plays a key role in energy-dependent mTORC1 regulation. During states of energy-deficient conditions like glucose deprivation, ATP levels fall and AMP levels rise, resulting in AMPK activation. AMPK phosphorylates TSC2 and Raptor, thereby suppressing mTORC1 activity [15, 16]. Abundant cellular energy provided by hypercaloric Western diet with high glycemic load thus reduces AMPK activity and stimulates mTORC1 signaling.

There is convincing evidence that other important nutrient and growth factor-sensors, especially the FoxO transcription factors, modulate mTORC1 signaling [17]. Increased insulin/IGF-1 signaling and activation of the PI3K/Akt-pathway results in Akt-mediated nuclear phosphorylation of FoxO proteins, thereby promoting their extrusion from the nucleus into the cytoplasm. This FoxO shuttling mechanism functions as a molecular switch for FoxO-mediated gene regulation. Like mTORC1,

FoxOs are involved in the regulation of cell proliferation, apoptosis, anti-oxidative stress responses and regulation of metabolism [18]. Intriguingly, FoxOs have emerged as important rheostats that coordinate the activity of Akt and mTORC1 [17]. Activated FoxOs (FoxO1, FoxO3, FoxO4) induce the expression of Sestrin3, which activates AMPK to inhibit mTORC1 in a TSC2-dependent manner [19]. Moreover, AMPK has been shown to phosphorylate FoxO3 and facilitate its nuclear localization [20]. It has been demonstrated that Akt-phosphorylated cytoplasmic FoxO1 is able to associate with the C terminus of TSC2 thereby dissociating the TSC1/TSC2 complex leading to activation of mTORC1 [21].

Remarkably, in response to amino acid depletion, mTORC1 activity is rapidly abolished [22]. Amino acid starvation impairs binding of mTORC1 to Rheb [23]. From all essential amino acids, leucine exerts the greatest effects on mTORC1 signaling [3, 6, 22]. Recent evidence has been provided that amino acids and especially leucine promote the cellular translocation of inactive mTORC1 to lysosomal compartments enriched in activated Rheb [9, 10]. This spatial regulation of inactive mTORC1 by amino acids is mediated by an active Rag heterodimer and is of utmost biological importance as it explains the complete mechanism of nutrient sensing of mTORC1. Thus, mTORC1 integrates not only growth factor/energy-derived signals to Rheb, but requires parallel signaling of leucine for final mTORC1 activation by translocation of inactive mTORC1 to cell compartments enriched in activated Rheb (Fig. 37.1). These two independent major pathways of mTORC1 activation explain why either insulin/IGF-1 signaling or amino acid signaling alone is not sufficient to reach maximal mTORC1 activation. Insulin is not able to activate the mTORC1 pathway when cells are deprived of amino acids [24]. In fact, recent experimental evidence confirmed that both insulin- and amino acid signaling are required for maximal mTORC1 activity in rat liver [24].

Activated mTORC1 finally phosphorylates important substrates involved in the regulation of the translational machinery, the S6 kinases (S6Ks), which phosphorylate ribosomal protein S6, and eukaryotic initiation factor (eIF) 4E-binding proteins (4E-BPs), which control the activity of the translation factor eIF4E that binds to the 5′-cap structure of eukaryotic mRNAs, thereby facilitating ribosome recruitment. Intriguingly, the downstream target of mTORC1, S6K1, phosphorylates insulin receptor substrate proteins (IRS), mediating an important feedback mechanism, which downregulates insulin/IGF-1 signaling. This is the molecular basis for insulin resistance, a characteristic feature of obesity and type 2 diabetes [25]. Cell growth not only requires increased amounts of protein but also adequate amounts lipids. It is thus not surprising that the key transcription factor of lipid biosynthesis SREBP (sterol regulatory element binding protein) is dependent on mTORC1 activation [26].

Activated mTORC1 signaling has already been implicated to play important roles in the development of Western diseases especially obesity, type 2 diabetes, cancer, and neurodegenerative diseases [27–30]. Furthermore, hyper-activated mTORC1 signaling due to mutations of genes encoding upstream regulators of mTORC1 promote the development of the syndromes tuberous sclerosis, Peutz-Jeghers syndrome, Cowden syndrome, neurofibromatosis type 1 and other hamartoma syndromes important for dermatology [31].

Western Diet Stimulates All Major mTORC1 Activation Pathways

Western diet upregulates all three major pathways important for mTORC1 activation (Fig. 37.1). Western diet provides abundant energy, glucose, and fat to suppress AMPK activity. The high glycemic load increases glucose availability and stimulates increased glucose-dependent insulin signaling. High intake of insulinotropic food has been a matter of concern for more than a decade [32]. Milk proteins significantly contribute to high insulin/IGF-1 signaling of Western diet. Mammalian milk has been identified as an *endocrine signaling system* that upregulates mTORC1 activity by increasing insulin secretion, hepatic IGF-1 secretion, and mTORC1-mediated β-cell proliferation for neonatal

Table 37.1 Relative leucine content of leucine-rich proteins (adapted from [43])

Protein source	Leucine content (g/100 g protein) (%)
Whey protein concentrate	14%
Cow milk protein (mostly casein)	10%
Egg protein	8.5%
Muscle protein	8%
Soy protein isolate	8%
Wheat protein	7%

Table 37.2 Leucine-enriched animal-derived foods

Food	Leucine (mg/100 g food)
Beef (rump steak)	2,369
Gouda cheese (40% fat)	2,359
Coalfish, cooked	1,883
Broiler, cooked	1,806
Curd cheese (20% fat)	1,290
Yoghurt (3.5% fat)	410
Cow milk (1.5% fat)	381

Source: German Nutrient Database, BLS-version 3.01

Table 37.3 Plant-derived foods with low leucine content

Food	Leucine (mg/100 g food)
Corn (cooked)	394
Wheat (cooked)	274
Rice (cooked)	219
Broccoli (cooked)	193
Cauliflower (cooked)	185
Potato (cooked)	124
White cabbage (cooked)	56
Tomato	38
Apple	16

Source: German Nutrient Database, BLS-version 3.01

growth requirements [33]. Milk consumption not only stimulates the somatotropic axis but also activates *incretin signaling* by enteral stimulation of glucose-dependent insulinotropic polypeptide (GIP) [34, 35]. Cow milk's excessive insulinotropic activity is characterized by milk's high *insulinemic index* [36]. Notably, increased daily intake of milk but not meat significantly raised basal insulin- and IGF-1 serum levels and increased insulin resistance in 8-year old boys [37]. Milk-induced insulin resistance can be well explained by increased mTORC1/S6K1-mediated IRS phosphorylation and degradation [25]. Moreover, epidemiological data in adults confirmed the correlation between increased dairy protein consumption and raised IGF-1 serum levels [38, 39]. Thus, dairy protein consumption significantly contributes to exaggerated insulin/IGF-1-signaling and insulin resistance promoted by Western diet, appreciated risk factors involved in the development of type 2 diabetes and obesity [40–42].

Furthermore, to achieve the physiological requirements for adequate growth, milk proteins provide high amounts of leucine, the most effective essential amino acid required for mTORC1 activation. Whey proteins have thus to be regarded as *life starter proteins* that not surprisingly contain the highest amount of leucine (14%), followed by casein (10%), the major protein constituent of cow milk and cheese [43] (Table 37.1). For comparison, 100 g of rump steak contains approximately 2.4 g leucine comparable to 100 g of Gouda cheese (2.4 g), which is in strong contrast to 100 g white cabbage (0.056 g), or 100 g apple (0.016 g) (Tables 37.2 and 37.3). To reach the leucine intake provided by

Table 37.4 Signaling mechanisms of Western diet-mediated mTORC1 activation

Compound of Western diet	Mechanisms of mTORC1 activation
High total calories (=high energy)	Reduced activity of AMPK
High glycemic load (=high energy)	Reduced activity of AMPK Increased insulin/IGF-1 signaling
High fat intake (=high energy)	Reduced activity of AMPK, increased S6K1/2
High alcohol intake (=high energy)	Reduced activity of AMPK
High dairy protein intake (=high leucine)	Increased insulin/IGF-1 signaling *and* leucine-mediated mTORC1 activation
High meat intake (=high leucine)	Leucine-mediated mTORC1 activation

100 g Gouda cheese or steak, 4.2 kg white cabbage or 100 apples could be consumed. These calculations exemplify the extreme differences in leucine amounts provided by an animal meat/dairy protein-based diet in comparison to a *vegetarian* or *vegan diet*. Thus, the increased consumption of meat and dairy proteins, staples of Western diet, provide abundant amounts of leucine for mTORC1 activation. In comparison to meat, milk proteins provide two major activating signals for mTORC1, high insulin/IGF-1 and high leucine signals [44] (Table 37.4).

Taken together, Western diet provides all major signals for maximal mTORC1 activation. After understanding the important role of dietary signaling for mTORC1 regulation, the interaction of Western diet and mTORC1 signaling in common Western skin diseases like acne, psoriasis, and atopic dermatitis will be discussed in more detail.

Acne

Acne is an epidemic skin disease of industrialized countries, reaching prevalence rates of over 85% of teenagers [45]. In the United States, acne nowadays persists after adolescence into the third decade of life in nearly half of men and women [46]. Acne has been clearly identified as a disease of Western civilization and has been closely linked to Western diet [47]. Intriguingly, acne is absent in populations consuming a *Paleolithic diet* excluding sugar, grains, and dairy protein like the Kitava islanders who exhibit low basal insulin levels compared with age-matched Europeans and do not suffer from epidemic diseases of civilization [47, 48]. Remarkably, a randomized placebo-controlled Australian trial confirmed that a reduction of glycemic load improved the clinical symptoms of acne, sebum excretion, and free androgen index in male acne patients in the age range of 15–25 years [49–51].

Epidemiologic data derived from the *Nurses Health Study II* and the *Growing Up Today Study* in the United States provided epidemiological evidence for a correlation between milk, and especially skim milk consumption, and the prevalence of acne [52–54]. Moreover, positive associations between acne and the consumption of other dairy products like instant breakfast drink, sherbet, cream cheese, and cottage cheese have been reported [52]. The association between acne and food composition has recently been confirmed in 783 patients with acne and 502 control subjects in South Korea [55]. The frequency of vegetables and fish intake was significantly higher in the acne-free control group than in the acne group. Intake of instant noodles, junk food, carbonated drinks, snacks, processed cheeses, pork, chicken, nuts, and seaweed were significantly higher in acne patients than in the controls [55]. Thus, the food pattern of Western diet composed of high glycemic load, high fat intake, and high dairy and meat consumption played an important role in the exacerbation of acne in South Korea. Nearly half of the male and female acne patients reported that food intake was an aggravating factor of their acne. Remarkably, in the group of food-aggravated acne patients, serum IGF-1 levels (543.9 ± 56.4 ng/mL) were significantly higher than IGF-1 levels (391.3 ± 118.2 ng/mL) in the acne group not reporting

aggravation by food [55]. Accumulating evidence derived from epidemiologic and controlled dietary studies allows the conclusion that especially high glycemic load diets and increased consumption of dairy proteins are the major dietary factors of Western diet promoting the development or exacerbation of acne [56–60].

Acne coincides with the growth phase of puberty induced by increased pituitary secretion of growth hormone (GH) and GH-mediated hepatic secretion of IGF-1, which is intimately involved in the pathogenesis of acne [61]. IGF-1 is a strong stimulator of sebaceous lipogenesis and upregulates the PI3K/Akt and mTORC1 pathway resulting in increased expression of SREBP1, the key transcription factor of most lipid synthesizing enzymes [62]. IGF-1-mediated activation of the PI3K/Akt pathway results in nuclear extrusion of FoxO transcription factors, recently linked to acne pathogenesis [63, 64]. IGF-1 is also related to increased androgen signaling, as IGF-1 stimulates adrenal and gonadal androgen synthesis and increases 5α-reductase activity, the responsible enzyme for the conversion of testosterone to the tenfold more potent dihydrotestosterone (DHT) [61, 65]. Intriguingly, subjects with Laron syndrome and congenital IGF-1 deficiency due to loss-of-function mutations of GH receptor exhibit dwarfism and do not develop acne or clinical signs of hyperandrogenism, unless substituted with high doses of recombinant IGF-1 [66] Remarkably, the transcriptional activity of the androgen receptor itself is also linked to insulin/IGF-1 signaling. IGF-1 stimulates androgen receptor transactivation by nuclear extrusion of the androgen receptor cosuppressor FoxO1 from the androgen receptor complex [61, 67, 68]. Thus, there is substantial evidence for the role of insulin/IGF-1 signaling in the pathogenesis of acne, an IGF-1- and androgen-dependent disease. Insulin/IGF-1 signals are known to mediate mTORC1 activation.

There is more evidence for the involvement of activated mTORC1 in the pathogenesis of acne. FoxO1 has been recognized as an interacting partner of the mTORC1 pathway and inhibits mTORC1 signaling at the level of TSC1/TSC2 [17, 19–21]. Oral isotretinoin (13-*cis*-retinoic acid), the most powerful sebumsuppressive anti-acne agent, has been shown to reduce serum IGF-1 concentrations [69], thus counter-balancing the IGF-1 raising effects of Western diet. Isotretinoin promotes sebaceous gland apoptosis and inhibits the G1/S checkpoint of the cell cycle [70]. It has recently been proposed that isotretinoin operates by upregulation of nuclear FoxO1 activity [64]. It is thus conceivable that FoxO-mediated inhibition of mTORC1 induces the autophagy machinery leading to sebaceous gland apoptosis observed during isotretinoin treatment. In fact, both mTORC1 inhibition and isotretinoin lead to cell cycle arrest at the G1/S transition [3, 70]. Moreover, isotretinoin, the most potent inhibitor of sebaceous lipogenesis, downregulated SREBP1 expression in human sebocytes [62, 70]. In accordance, mTORC1 has been linked to the regulation of lipid metabolism and SREPB1 expression [26, 71]. It is thus conceivable that high insulin/IGF-1 signaling of Western diet activates Akt and mTORC1 resulting in increased SREBP-stimulated sebaceous lipid synthesis.

Moreover, the high intake of BCAAs provided by high dairy protein and meat consumption may be another mechanism stimulating sebaceous lipogenesis. Increased IGF-1-mediated conversion of testosterone to DHT, might stimulate increased uptake of BCAAs by sebaceous glands. It has recently been shown in muscle cells that DHT stimulated the uptake of essential amino acids in an mTORC1-dependent process [72]. It is of special concern, that especially male adolescents in the fitness and bodybuilding environment consume high amounts (60–80 g/day) of leucine-rich whey- or casein-based protein concentrates to gain muscle mass [73] (Table 37.1). Leucine not only contributes to the synthesis of muscle proteins but can be converted into lipids (fatty acids and cholesterol) and stored in adipose tissue [74]. Adipose tissue efficiently converts BCAAs carbon skeletons into newly synthesized fatty acids, a process that is stimulated by insulin [74]. Remarkably, it has been demonstrated that sebaceous glands like adipocytes are able to take up and convert leucine into major sebum lipid classes [75, 76]. In this regard, leucine-rich Western diet may have two major effects on sebaceous lipogenesis: (1) by increasing leucine-stimulated mTORC1/SREBP signaling, and (2) by raising the supply of leucine as a lipid precursor for *de novo* sebaceous lipid synthesis.

Comedogenesis is regarded as the primary process in the pathogenesis of acne and is induced by increased proliferation and retention of acroinfundibular androgen-dependent keratinocytes [77]. Recently, the PI3K/Akt/mTORC1 pathway has been shown to stimulate keratinocyte proliferation [78].

Table 37.5 Natural and pharmacological mTORC1 inhibitors in the prevention and treatment of diseases of civilization

Compound	Acne	Psoriasis	AD	Obesity	T2D	Cancer	NDD
Rapamycin						+	
Sirolimus		+					
Everolimus		+					
Resveratrol	+			+	+	+	+
EGCG	+		+	+	+	+	+
DIM						+	
Curcumin						+	
Genistein						+	
Caffeine					+	+	+
Metformin	+			+	+	+	

AD atopic dermatitis, *T2D* type 2 diabetes, *NDD* neurodegenerative diseases
+ = benefical effects suggested or proven in cell systems or clinical studies

In keratinocyte cultures of high cell density imitating states of hyperproliferation, isotretinoin decreased keratinocyte proliferation [79]. This finding can now be well explained by FoxO1-mediated inhibition of mTORC1 [21]. Further evidence points to the role of mTORC1 signaling for keratinocyte proliferation. Genetic excision of TSC1 activated mTORC1 signaling in keratinocytes [78]. Loss-of-function of either the TSC1 or TSC2 gene leads to persistent activation of mTORC1 resulting in the hamartoma syndrome tuberosus sclerosis complex [31]. Most recently, folliculocystic and collagen hamartoma of tuberous sclerosis complex with multiple comedones and keratin-containing cysts lined by infundibular epithelium have been described [80].

It should be emphasized that subjects exhibiting genetic variants featuring increased expression and responsiveness of certain components of the somatotropic axis (GH, GHR, IGF-1, IGF1R) and/or reduced activity of FoxO transcription factors will show increased susceptibility for mTORC1 activation [81]. Prototypically, obese women with polycystic ovary syndrome (PCOS) exhibit signs of hyperandrogenism, acne, insulin resistance and increased insulin and IGF-1 serum levels [82]. Treatment of PCOS with the anti-diabetic drug *metformin* improves overweight, insulin resistance and clinical signs of hyperandrogenism including acne [83]. Metformin is known to activate the AMPK resulting in mTORC1 inhibition [7, 16]. Recently, a second AMPK-independent mechanism of metformin-mediated mTORC1-inhibition has been identified. Metformin inhibited the leucine-induced translocation of inactive mTORC1 to Rheb-enriched lysosomal membrane compartments, thereby reducing mTORC1 activation [84] (Fig. 37.1). Metformin is thus an effective mTORC1 inhibitor, reduces overweight, insulin resistance (via mTORC1/S6K1-driven IRS-phosphorylation) and hyperandrogenism (Table 37.5). Thus, metformin counterbalances the adverse effects of Western diet mediated by high insulin/IGF-1 and leucine signaling. It is well known that PCOS is associated with an increased risk of cancer. Intriguingly, recent evidence confirms the cancer-protective effects of metformin treatment [85–89]. An epidemiologic association between the prevalence of severe long-lasting acne and increased risk of prostate cancer later in life has been reported [90].

Acne Treatment with Plant-Derived mTORC1 Inhibitors

Resveratrol

It has been demonstrated that *resveratrol*, a polyphenolic flavonoid from grapes and red wine with potential antiinflammatory, antioxidant, neuroprotective and anticancer properties downregulates PI3K/Akt/mTORC1 signaling [91, 92]. In human glioma cells, resveratrol attenuated PI3K/Akt/mTORC1 signaling, and in combination with rapamycin enhanced resveratrol-induced cell death [93].

Resveratrol inhibited the proatherogenic oxidized LDL-induced activation of the PI3K/Akt/mTORC1/S6K pathway and remarkably suppressed DNA synthesis and proliferation of smooth muscle cells [94]. Resveratrol has been demonstrated to activate AMPK in breast cancer cells, and subsequently inhibited mTORC1 [95]. Notably, resveratrol has been shown to directly inhibit PI3K and targeting the class IA PI3K ATP-binding site in a competitive and reversible fashion [96]. Thus, there is convincing evidence for the role of resveratrol as a direct and indirect inhibitor of mTORC1.

These recent insights imply that resveratrol may exert therapeutic effects in the treatment of acne. In fact, resveratrol has been shown to inhibit the proliferation of *Propionibacterium acnes* [97]. Recently, it has been reported that topical treatment of facial acne vulgaris in 20 patients with a resveratrol-containing gel (0.01% weight/volume) significantly reduced the number of microcomedones, papules and pustules compared to vehicle control [98].

Epigallocatechin-3-Gallate

Plant extracts and isolated compounds are increasingly used in cosmetics and food supplements to improve skin conditions [99–101]. Green tea extract and tea tree oil have been investigated in the treatment of acne and green tea extract has been introduced for the treatment of condylomata acuminata [99]. The specific green tea catechin, (−)epigallocatechin-3-gallate (EGCG), is regarded as the active antiinflammatory and antiproliferative compound of green tea extracts [99–102]. Recently, it has been demonstrated that topical 2% green tea lotion was effective in the treatment of mild-to-moderate acne vulgaris [103]. After 6 weeks, the mean total lesion count and mean severity index of acne showed significant reductions of 58% and 39%, respectively [103]. Furthermore, a 3% green tea emulsion significantly reduced sebum production in ten healthy male volunteers after 8 weeks of treatment [104].

Green tea extract and EGCG have been shown to inhibit mast cell-stimulated type I collagen expression in keloid fibroblasts. EGCG-mediated inhibition of the PI3K/Akt/mTORC1 signaling pathway has been suggested as the most likely mechanism [105]. In fact, EGCG in physiologically relevant concentrations has been proven to function as an ATP-competitive inhibitor of both PI3K and mTORC1, respectively [106]. Resveratrol and EGCG are thus natural PI3K and mTORC1 lipid kinase inhibitors.

Taken together, epidemic acne should be considered as a visible model disease of exaggerated mTORC1 signaling promoted by Western diet. Especially the time period of puberty, characterized by high IGF-1/mTORC1 activity (growth phase), appears to be a most vulnerable phase for the aggravating effects of Western diet-mediated mTORC1 signals, which are superimposed on puberty-stimulated IGF-1/mTORC1 signaling.

Exaggerated mTORC1 signaling of Western diet is just the opposite endocrine constellation observed in non-Westernized populations consuming a *Paleolithic diet* or individuals consuming *vegan diets* as well as untreated subjects with Laron syndrome [42, 47, 66]. Metformin, an old drug for the treatment of type 2 diabetes, has been recognized as a powerful inhibitor of mTORC1-signaling, antagonizing the signaling of Western diet. Plant-derived naturally occurring mTORC1 inhibitors like resveratrol and EGCG may be useful new therapeutic plant-derived agents for the treatment of acne, especially when combined with dietary restriction of Western diet.

Psoriasis

Psoriasis and atopic dermatitis, both common inflammatory skin diseases, are observed with increasing prevalence rates in westernized societies. They are influenced by nutrition and metabolic status, most obvious in the obesity–psoriasis relationship. They share some similarities in that they are complex inherited diseases involving genes that encode immune components and structural proteins that regulate differentiation of epidermal cells. Both diseases are characterized by proliferation of epidermal

keratinocytes, abnormal cornification or disturbed differentiation of the epidermis, lesional infiltrates of T cells, dendritic cells, and other types of leukocytes [107].

Chronic plaque psoriasis is frequently associated with metabolic diseases including type 2 diabetes, obesity, dyslipidemia, metabolic syndrome, cardiovascular diseases, nonalcoholic fatty liver disease, depression, malignancy as well as psoriatric arthritis [108–110]. Both psoriasis and obesity show many shared cytokines that are known to contribute to features of the metabolic syndrome, hypertension, dyslipidemia, and insulin resistance [111]. The overlap of clinical associations between psoriasis and common Western diseases of civilization already points to the existence of a fundamental pathobiochemical mechanism: exaggerated mTORC1 signaling.

In the pathogenesis of psoriasis, deviations of immune cell and keratinocyte function potentiate the development of chronic skin inflammation through the production of cytokines, especially tumor necrosis factor-α (TNFα), the major proinflammatory cytokine of psoriasis [112–114]. Immunosuppressive therapy is an effective mode of treatment for psoriasis, because psoriasis is sustained by proinflammatory CD4+ T helper cells mainly belonging to the Th1, Th17, and Th22 lineage [115]. It is known for a long time that rapamycin (sirolimus) inhibits mTORC1 and exerts immunosuppressive effects [116]. Preliminary studies on the effects of topical treatment of psoriasis with sirolimus, systemic treatment of sirolimus, or everolimus combined with low-dose cyclosporine A showed beneficial effects of these rapalogs in the treatment of psoriasis [117–120].

mTORC1 signaling has recently been appreciated to play a fundamental role in the regulation of T-cell homeostasis and mTORC1 has been linked to T-cell differentiation, function, and metabolism [121]. mTORC1 integrates signals in the immune microenvironment and programs the generation of CD4+ effector versus regulatory T cells, the generation of CD8+ effector versus memory cells, T cell trafficking, and T cell activation versus anergy. Thus, mTORC1 provides a direct link between T cell metabolism and T cell function [122]. Remarkably, the tumor suppressor TSC1 established a quiescence program in naive T cells by controlling cell size, cell cycle entry, and responses to stimulation of the T cell antigen receptor. TSC1-deficient T cells exhibited higher mTORC1 activity, which was essential for the disruption of immune homeostasis [123]. TSC1-dependent control of mTORC1 is crucial in actively maintaining quiescence of naive T cells to facilitate adaptive immune function [123]. Thus, stimulation of T cell mTORC1 activity by exaggerated insulin/IGF-1 and leucine signaling of Western diet may disturb T-cell homeostasis and promote deviations in T-cell signaling in psoriasis and other immunopathologies.

Augmented mTORC1 signaling plays not only an important role for disturbing T cell functions in psoriasis but appears also to be involved in proinflammatory signaling of keratinocytes. In primary human keratinocytes treated with TNFα, mTORC1-activated proinflammatory NF-κB signaling, whereas the mTORC1 inhibitor rapamycin inhibited TNFα-induced IκB degradation, thus reducing the transcriptional activity of NF-κB [124]. In primary human keratinocytes, TNFα-mediated activation of mTORC1 and pro-inflammatory NF-κB signaling resulted in increased transcription of psoriasis-associated cytokines TNFα, IL-6, IL-8, IL-17, IL-20, IL-22, and IL-23 [110, 125]. Intriguingly, IKKβ, a major downstream kinase in the TNFα-signaling pathway, has been demonstrated to physically interact with and phosphorylate TSC1, thereby suppressing TSC1, which results in mTORC1 activation [126]. TNFα/IKKβ/TSC1/Rheb-mediated activation of mTORC1 is thus a most conceivable mechanism for TNFα-mediated keratinocyte proliferation in psoriasis. Treatment of psoriasis with TNFα-antagonists may inhibit keratinocyte hyperproliferation as well as T cell activity of skin lesions overexpressing TNFα.

mTORC1 and the Obesity–Psoriasis Relationship

Not only locally overexpressed TNFα in psoriatic skin lesions or mucosa of psoriatic arthritis may activate mTORC1, but also increased levels of systemic circulating TNFα released from visceral fat

of obese individuals [127] The accumulation of fat around abdominal viscera and inside intraabdominal solid organs is strongly associated with obesity-related complications like type 2 diabetes and coronary artery disease. Visceral adipose tissue and its adipose-tissue-resident macrophages produce significant amounts of TNFα [127]. TNFα derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. Notably, saturated fatty acids, which are released from hypertrophied adipocytes via macrophage-induced lipolysis, serve as a naturally occurring ligand for the Toll-like receptor-4 (TLR4) complex, thereby activating macrophages, an important process for adipocyte tissue remodeling in metabolic obesity [125]. On the contrary, weight loss and reduction of visceral fat improves inflammation in terms of both the inflammatory (TNFα, IL-6 and leptin) and antiinflammatory (adiponectin) obesity-related inflammatory markers [128]. Clinical evidence has been provided, that weight loss improved psoriasis and contributed to a dose reduction for systemic medication (lowered the dose of cyclosporin A) [129]. Furthermore, there is evidence for a risk reduction for myocardial infarction in patients with rheumatoid arthritis who responded to anti-TNFα therapy [109].

Visceral obesity thus augments mTORC1 signaling in psoriasis via increased systemic release of TNFα from visceral adipose tissue and visceral fat macrophages, thus amplifying cutaneous local TNFα/mTORC1-signaling of psoriatic lesions. Moreover, adipose tissue in obesity contributes to insulin resistance of adipose tissue with subsequent hyperinsulinemia, thereby augmenting insulin-mediated mTORC1 activation of non-insulin-resistant epithelial cells.

Intriguingly, adipose tissue in obesity exhibits defects in BCAA uptake and metabolism [74]. Furthermore, overactivation of the mTORC1/S6K1 pathway and inhibitory phosphorylation of IRS-1 underlie insulin resistance in amino acid infused humans [130]. The deficient uptake and metabolism of leucine by adipose tissue in obese subjects leads to hyperleucinemia, which may further stimulate mTORC1, thereby augmenting TNFα/mTORC1-driven signaling in obesity-associated psoriasis.

Atopic Dermatitis

Over the past decades, the incidence of atopic diseases such as asthma, atopic dermatitis (AD), and food allergies has increased dramatically [131]. AD is a chronic relapsing, pruritic, inflammatory skin disease characterized by cutaneous hyperreactivity to environmental triggers. Two different forms of AD, an extrinsic AD with elevated IgE involving 70–80% of the patients and an intrinsic AD with serum IgE not elevated and no specific IgE are differentiated. Extrinsic AD exhibits elevated Th2- and decreased Th1-expressing cells in the peripheral blood, and elevated expression of IL-4, IL-5, and IL-13. Remarkably, genetic factors in intrinsic AD involve chromosomal regions associated with psoriasis susceptibility [132]. AD is characterized by various immunological alterations involving interactions between IgE-bearing antigen-presenting cells, T-cell activation, mast-cell degranulation, keratinocytes, eosinophils, and inflammatory dendritic epidermal cells orchestrating immediate and cellular immune responses. Patients with AD demonstrate compromised skin barrier function that leads to activation of keratinocytes and immune cells, which favor a strong Th2 bias [133].

There are intriguing overlapping common pathologies in AD and psoriasis, the association with genetic psoriasis susceptibility loci, the involvement of the involvement of polarized Th17 cells, clinical response to the immunosuppresive agents, especially topical glucocorticoids.

Glucocorticoids Inhibit mTORC1 Signaling

Recently, the molecular crosstalk between glucocorticoid receptor (GR) and mTORC1 signaling has been elaborated [134]. A well known adverse effect of prolonged systemic glucocorticoid treatment is muscle atrophy. In skeletal muscle, direct target genes of the GR signaling involve the

protein REDD1 (regulated in development and DNA damage responses) and the transcription factor KLF15 (Krüppel-like factor-15). Both inhibit mTORC1 activity, although via distinct mechanisms. The REDD1 gene is activated at the promotor level by ligand-bound GR and is transcriptionally induced under stress conditions like hypoxia (via HIF1α), which appears necessary for the downregulation of mTORC1 signaling during stress conditions [135]. REDD1 functions upstream of TSC2 and Rheb in order to downregulate mTORC1 signaling in response to glucocorticosteroids [135–137].

KLF15 upregulates gene expression of branched-chain aminotransferase-2 (BCAT2), a mitochondrial enzyme, catalyzing the first step in the catabolism of BCAAs to accelerate BCAA degradation [138]. The glucocorticoid-driven GR-KLF15-BCAT2 axis may negatively modulate the intracellular availability of BCAAs resulting in a negative impact on mTORC1 function in skeletal muscle. Glucocorticoid-mediated downregulation of mTORC1 is not only a superb explanation for glucocorticoid-induced muscle atrophy, but also for skin atrophy after long-term systemic or topical glucocorticoid use.

Leucine and T Cell Activation

Both psoriasis and AD are hyperproliferative skin disorders affecting T cell regulation and T cell homeostasis. While research of the last decades has primarily focused on the role of antigenic and cytokine signals and costimulation in guiding T cell responses, the fundamental aspect of cellular metabolism that regulates T cell function and differentiation has been neglected [139]. Recently, the pivotal role of mTORC1 in T cell metabolism affecting T cell function and differentiation has been appreciated [122]. The metabolic demands of T cells are extraordinarily high and an increase in T cell metabolism has been recognized as a pivotal contribution to T cell activation [140, 141]. The transcription factors KLF2 and FoxO have been implicated in regulating T cell metabolism [122]. Whereas quiescent T cells are in a resting state of metabolism characterized by catabolism driven by autophagy, activated T cells have high demands for adequate amounts of the essential components for protein, amino acids, lipid, and DNA biosynthesis involving mTORC1 function.

Leucine plays an important functional role in T cell activation and function [142]. Notably, the leucine-antagonist *N-acetylleucine amide* (NALA) inhibited T cell function and T cell receptor (TCR) engagement in the presence of NALA and promoted T cell anergy [143, 144]. NALA inhibited leucine-induced S6K phosphorylation and was capable of inhibiting amino acid-mTORC1 signaling in Jurkat cells, caused cell cycle arrest at G1 concomitant with the inhibition of S6K activation and inhibition of p27 degradation [143]. Inasmuch as a lack of leucine inhibits mTORC1 activation, these latter findings are consistent with the observation that TCR engagement during rapamycin-mediated mTORC1 inhibition promoted anergy [145]. Similarly, the glucose analog 2-deoxyglucose (2DG) inhibited mTORC1 function most likely via the AMPK pathway and promoted T cell anergy [146]. Furthermore, the AMPK activator AICAR inhibited mTORC1 and T cell function and mitigated experimental autoimmune encephalomyelitis [122]. Intriguingly, regulatory T cells can inhibit T cell function by expressing amino acid degrading enzymes that deplete the environment of essential amino acids, which are important for mTORC1 function [147].

In AD, a dysregulated and Th2-biased immune response appears to be a key pathogenetic factor [133]. Remarkably, an important regulatory role of mTORC2 in the differentiation of Th1 and Th2 cell subsets via distinct signaling pathways has been reported [148]. Notably, the mTORC1 inhibitor EGCG has been shown to improve *Dermatophagoides pteronissinus* extract-induced AD-like skin lesions in NC/Nga mice [149]. Western diet-mediated overstimulation of the nutrient-sensitive mTORC1 may thus result in cellular imbalances of the functionally interacting mTORC1 and mTORC2 system, which may disturb immunological homeostasis and may promote changes in T cell subset distribution.

High Leucine of Infant Formula Compared to Human Milk

There is evidence that breastfeeding for at least 4 months, compared with formula feeding prevents or delays the occurrence of AD [131]. Allergologists primarily focused on the allergic mechanisms of cow milk-based infant nutrition. This was the reason for hydrolyzing cow milk proteins in order to reduce protein allergenicity. However, this procedure has no effect on the amount of total amino acids in the hydrolysate. Notably, the *Committee on Nutrition and Section on Allergy and Immunology* of the *American Academy of Pediatrics* came to the conclusion that in infants at high risk of atopy and who are not exclusively breastfed for 4–6 months, there is "only modest evidence" that the onset of atopic disease may be delayed or prevented by the use of hydrolyzed formulas compared with formula made with intact cow milk protein, particularly for AD [131]. With respect to the important role of leucine in T cell regulation, the question arises whether the preventive effect of breastfeeding is mediated by the nearly twofold lower content of leucine in human milk compared to most cow milk-based infant formula.

The average protein content of human milk during the first 12 months of lactation has been determined as 1.2 g protein/100 ml of whole milk [150]. This is the lowest protein concentration found in the milk of any mammalian species in which this value has been measured. In comparison, cow milk contains 3.4 g protein/100 g. It has been shown that the protein content of mammalian milk of various species is inversely related to the rate of growth of the offspring [151]. Human newborns receive the lowest protein content of milk among mammalian species and require 180 days to double their birth weight in comparison to rat or rabbit with milk protein concentrations of 9 g/100 mL and 10 g/100 mL, who double their birth weight already after 5 days. It is known that premature infants fed formula containing a higher protein concentration gained weight faster than those fed formulas with a protein concentration closer to that of human milk [152, 153]. Remarkably, the leucine amount per g milk protein appears to be a mammalian species-independent constant in the range of 100 mg leucine/g milk protein for man, various primates and non-primate species including cow [154]. Thus, the total amount of milk protein fed to infants is the critical determinant for the total leucine uptake, an important signal for mTORC1 activation.

It is thus of most serious concern that currently available cow milk-based infant formula provides more than double amounts of leucine/feeding volume in comparison to the physiological leucine content of human breast milk. Thus, artificial infant formula does not meet the physiological leucine signaling axis required for adequate mTORC1 regulation in the newborn. These aberrations of leucine-mediated mTORC1 signaling are in excellent accordance with the *early protein hypothesis* linking high protein intake during the neonatal period to increased weight gain and childhood obesity. In fact, a higher protein content of infant formula has been associated with a higher weight in 2 year old infants [155, 156]. The markedly higher protein and leucine intake with infant formula feeding, as compared with the protein supply in breastfed babies, may play an important role in predisposing infants to an increased obesity risk in later life [155, 156]. It has been recently confirmed that especially BCAAs (leucine, isoleucine and valine), total IGF-1 as well as C-peptide (a measure of insulin secretion) were significantly higher in infants fed a high-protein formula (2.9 and 4.4 g protein/100 kcal) in comparison to infants fed a low-protein formula (1.77 and 2.2 g protein/100 kcal) and breastfed infants, respectively [157]. Median serum concentrations of leucine at 6 month of age were lowest in breast-fed infants (106 μmol/L) compared to infants either fed low-protein formula (120 μmol/L) or high protein formula (165 μmol/L) [157]. Furthermore, this important study showed a correlation between total serum IGF-1 and growth (weight-for-length) at 6 months of age. These data clearly confirm that increased dairy protein consumption during the neonatal period raised serum leucine and IGF-1 levels, which will have an impact on postnatal mTORC1 signaling promoting general growth as well as S6K1-mediated adipogenesis. Thus, overfeeding of human neonates with leucine by inadequately high protein amounts provided by current infant formula may not only lay the basis for the obesity epidemic of Western countries, but may also may promote the epidemic of atopic diseases.

Remarkably, it has been shown in human B-cells that IGF-1 induces IgE and IgG4 production by class switching in an IL-4- and IL-13 independent manner [158]. Prenatal exposure to a farm environment and milk consumption of pregnant mothers has been shown to have an influence on the infant's atopic sensitization at birth [159]. The higher rate of cord blood food sensitization in farm children was rather unexpected. This finding was attributed to a higher consumption of boiled farm milk in farm mothers (337 mL/day) compared with mothers not living on a farm (226 mL/day).

Recently, a higher maternal intake of green and yellow vegetables, citrus fruit, and beta-carotene during pregnancy was significantly associated with a reduced risk of eczema in the offspring [160]. The increased risk for obesity in leucine-rich formula fed infants [157] and the atopy-protective and obesity-protective effects of physiological breastfeeding associated with lower leucine supply to the infant, may just reflect a common leucine/mTORC1-driven signaling pathway in the pathogenesis of both obesity and atopy. Remarkably, there is an increasing body of evidence pointing to the possible association of allergic diseases with obesity and overweight [161–164]. Thus, there is accumulating evidence for the contribution of diet-induced mTORC1 hyperactivity as the common cause of the observed psoriasis-obesity and atopy–obesity relationship already triggered by inappropriate infant feeding.

Natural mTORC1 Inhibitors: Signaling Antagonists of Western Diet

Increasing studies have demonstrated that some plant-derived natural products, including curcumin, resveratrol, epigallocatechin gallate (EGCG), genistein, 3,3′-diindolylmethane (DIM), and caffeine, may all inhibit mTORC1 signaling directly or indirectly (Table 37.5) [91–106, 165–172].

EGCG, the most studied polyphenol component in green tea, is a potent antioxidant and PI3K- and mTORC1 inhibitor [106]. EGCG treatment of cocultured keloid fibroblasts and HMC-1 cells dose-dependently reduced the phosphorylation of the mTORC1 downstream targets S6K and 4E-BP1, respectively [105]. In both p53 positive and negative human hepatoma cells, EGCG activated AMPK and suppressed mTORC1 and 4E-BP1 [173]. Furthermore, EGCG upregulated nuclear FoxO levels in the worm *Caenorhabditis elegans*, known to attenuate mTORC1 signaling and explaining the worm's life span extension [174]. EGCG has been shown to modulate amyloid precursor cleavage and reduced cerebral amyloidosis in Alzheimer transgenic mice [175], confirming further evidence of EGCG-mediated neuroprotective effects and risk reductions for neurodegenerative diseases [175–178]. Furthermore, there is substantial evidence for antiobesity effects of green tea catechins [179].

Resveratrol has already been suggested for the treatment and prevention of several Western diseases of civilization and showed beneficial effects in the treatment of acne [98], the treatment and prevention of obesity [180–183], type 2 diabetes [180, 184], metabolic syndrome [185], cardiovascular diseases [186], cancer [187, 188], neurodegenerative diseases [189, 190] and life extension [191]. The potential effectiveness of the mTORC1 inhibitor resveratrol clearly elucidates that all age-related Western diseases arise from a common fundamental signaling pathway, high-tuned mTORC1 signaling by Western diet.

Curcumin, a natural polyphenol product isolated from the rhizome of the plant *Curcuma longa* exerts antiproliferative effects and may present another class of mTORC1 inhibitors already undergoing clinical trials as anti-cancer agent [192]. Curcumin inhibited the growth of a variety of cancer cells and showed effectiveness as a chemopreventive agent in animal models of carcinogenesis [170–172]. Curcumin inhibited mTORC1-mediated signaling pathways in cancer cells [172]. In numerous cancer cell lines, curcumin inhibited phosphorylation of mTORC1 and its downstream targets, S6K1 and 4E-BP1, suggesting that curcumin may execute its anticancer effect primarily through blocking mTORC1 signaling pathways [170–172]. Most recently, it has been demonstrated that curcumin dissociated Raptor from mTORC1, thus leading to inhibition of mTORC1 activity [165].

3,3′-Diindolylmethane (DIM). Emerging evidence provides credible support in favor of the potential role of bioactive products derived from ingesting cruciferous vegetables such as broccoli, brussel sprouts, cauliflower, and cabbage. Among many compounds, DIM is generated in the acidic environment of the stomach following dimerization of indole-3-carbinol monomers present in these classes of vegetables. DIM suppresses signaling through Akt/mTORC1 pathways resulting in cell cycle arrest [193]. Platelet-derived growth factor-D (PDGF-D)-overexpressing prostate carcinoma (PC3) cells exhibited a rapid growth rate and enhanced cell invasion that was associated with mTORC1 activation. DIM significantly inhibited both mTORC1 and Akt in PC3 PDGF-D cells, which was correlated with decreased cell proliferation and invasion [168].

Genistein. The soy-derived isoflavone and phytoestrogen genistein has been shown to upregulate the expression of p27, and inhibits PI3K/Akt/mTORC1 signaling in mouse epidermal and human breast cancer cells [194]. Genistein is a natural protein tyrosine kinase inhibitor that exerts anti-cancer effect by inducing G2/M arrest and apoptosis. Core signaling molecules inhibited by genistein can be functionally categorized into the canonial receptor-MAPK or receptor-PI3K/Akt-cascades, the later controls the downstream activity of mTORC1 [195]. In glioblastoma multiforme, a malignant primary brain tumor with upregulated PI3K/Akt signaling due to deletion or mutation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) mTORC1 was upregulated. In human glioblastoma cells, the combination treatment of rapamycin with isoflavones such as genistein and biochanin A decreased mTORC1 activity [196].

Caffeine, a xanthine alkaloid, is found in varying quantities in the seeds, leaves, and fruit of various plants, where it acts as a natural inhibitor that paralyzes and kills certain insects feeding on the plants. Caffeine is the most commonly consumed neurostimulatory compound by humans in infusions extracted from the bean of the coffee plant, tea leaves, various products derived from the kola nut, yerba maté, guarana berries, guayusa, and the yaupon holly. Many research investigations, epidemiological studies, and meta-analyses regarding coffee consumption revealed its inverse correlation with that of type 2 diabetes, various cancer lines, and neurodegenerative diseases [197]. Caffeine has been reported to inhibit PI3K kinase including mTORC1 [198, 199]. Caffeine decreased the phosphorylation of the mTORC1 downstream targets S6K kinase, S6 ribosomal protein, 4E-BP1, thus elucidating caffeine's mode of action as an inhibitor of mTORC1. Caffeine-induced autophagy has been shown to be mainly dependent on the PI3K/Akt/mTORC1 pathway [200].

Conclusion and Future Perspectives

Western diet, enriched in carbohydrates, fat and animal and dairy protein, boosts mTORC1 signaling by high energy levels (glucose, fat-, and alcohol-derived carbons), high insulin/IGF-1 signaling (preferentially mediated by glucose, dairy, and meat proteins) and high levels of leucine (provided by meat and dairy proteins). In comparison to Western diet, a *vegan diet* has a much lower energy density, is far less insulinotropic, and contains much less leucine and other BCAAs, which could drive mTORC1 activation.

Thus, a vegan diet exerts much less mTORC1 stimulation in comparison to typical Western diet. Moreover, a vegan diet provides more natural plant-derived mTORC1 inhibitors and contains thus less activating and more inhibiting stimuli attenuating mTORC1 signaling. All Western diseases, including acne, obesity, obesity-related psoriasis and atopic diseases, type 2 diabetes, cancer, and neurodegenerative diseases, appear to have a common fundamental Western diet-driven signaling mechanism, i.e., exaggerated activation of the cell's central nutrient sensor mTORC1. Inhibition of hyper-activated mTORC1 with various natural plant-derived mTORC1 inhibitors like resveratrol, EGCG, and others is a rational approach for the treatment of Western diseases. However, a reduction

of the causative dietary factors of exaggerated mTORC1 signaling should be recognized as the most important target for successful prevention and treatment of Western diseases.

It will be of pivotal importance to (1) restrict the total daily energy intake and (2) to reduce the glycemic load and especially—not yet realized in the field of nutrition research—(3) to balance the high intake of leucine provided by dairy protein and animal meat. The presented insights in leucine-regulated mTORC1 signaling clearly vote against further increases in daily protein consumption as proposed during the ongoing protein debate. It is of great concern that infants who are not breastfed obtain more than twofold increased amounts of leucine provided by infant formula or cow milk-based feeding in a sensitive priming period of postnatal life.

The presented comprehensive *concept of Western diet-induced hyperactive mTORC1 signaling* explains the attenuating effects of natural plant-derived mTORC1 inhibitors on mTORC1 signaling. These compounds have been created in the course of evolution to antagonize metabolic activity of mTORC1-sensitive plant invaders. The mTORC1 concept provides a great chance for the treatment and prevention of common diseases of civilization. We are just at the beginning to understand the complex molecular signaling pathways of our diets. Nevertheless, we are closer than ever to understand Hippocrates of Kós who stated about 2,400 years ago "*your diet should be your medicine, and your medicine should be your diet*".

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Chapter 38
Legumes and Preventive Dermatology

Jesus M. Porres and Wen-Hsing Cheng

Key Points

- Skin is the organ with the greatest surface area in the human body. Its main function is to act as a physical barrier that protects the organism against potential hazards from the environment.
- However, skin is also subjected to numerous endogenous or exogenous aggressions that can severely damage its structure and cause aging or cancer. Among the most dangerous hazards on skin is solar irradiation and, in particular, ultraviolet irradiation that act through direct formation of DNA dimmers or enhanced oxidative stress, which can in turn lead to the activation of different MAPKs in association with the development of cancer and photoaging.
- Legumes contain a variety of nutrients and bioactive compounds including selenium, vitamins, polyunsaturated fatty acids, phytic acid, and flavonoids that have shown great potential to improve skin health and protect against photoaging and cancer.
- Such bioactive food compounds may exert their biological actions through their antioxidant properties or direct effect on several MAPK regulated pathways. According to the US National Cancer Institute, soybean is classified as a plant-derived food carrying cancer-preventive properties. The bioactive components in soybeans with anticancer activities are mainly attributed to the two major phenolic phytochemicals, genistein and daidzein. However, while experimental data show that soybean is effective in cancer prevention, recent ideas developed in the field point to the "cocktail" approach that includes chemoprevention agents of various functions to improve cancer prevention efficacy.
- On the other hand, although soybean and its isoflavones have been the most studied members of leguminous family with regard to skin protection, other polyphenolic and flavonoid compounds in this family with enormous potential for optimal skin health remain to be studied.

Keywords Legumes • Skin • UVA • UVB • Skin cancer • Oxidative stress • MAPKs • Flavonoids • Selenium • Phytic acid • Allergy

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Introduction

In the human body, skin is the organ with the greatest surface area that acts as a physical barrier against potential hazards from the environment. Other important physiological functions of skin include temperature homeostasis, fluid and electrolyte retention, sensation, mediation of immune responses to exogenous antigens or endogenous tumors, and vitamin D synthesis. To carry on these functions, skin is composed of three main layers, namely, epidermis, dermis, and hypodermis. Epidermis is the outermost layer that serves as a physical and chemical barrier; it comprises, from lower to upper level, stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The epidermis is a stacked layer of cells in transition, from the basal stratum composed of parent cells to the last layer, the stratum corneum, formed by dead corneocytes filled with keratin. The stratum corneum is the thickest of all epidermal layers and serves as main protection against chemical and biological attacks. The dermis is an inner layer providing structural support. It contains collagen, elastic, and reticular fibers and is rich in blood and lymph vessels, nerves, and receptors. The hypodermis is the innermost layer of skin, mainly composed of adipose tissue with insulation functions.

Sunlight coupled with living in an oxygen-rich environment may pose potential hazards for the skin [1]. Radiation may severely affect the structure and functionality of the skin through direct DNA damage or oxidative stress that will cause photoaging or skin cancer. Photoaging of the skin can be described as accelerated aging after long-term exposure to ultraviolet (UV) radiation in sunlight [2], which can be characterized by wrinkling and furrowing of the skin, increment of skin fold thickness, irregular pigment distribution, and trans-epithelial water loss that leads to dryness. Skin cancer is mainly of non-melanoma type represented by basal or squamous cell cancer, although melanoma with stronger invasiveness and that is potentially more lethal must also be considered [3]. For a photochemical reaction to take place in the skin, UV light must be absorbed by a chromophore like DNA or urocanic acid, initiating a series of photochemical reactions that will in turn cause DNA, protein, and lipid alterations [1]. UVB (290–320 nm) is the major type of UV that causes sunburn and direct damage to the cellular DNA, leading to the appearance of 6-4 cyclobutane pyrimidine dimers. UVA (320–400 nm) penetrates deeper into the skin layer and causes indirect damage to DNA through the production of reactive oxygen species (ROS) [4]. UV irradiation can also activate different signaling cascades that can contribute to carcinogenesis. There are several strategies for the mitigation of genomic instability induced by irradiation [3]: free radical scavenging, increased expression of endogenous antioxidant enzymes, maintaining DNA structure in a form less prone to be damaged, and eliminating ROS by hypoxia. In recent years, several phytochemicals present in different plant species have become the focus of intense research for their potential antioxidant, anticancer, or antiaging properties. Some of these compounds can be supplied to skin through the diet in a way that oral photoprotection seems to be interesting as it is easy to combine with clothing or sunscreens [5]. However, bioactive polyphenols that can reach the skin may be limited due to low bioavailability or reduced biological action and circulation time after being metabolized in the liver, thus increasing the lag time for the appearance of benefits. That is the main reason why topical applications in general are advantageous in attaining greater concentrations of the compounds in the target zone of the skin. Among some of the most promising phytochemicals are flavonoids and other polyphenolic compounds, although other bioactive compounds such as selenium and phytic acid should also be considered. The leguminosae family comprises a wide variety of genuses with enormous nutritional and functional importance due to numerous bioactive components with great potential to act as antioxidant or antigenotoxic factors. Among such non-nutritional compounds are substances such as phytic acid and flavonoids. The latter substances are ubiquitous among the different legume species and their protective effect has been tested systemically as food components that act coordinately with other compounds like dietary fiber, resistant starch, and saponins, or else isolated after a selective extraction and purification process. Once extracted, flavonoids can be used as a crude extract or follow a more laborious separating process to isolate individual compounds.

Among the most extensively studied flavonoids in the leguminose family are those isolated from soybean (*Glycine max*). According to the US National Cancer Institute, soybean is classified as a plant-derived food carrying cancer-preventive properties. The bioactive, anticancer properties of soybeans are mainly attributed to the two major phenolic phytochemicals, genistein and daidzein [6]. Genistein is a protein-tyrosine kinase inhibitor and mitigates tumorigenesis through inhibition of angiogenesis and stimulation of apoptosis [7, 8]. Daidzein is converted to equol by bacterial fermentation in the large intestine of a relatively low percentage of Westerners compared to Asian populations [9, 10]. Equol has been demonstrated to be more effective than daidzein intrinsically present in soybean [11], and protects against UV-induced cancer in the skin of hairless mice [12]. In addition to the two well-studied isoflavones, other soybean components may play critical roles in rejuvenation of the skin after UV exposure [13] and in the protection against UVB-induced keratinocyte death [14]. While experimental data show that soybean is effective in cancer prevention, recent ideas developed in the field point to the "cocktail" approach that includes chemoprevention agents of various types to improve cancer prevention efficacy. On the other hand, other well-researched flavonoids like quercetin and kaempferol are present in raw and processed food legumes different from soybean [15, 16], and their potential health benefits on skin health, mainly through dietary intake, should not be underestimated.

Mechanisms of Skin Cancer and Photoaging

Ultraviolet exposure from sunlight accounts for the majority of skin changes in association with cancer and photoaging. The genome is constantly subjected to endogenous and exogenous DNA damage. Failure to repair these DNA lesions can lead to genome instability and accelerate the onset of aging and cancer of the skin. Solar UVB irradiation-induced skin cancer development is a multistage process involving three different stages exemplified by initiation, promotion, and progression in which various cellular, biochemical, and molecular changes take place [17]. Upon exposure to UVB irradiation, cells can develop intrastrand cross-link between two adjacent pyrimidines and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-G) [18]. Likewise, UVA primarily induces oxidative DNA lesions such as 8-oxo-G and to a less extent in other forms such as cyclobutane pyrimidine dimers. After exposure to oxidative radicals and UV irradiation, base excision repair (BER) can correct non-bulky damage [19]. There are two sub-pathways of BER, short-patch and long-patch BER, depending on the length of nucleotides needed to be repaired [20] or on tissue specificity [21]. To date, there is no known genome instability syndrome associated with mutations in BER proteins. This is likely attributed to the notion that BER proteins are essential, as knockout of these proteins in mice is embryonic lethal.

The DNA bulky adducts and DNA intrastrand cross-links generate helical distortion of the DNA duplex, which can be fixed by nucleotide excision repair (NER) pathway. There are genetic disorders associated with deficiency in NER pathway, such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS). Both XP and CS type B (CSB) patients are hypersensitive to UV irradiation and show features of premature aging [22]. Interestingly, XP, but not CSB, patients are predisposed to skin cancers. Nonetheless, CSB-deficient mice show increased susceptibility to UV and skin cancer [23]. The NER process includes the sequential recruitment of proteins for damage recognition, DNA duplex opening, incision on the damage strand, gap synthesis, and ligation. There are two sub-pathways of NER: global genomic NER (GGR) and transcription-coupled NER (TCR) [24]. Both GGR and TCR rely on XPC/HR23B complex for damage recognition. For TCR, TFIIH and XP complementation group G (XPG) are recruited to the site of stalled transcription by CS type A (CSA) and CSB. Next, XPA and replication protein A (RPA) are recruited and facilitate the loading of DNA duplex unwinding proteins, including XPB and XPD, around the lesion site. The endonucleases XPG and ERCC1/XPF then cleave one strand of approximately 30 bases of oligonucleotide containing the lesion 3′ and 5′ to the damage, respectively. Using the intact complementary strand, DNA Pol ε/δ and other replication factors synthesize the missing DNA in the gap. Finally, the nick is sealed by a DNA ligase.

In addition to its direct effect on DNA, UV irradiation exerts its action through other mechanisms such as the generation of oxidative stress that can lead to protein or lipid oxidation and the activation of different cellular signaling pathways. For example, pRB-cyclin D1-CDK4 and cyclin E-CDK2 complexes are involved in cell cycle progression from G1 to S phase [53]; activator protein-1 (AP-1), a well-characterized transcription factor formed by homodimers and/or heterodimers of the *fos* and *jun* families, plays an important role in preneoplastic to neoplastic transformation and proliferation [26]; UV irradiation modulates the expression of ornithine decarboxylase [12], inducible COX2 [27], or AP-1- and nuclear factor kappa β (NF-κβ)-dependent transcription of genes for the metalloproteinases (MMPs) [28].

Protective Effect of Legumes Against Skin Cancer and Photoaging

The interaction between nutrients and skin health and, particularly, the importance of essential nutrients such as vitamins, carotenoids, selenium, retinol, and dietary fats for cutaneous immune response and photoprotection or their therapeutic potential in skin disorders are well known [29, 30]. In addition, diverse natural agents present in foods with potential antioxidative, anti-inflammatory, antimutagenic, anticarcinogenic, and immunomodulatory properties can exert striking inhibitory effects on diverse cellular and molecular events [17]. Therefore, individuals can modify their dietary habits and lifestyles in combination with a careful use of skin care products. Furthermore, the use and potential benefits of natural phytochemicals have been suggested not only in the usual levels of exposure to environmental radiation but also in particular situations such us those currently found in astronauts [31]. Legumes and their derived food products contain numerous compounds that can protect skin against numerous physical and chemical hazards by either oral ingestion or topical administration. Therefore, they can be considered as nutraceutical agents in preventive dermatology and play a major role in the development of new strategies to reduce the adverse effects of solar irradiation on the skin.

Legume Components Active in the Prevention and Treatment of Skin Disorders

Polyphenols and, more specifically, flavonoids are the legume compounds to which more intensive research has been dedicated for the treatment of skin disorders. However, it should be mentioned that other nutrient and non-nutrient components present in legumes, including vitamins, polyunsaturated fatty acids, phytic acid, trypsin inhibitors, and saponins, could also play important roles in the dietary treatment of skin alterations. Flavonoids have low oxidation potential and are easily oxidized by ROS. Nevertheless, their antioxidant capacity varies considerably depending on different backbone structures and functional groups as well as the number and type of functional groups attached to the main nucleus [3]. Two different aspects of the biological effects of flavonoids have been assessed: increased tumor cell death and preventive effects in normal cells as a result of pre- or co-administration of flavonoids and irradiation or administration of genotoxic agents.

Dietary Administration

The effect of food legumes, their by-products, and isolated extracts has been tested after systemic dietary administration in several experimental models. In this regard, Barnes [32] has reviewed the protective effects of soybean against cancer of various origins and pointed out that dietary whole soybean or genistein administration protects ICR or CD1 mice against skin cancer induced by either

12-*O*-tetradecanoyl phorbol-13-acetate (TPA) or 7,12-dimethylbenz[a]-anthracene (DMBA). Likewise, Limtrakul et al. [33] have reported that dietary soymilk supplementation together with soy protein extract lowered tumor-bearing and the volume of tumors in a mouse model of two-stage carcinogenesis when compared to soy protein extract only. Cai and Wei [34] have reported a considerable increase in antioxidant enzyme activities of the skin in SENCAR mice after dietary administration of genistein (50 or 250 ppm) for 30 days, whereas Wei et al. [35] described a significant reduction in tumor multiplicity caused by oral administration of genistein in the drinking water of hairless mice for 2 weeks before a 3-week UVB exposure during which genistein was also supplemented. Kim et al. [28] have also found a protective effect of oral administration of an isoflavone extract to hairless mouse during the course of UV irradiation for 4 weeks. The isoflavone-administered animals exhibited a skin with better appearance and less wrinkling than that of the control group. Moreover, the amount of collagen deposition was higher in the isoflavone group, a phenomenon that was correlated to the inhibition of UV-induced metalloproteinase expression in human skin fibroblasts.

Topical Administration

The soybean isoflavone genistein is one of the most studied flavonoids for its potent inhibitory effect on UV-induced skin cancer and photoaging. In their review covering the effects of dietary or topical administration of genistein, Wei et al. [35] summarized the possible mechanism of its biological action that may represent a clear paradigm of how this ample group of phenolic substances work: scavenging of ROS, blocking of oxidative and photodynamic DNA damage, inhibition of tyrosine kinase, down regulation of EGF-receptor phosphorylation and MAPK activation, and suppression of oncogene expression in UVB-irradiated cells. Sharma and Sultana [36] have tested the protective effects of soy isoflavones against biochemical alterations induced topically by TPA in a Swiss albino mouse model. The authors found that topical applications of soybean isoflavones 30 min prior to the application of TPA prevented the induction of ornithine decarboxylase and DNA synthesis mediated by TPA; recovered the contents of reduced glutathione and antioxidant enzymes catalase, glutathione peroxidase, and glutathione *S*-transferase; and decreased the content of H_2O_2. In another set of experiments, Bonina et al. [37] tested the protective effects of soybean germ oil against UVB-induced erythema. By employing reflectance spectroscopy to estimate erythema index values, they showed that the degree of skin redding in human volunteers after exposure to irradiation was inversely associated with the inhibitory activity of tested substances. Soybean germ oil proved to be more effective in the protection of skin than tocopherol, although the authors imply that the effects were mainly related to radical scavenging components different from isoflavones.

The protective effect of the isoflavone equol against photoaging induced by chronic irradiation in hairless mice for 30 weeks has been studied by Reeve et al. [2] who found that such chronic irradiation treatment induced a marked increase in epidermal hyperplasia, dermal mast cell number, pronounced focal elastotic deposits, degraded dermal collagen, and deposition of glycosaminoglycans in the lower dermis. Daily postirradiation application of equol lotion caused a reduction in epidermal hyperplasia and a transient increase in dermal mast cell number after 1 week of treatment, and was consistently protective against all the above-mentioned markers of photoaging by the end of the study. Widyarini et al. [12] have assayed the protective effect of daily topical applications of equol against the combined chronic UV exposure and carcinogen-induced development of tumors. In the short term, equol inhibited the tumor-promoting biomarker enzyme ornithine decarboxylase in a dose-dependent manner. In the long term, the latent period to detect the first tumor was not affected by topical equol, but the progressive tumor prevalence in hairless mice was significantly delayed by equol treatment in each of the carcinogenesis models applied.

Lin et al. [38] have carried out an extensive study to differentiate the protective action against solar-simulated UV irradiation of five well-known isoflavone compounds (genistein, equol, daidzein,

biochanin A, and formononetin) in a skin model of pig. The authors evaluated colorimeter-measured erythema and sunburn cell numbers, and found that genistein, daidzein, and biochanin A had a stronger action than equol and formononetin, but lower than a mixture of ascorbic acid (15%)/α-tocopherol (1%)/ferulic acid (0.5%). Huang et al. [14] tested the effect of another isoflavone extract isolated from soybean cake that contains daidzein, genistein, and glycitein as well as their acetyl glucoside groups on UVB-induced death of human keratinocytes, desquamation, transepidermal water loss (TEWL), erythema, and epidermal thickness of mouse skin. The isoflavone extract significantly inhibited the above-mentioned alterations and also caused a significant increase in catalase activity of mouse skin and decreased the levels of COX2 and proliferating cell nuclear antigen (PCNA), a marker of DNA replication signaling the production of UVB-induced damage in the S phase of the cell cycle.

Other Bioactive Components

The protective effect of other flavonoids present in legumes has been reported. Kaempferol has been shown to decrease UVB-induced COX2 protein expression and transcriptional activities in different experimental models such as ex vivo mouse skin epidermal JB6 P+ cells or in vivo mouse skin. The inhibitory effect of kaempferol is achieved via direct blockade of Src kinase activity [39]. Likewise, quercetin caused the suppression of UVB-induced transactivation of AP-1, NF-κβ, and phosphorylation of MAPK [40].

Selenium is another promising bioactive food component that can protect against UV damage of the skin. Selenium is an essential nutrient that is widely distributed in inorganic form in soil and in organic form in certain foods. Dietary selenium deficiency promotes, while supranutritional selenium (0.5 mg/kg, sodium selenite) suppresses, skin carcinogenesis in the Skh:HR-1 hairless mice exposed to UVB irradiation [41]. Furthermore, adding selenite at a concentration of 2–8 ppm to drinking water suppresses UVB-induced skin carcinogenesis in the hr/hr strain of hairless mice [42]. However, the randomized Nutrition Prevention of Cancer (NPC) trial did not demonstrate protection of a selenized yeast product (200 μg selenium/day for 8 years) against development of skin cancer in old individuals (mean age, 63 years) with a history of basal cell or squamous cell carcinoma of the skin. Interestingly, the NPC trial showed that the oral selenium supplementation for 13 years significantly decreased incidence of prostate, colon, and lung cancer in skin cancer patients [43]. On the other hand, Van der Pols et al. [44] have reported that relatively high serum selenium concentrations are associated with a 60% decrease in subsequent tumor incidence of basal and squamous cell carcinoma. Intriguingly, a viral selenoprotein reminiscent of glutathione peroxidase protects against UV-induced viral death, thus promoting skin neoplasm in humans [45]. Recent results shed light on the mechanism by which selenium mitigates tumorigenesis. Pretreating murine keratinocytes with selenite or selenomethionine protects against UVB-induced interleukin 10 expression, suggesting that selenium compounds act as an antioxidant to inhibit the release of cytokines upon oxidative stress [46]. The antioxidative property of selenium is most likely attributed to selenoproteins, as mice with selenoprotein deficiency specific in the skin cells show hyperplastic epidermis, aberrant hair follicle morphogenesis, and premature death [47]. In conclusion, the efficacy of selenium being a chemoprevention agent against skin depends on the stage of carcinogenesis and selenium formulation and dosages. Future studies are needed to determine the expression profile of selenoproteins in various skin sub-tissues and stage of tumorigenesis.

Phytic acid (myo-inositol hexakis dihydrogen phosphate, IP_6) is ubiquitously present in legume and cereal foods where it represents the major storage form of organic phosphorus. In addition, its anticancer effect has been extensively tested in different models like prostate, large intestine, and skin cancer. Kolappaswamy et al. [48] have reported the benefits of IP_6 supplementation in drinking water to decrease the incidence (fivefold) and multiplicity (fourfold) of UVB-induced skin cancer in SKH1 mice. Phytic acid has been known to suppress carcinogenesis by mediating cell proliferation, cell cycle

progression, metastasis, invasion, angiogenesis, and apoptosis. In fact, Jaya and Krisha [49] have reported that topical application of IP_6 to DMBA-treated mice led to a decrease in DMBA-induced p53 mutant and overexpression of Bcl-2. In addition, IP_6 enhanced the activity of caspases, bringing it back to normal or inducing it above the normal levels. The pro-apoptotic effect of IP_6 appeared to be dependent on doses and duration of the exposure.

Mechanisms Involved in the Health Benefits of Legume Flavonoids for Skin

Although flavonoids show a considerable antioxidant activity, scavenging of free radicals is not the sole action performed by these compounds. They are also known to be involved in the regulation of different cellular signaling pathways and inhibition of metalloprotease activity or expression [50]. As for the antioxidant potential, Reeve et al. [2] have reported that equol exhibited a strong protective effect against acute UVA (320–400 nm)-induced lipid peroxidation in mouse skin, which may account for its anti-photoaging mechanism. On the other hand, Widyarini et al. [12] suggested that oxidative metabolic pathways essential for the activation of polycyclic hydrocarbon carcinogens might be susceptible to the antioxidant actions of isoflavones like equol. In contrast, Kang et al. [26] have suggested that most of the cancer prevention pathways activated by phenolic phytochemicals are not attributed to their antioxidant potential, but rather to their binding capacity to selected target molecules like protein kinases, matrix metalloproteases, or cyclooxygenases. These authors studied the inhibitory effect of equol on TPA-induced transformation of JB6 P+ mouse epidermal cells via targeting the MEK/ERK/p90RSK/AP-1 signaling pathway and found that equol directly bound to the glutathione *S*-transferase–MEK1 complex to inhibit MEK signaling without competing with ATP, thus causing the attenuation of TPA-induced activation of *c-fos* that in turn suppressed AP-1 transactivation. Furthermore, equol suppressed TPA-induced ERK-1 and ELK1 pathway activation in JB6 P+ cells in a dose-dependent manner. Equol also suppressed epidermal growth factor (EGF) or H-Ras-induced neoplastic transformation of JB6 P+ cells by targeting the MEK/ERK cascade. Chen et al. [51] have reported that pretreatment of skin with non-denatured soy extracts could prevent or reduce UVB-induced skin damage through different mechanisms including the inhibition of thymine–thymine dimer formation, enhanced checkpoint kinase-1 activity (Chk1) that delays cell cycle progression at the G2 phase and enables longer time for DNA repair, reduction of UVB-induced COX2 expression and prostaglandin E2 secretion, suppression of p38 MAPK activation, and inhibition of VEGF-induced endothelial tube formation in matrigel (angiogenesis). Chiu et al. [52] have investigated the in vitro and in vivo anti-photoaging effects of an isoflavone extract from soybean cake on the inhibition of HaCaT cell death and phosphorylation of p38, JNK, and ERK1/2. According to the authors, such biological effects would contribute to decreasing epidermal thickness, erythema, and TEWL as well as decreasing expression of COX2 and PCNA, markers of inflammation and DNA replication, caused by UVB-induced skin damage.

Different signaling pathways through which flavonoids can also act to block cell progression have been described by Lee et al. [25], who reported that 7-3′-4′-trihydroxyflavone (7,3′,4′-THIF), a metabolite of the soybean isoflavone daidzein synthesized in the liver, arrested EGF-induced JB6 P+ mouse epidermal cells at the G1 phase by suppressing cyclin-dependent kinase-4 (CDK4)-mediated phosphorylation of retinoblastoma protein. 7,3′,4′-THIF binds to phosphatidylinositol 3-kinase (PI3K), CDK2, and CDK4, thus inhibiting their kinase activity, suppressing the Akt/GSK-3β/AP-1 pathway and subsequently attenuating the expression of cyclin D1. Since PI3K-dependent phosphorylation of glycogen synthase kinase (GSK-3β) inhibits its activation and consecutively stabilizes cyclin D1, inactivation of PI3K can cause cyclin D1 instability and G1 phase arrest. It appears that 7,3′,4′-THIF competes with ATP for PI3K binding, thus reducing the activity of the later enzyme. Finally, Lee et al. [27] have studied the suppression of UVB-induced skin cancer by 7,3′,4′-THIF via targeting the signaling pathway in which Cot and MKK4 are involved. Cot and MKK4 are kinases

upstream of the p38 and JNK, phosphorylation of which mediates the inflammatory response and activates the transcription of eukaryotic NF-κB. 7,3',4′-THIF easily docked to the ATP binding site of Cot and MKK4, thus inhibiting their activity and suppressing the UVB-induced phosphorylation of JNK and p38 MAPK, which in turn caused an inhibition of COX2 activation in either mouse epidermal JB6 P+ cells or SKH-1 hairless mice. All these events resulted in the suppression of the incidence and multiplicity of UVB-induced tumors.

Allergic Reactions Caused by Legume Consumption

In contrast to their tremendous potential for skin health, legumes may also pose some potential hazards for skin physiology, mainly those derived from their allergenic potential and the cross-reactions of immune responses between legume seed proteins [53, 54]. Allergic reactions to legumes may range from oral pruritis, urticaria, or eczema to more potentially dangerous systemic anaphylactic reactions, and the degree of which may be tested by using different methodologies like the skin prick test, indirect histamine release, enzyme-allergosorbent-test (EAST), EAST inhibition, or immuno-CAP test [55]. Legumin-like basic subunit of legume storage proteins has been pointed out as the one with the greatest immune-reactivity [56], whereas processing of legumes shows different responses in legume reactivity [57]. Some thermal treatments do not affect reactivity while others decrease it considerably, possibly depending on the degree of protein denaturation. On the other hand, protein hydrolysis appears to be an efficient means to decrease the allergic potential of legumes.

Conclusions and Future Perspectives

There is much evidence that suggests the efficacy of legumes and their intrinsic bioactive compounds in the prevention of skin disorders including cancer and aging. Recent advances have witnessed some progress in understanding the mechanisms by which these key compounds act at the molecular level. However, it would still be far from a full understanding of the mode of actions. It is of future interest to elucidate (1) the potential of an adequate dietary intake of legumes for skin protection or its combination with topical application of isolated products; (2) the synergistic effect of different legume compounds, their metabolites, and other bioactive compounds on the protection of skin, again cancer and aging; and (3) how legume bioactive compounds function in DNA repair and genome maintenance. Understanding the molecular basis will provide a rationale for subsequent clinical trials of topical or oral administrations to protection of "skin against" cancer and aging.

Glossary

Genomic instability A biological process of increased tendency of the genome to acquire mutations due to dysfunctional DNA replication and damage response, which are often seen in cells prone to cancer and aging such as ataxia telangiectasia and Werner syndrome.

MAPKs Mitogen Activated protein kinases. Enzymes that take part in different signaling pathways involved in cell proliferation and tumor progression.

Non-melanoma skin cancer Most prevalent form of skin cancer corresponding to basal or squamous cell cancer.

Oxidative stress An imbalance when the production overruns the removal of ROS, resulting in overproduction of peroxides and free radicals that damage lipids, proteins, and DNA of the cells.

Phytochemicals Compounds present in plant foods or medicinal plants that show a well-recognized biological action and have the potential to beneficially affect human health.

Skin photoaging Rapid aging of the skin as a result of chronic exposure to ultraviolet radiation present in sunlight.

UV radiation Portion of the electromagnetic spectrum between X rays and visible light (40–400 nm).

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Chapter 39
Licorice: *Glycyrrhiza glabra* Linn. Used for Dermatitis

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Key Points

- Licorice rhizomes and roots are normally used for their medicinal properties.
- Traditionally *Glycyrrhiza glabra* roots were used for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases.
- Glycyrrhizin and glycyrrhetinic acid (GA) have a significant inhibitory effect on melanogenesis.
- Hypertension is a commonly observed side effect with licorice supplementation because licorice has a direct effect on the renin–angiotensin–aldosterone system.

Keywords *Glycyrrhiza glabra* • Contact dermatitis • Psoriasis • Melanogenesis

Introduction

Glycyrrhiza glabra Linn is well known as licorice or sweetwood and belongs to Leguminaceae (Fabaceae) family, subfamily Papilionaceae [1]. Licorice rhizomes and roots (Fig. 39.1) are normally used for their medicinal properties. The plant is native to the certain Asian and Mediterranean areas. Traditionally, dried root and rhizome of licorice were employed medicinally by many civilizations such as Egyptian, Chinese, Greek, Indian, and Roman as an expectorant and carminative. In modern medicinal system, to mask the bitter taste of certain preparations, licorice extracts are often used as a flavoring agent [2]. The dried rhizome and root of the plant are used as flavoring agent and the taste coorigent in the pharmaceutical and confectionery industries and its products are widely reported to be useful in ulcer therapy. Glycyrrhizin, a triterpene glucoside, is the principal constituent of *G. glabra* which is 50 times sweeter than sugar [3].

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Fig. 39.1 Licorice roots

The Japanese used licorice extracts for more than 60 years to treat chronic hepatitis, various viral infections such as human immunodeficiency virus (HIV), cytomegalovirus (CMV), and *herpes simplex*. Deglycyrrhizinated licorice (DGL) preparations are useful in treating various types of ulcers, while topical licorice preparations have been used to sooth and heal skin eruptions, such as psoriasis and herpetic lesions [1]. The licorice shrub is a member of the pea family and grows in subtropical climates in rich soil to a height of 4 or 5 ft. It has oval leaflets, white to purplish flower clusters, and flat pods. Below ground, the licorice plant has an extensive root system with a main taproot and numerous runners. The main taproot, which is harvested for medicinal use, is soft, fibrous, and has a bright yellow interior. *G. glabra* has found to be an effective agent for the treatment of atopic dermatitis [4].

Classification

Kingdom*:* Plantae
Division: Angiospermae
Class: Dicotyledoneae
Order: Rosales
Family: Leguminaceae
Genus: *Glycyrrhiza*
Species: *glabra* Linn.
Synonym: *Loquiritae officinalis* Moench

Vernacular Names [3]

English: Licorice, sweet root
French: *Reglisse*, *reglisse glabre*, *reglisse officinale*
Arabic: *Shagaret es-sus*
Berber: *Azrar azidane*
Sanskrit: *Yashti-madhuh*, *madhuka*
Hindi: *Jothi-madh*, *mulhatti*

Botany

The plant is native to the Mediterranean regions. Now it is also grown in Punjab, Jammu and Kashmir, and South India. Underground portion of the plant consists of a slender branching rhizome with a number of rootlets. The stems are herbaceous, erect, and up to 1–1.3 m high. Leaves are alternate,

bearing several pairs of blunt, ovate, and petiolate leaflets. The flowers are arranged in axillary spikes and have long peduncles. Flowers are pale blue arranged in a raceme and 1.25 cm long. Calyx is glandular and pubescent. The pods are glabrous, and red to brown having three to four seeds. Rhizome is soft, flexible, and fibrous with light yellow color and a characteristic sweet taste.

Sandy or clay soil in valley ideally suits the growth pattern of plant. The species *G. glabra* has many varieties like var. *B. violacea* Boiss (Persian licorice), which is collected in Iran and Iraq and bears violet flowers. The *G. glabra* var. *typical* Reg. and Herd is grown in Spain, Italy, England, France, Germany, and the USA. The var. *glandulifera* Reg. and Herd is abundant in the wild state in Galicia and central and southern Russia. Asian licorice is obtained from *Glycyrrhiza uralensis* Fisch, which is found in Siberia, Turkey, Mongolia, and China [5].

Chemical Compounds (Table 39.1)

Numerous phytoconstituents have been isolated from licorice. A water-soluble, biologically active complex accounts for 40–50% of total dry material weight of licorice. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances.

A triterpenoid compound, glycyrrhizin, accounts for the sweet taste of licorice root. Glycyrrhizin represents a combination of potassium–calcium–magnesium salts of glycyrrhizic acid. The natural saponin, glycyrrhizic acid, is composed of a hydrophilic part (glucuronic acid) and a hydrophobic fragment (glycyrrhetic acid). Yellow color of licorice roots and rhizomes is due to the flavonoids liquiritin, isoliquiritin (a chalcone), and other compounds.

Types of Dermatitis

Contact Dermatitis

It is one of the most common causes of occupational skin disorders. There are two types of contact dermatitis, irritant and allergic. Irritant contact dermatitis is the most prevalent form of contact dermatitis.

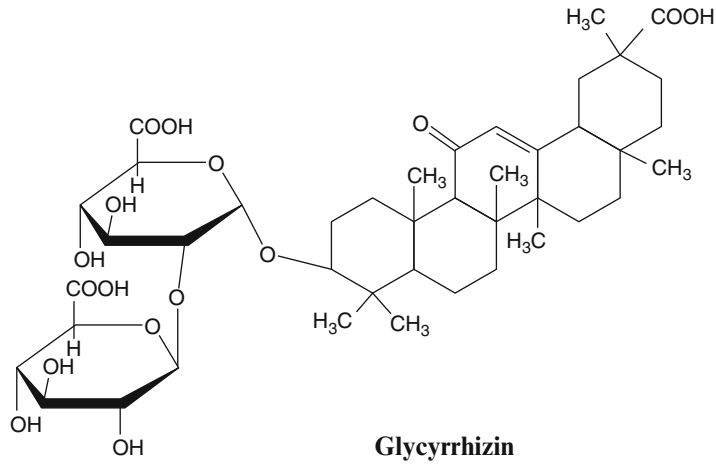
Irritant Contact Dermatitis

If a compound directly comes in contact of the skin, then site of contact causes irritant contact dermatitis. The compound may act immediately (such as strong acids or bases) or may act over a longer time (such as detergents).

Table 39.1 Summary of *Glycyrrhiza glabra* Linn. compounds and their therapeutic actions

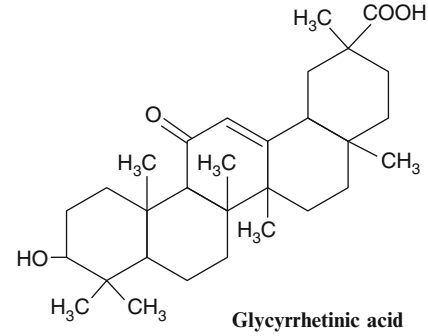
Compound	Action	References
Glycyrrhizin (Fig. 39.2)	Skin lightening	[9]
	Atopic dermatitis, pruritis, and cysts	[10]
Glycyrrhetinic acid (Fig. 39.3)	Herpes, eczema, psoriasis	[7]
Isoliquiritigenin (Fig. 39.4)	Skin lightening	[9]
Glabrene (Fig. 39.5)	Melanogenesis	[9]
Stearyl glycyrrhetinate (Fig. 39.6)	Sun care, sunscreen agent	[9]
Deglycyrrhizinated licorice (DGL) preparations	Sooth and heal skin eruptions, psoriasis, and herpetic lesions	[1]

Fig. 39.2 Glycyrrhizin



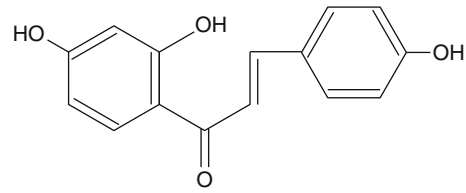
Glycyrrhizin

Fig. 39.3 Glycyrrhetinic acid



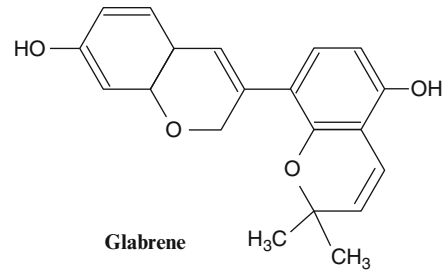
Glycyrrhetinic acid

Fig. 39.4 Isoliquiritigenin



Isoliquiritigenin

Fig. 39.5 Glabrene



Glabrene

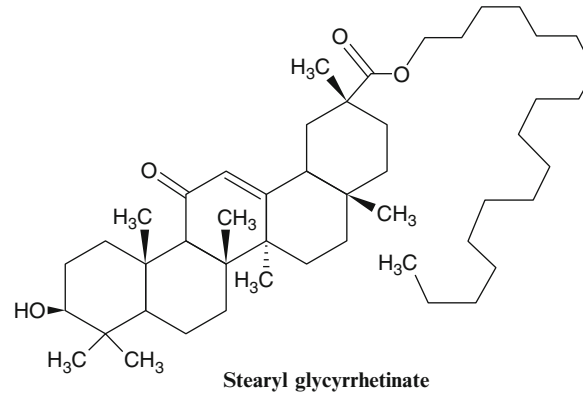


Fig. 39.6 Stearyl glycyrrhetinate

Stearyl glycyrrhetinate

Allergic Contact Dermatitis

Sensitizing agents cause allergic contact dermatitis. In this form of dermatitis, the skin's reaction is allergic. An antigen elicits an antibody immune reaction (antigen–antibody reaction). Other occupationally related skin conditions include *occupational acne* usually caused by petroleum-based products; *contact urticaria* or hives; and *photosensitivity* where the skin becomes more sensitive to sunlight.

Dermatitis and Licorice

Traditionally *G. glabra* roots were used for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases [6]. Certain oral licorice preparations, containing glycyrrhetinic acid, are used for the treatment of viral infections such as viral hepatitis, common cold. The topical preparations containing glycyrrhetinic acid are used for herpes, eczema, and psoriasis treatment [7].

A unique compound, glabrene, from the plant possesses anti-inflammatory and melanogenesis inhabiting property. Glabrene specifically inhibits the T1 and T3 tyrosinase isoenzyme activity and, hence, isoliquiritigenin and glabrene may serve as skin-lightening agent for the medicinal and cosmeceutical purposes. Glycyrrhizin and glycyrrhetinic acid have been a significant inhibitory effect on melanogenesis. Glycyrrhetinic acid in the form of stearyl glycyrrhetinate can be used as a sun care and sunscreen agent. A synthetic derivative of GA has been found to have a prominent inhibitory potential to melanogenesis. However, there is a need to establish the toxicological safety of the analog of GA for skin applications [8].

Glycyrrhizinic acids have been used to cure atopic dermatitis, pruritis, and cysts due to paracytic infestation of the skin [9].

The role of *G. glabra* extract on skin is mainly credited to its antioxidant property, particularly to its potent antioxidants triterpene saponins and flavonoids. Skin whitening; skin depigmenting, skin lightening, antiaging, anti-erythemic, emollient, antiacne, and photoprotection effects are mainly attributed to *G. glabra* extract [10].

Drug–Botanical Interactions

When digoxin in combination with licorice is administered in patients with ischemic heart disease, an increased risk of cardiac arrhythmias can be observed. In susceptible individuals, the mineralocorticoid side effect of licorice is enhanced by estrogen-based oral contraceptives. Hypertensive patients using diuretics and licorice simultaneously can exhibit hypokalemia which is commonly associated with metabolic acidosis.

Dosage

It is extremely difficult to calculate a proper dose for all individuals, because every individual has varying susceptibility to different licorice preparations. A daily oral intake of 1–10 mg of glycyrrhizin which corresponds to 1–5 g licorice (2% glycyrrhizin) has been estimated to be safe for most healthy adults. Studies of DGL for peptic ulcers employed dosages ranging from 760 to 2,280 mg DGL daily.

Side Effects and Toxicity

Hypertension is a commonly observed side effect with licorice supplementation. This is because of the effect of licorice on the renin–angiotensin–aldosterone system. Licorice saponins are able to elevate aldosterone action while binding to mineralocorticoid receptors in the kidneys. This phenomenon is known as pseudoaldosteronism. Along with hypertension, patients may experience hypokalemia (potassium loss) and sodium retention, resulting in edema. All these symptoms disappear with discontinuation of the therapy. Normally, the commencement and severity of symptoms depend on the dose and duration of licorice intake, as well as individual susceptibility. Patients with delayed gastrointestinal transit time may be more vulnerable to these side effects due to entero-hepatic cycling and reabsorption of licorice metabolites. The amount of licorice ingested daily by patients with mineralocorticoid excess syndromes appears to vary over a wide range, from as little as 1.5 g daily to as much as 250 g daily [11].

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Chapter 40
Role of *Emblica officinalis* in Prevention of Skin Disease

Anand A. Zanwar, Sachin L. Badole, and Rashmi Saini

Key Points

- *Phyllanthus emblica* Linn. syn. *Emblica officinalis* Gaertn. belonging to Euphorbiaceae family.
- The species is native to India and also grows in tropical and subtropical regions.
- *E. officinalis* mainly contains vitamin C which is responsible for its antioxidant activity.
- *E. officinalis* have been used in traditional medicine to treat broad-spectrum disorders.
- Toxicological studies have shown it to be safe and nontoxic.

Keywords Amla • Antioxidant activity • *Emblica officinalis* • Indian gooseberry • *Phyllanthus emblica*

Introduction

Indian gooseberry or *Emblica officinalis* enjoys a hallowed position in Ayurveda—an Indian indigenous system of medicine. According to the belief in ancient Indian mythology, it is the first tree to be created in the universe [1]. The species is native to India and also grows in tropical and subtropical regions. Indian gooseberry is a medium-sized tree, the fruit of which is used in many ayurvedic preparations from time immemorial. The parts of *Emblica officinalis* (*E. officinalis*) such as leaf, roots, stem, fruit, flower, and bark have been used in traditional medicine to treat broad-spectrum disorders [2].

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Fig. 40.1 Photograph of *Emblica officinalis* fruit, fleshy, globose, and shining and changed to light yellow or red-brick when mature

Botanical Descriptions

Synonym: *Phyllanthus emblica* Linn.
Family: Euphorbiaceae
Habitat: Native to tropical Southeast Asia; distributed throughout India.
English: Emblic, Indian gooseberry
Ayurvedic: Aaamalaki, aaamalaka, dhaatri, kaayasthaa, amoghaa, amritaphala, amla, aaamalaa, dhaatriphala, Vayasyaa, vrshya, shiva, hattha
Unani: Aamalaa, amlaj
Siddha/Tamil: Nellikkaai, nelli
Distribution: Tropical and subtropical countries

It flowers during March–April and has an extended fruiting period from October to March. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex. Leaves are simple, many sub-sessile, closely set along the branchlets, distichously light green having the appearance of pinnate leaves [2]. Leaves measure about 1.8×0.5 cm which are closely set in pinnate fashion, making the branches feathery in general appearance. Bark is thick (12 mm), shining grayish brown or grayish green. The fruits are yellowish green (Fig. 40.1: Photograph of *E. officinalis* fruit), fleshy, globose, and shining and changed to light yellow or red-brick when mature. The average yield of wild trees growing in the forests is 23.5 kg.

Cultivation and Collection

Indian gooseberry is quite hardy and it prefers a warm dry climate. It needs good sunlight and rainfall. It can be grown in almost all types of soils, except very sandy type. The seeds are enclosed in a hard seed coat which renders the germination difficult. Seeds are soaked in water for 3–4 h and sown on previously prepared seed beds and irrigated. Excess irrigation and water logging are harmful. One-month-old seedlings can be transplanted to polythene bags and 1-year-old seedlings can be planted in the main field with the onset of monsoon. Pits of size 50 cm^3 are dug at 6–8 m spacing and filled with a mixture of top soil and well-rotten planting is done. Irrigation and weeding are required during the first year. Application of organic manure and mulching every year are highly beneficial. Fruit yield ranges from 30 to 50 kg/tree/year when fully grown [2].

The fruiting season is exceptionally long. The fruit in this area becomes fit for harvesting in December. They can be retained on the tree up to March without any significant loss in quality or yield. The picking of fruits is generally done by the villagers in February and March. Indian gooseberry is being cultivated on a large scale due to increase in demand.

Pharmacology and Phytochemistry of *E. officinalis*

Traditionally, the fruit is useful as astringent, cardiotonic, diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, antipyretic, anti-inflammatory, hair tonic, and digestive medicine. It is used for a variety of ailments such as anemia, hyperacidity, diarrhea, eye inflammation, anomalies of urine, leucorrhea, jaundice, nervine debility, liver complaints, and cough [3]. The taste of Indian gooseberry is sour, bitter, astringent, and is quite fibrous. In India, it is common to eat gooseberries steeped in salt water and turmeric to make the sour fruits palatable.

Seeds contain fixed oil, phosphatides, and a small quantity of essential oil. The fixed oil yields about 16% and has the following physical and chemical characteristics: acid value 12.7; saponification value 185; iodine value 139.5; acetyl value 2.03; unsaponifiable matter 3.81%; sterol 2.70%; and saturated fatty acid 7% [1].

The *E. officinalis* fruits are not good for fresh consumption because of astringency and acidic taste. These fruits are used in huge quantities for making pickles and preserves, both in the villages and in the towns. They are offered for sale in the towns for this purpose. The Indian gooseberry-fruits are dried for making triphala. They are also used as a principal ingredient in making another famous ayurvedic tonic, chyavanprash [2].

The norsesquiterpenoid glycosides isolated from roots of *Phyllanthus emblica* include 4′-hydroxyphyllaemblicin B, phyllaemblicins E and F, phyllaemblic acid, phyllaemblicin B, and phyllaemblicin C [4].

There are different types of compounds which are responsible for antioxidant activity of *E. officinalis. Emblica* fruit contains vitamin C (478.56 mg/100 ml), and emblicanins A and B. The medicinally important compounds such as ellagic acid, gallic acid, tannins, methyl gallate, corilagin, furosin and geraniin were isolated from ethyl acetate extract of *E. officinalis* and the potency of nitric oxide scavenging activity was found to be in following manner: geraniin > corilagin > furosin > gallic acid > methyl gallate [5].

The total antioxidant capacity in terms of the ascorbic acid equivalents by cyclic voltammeter is 94 mg/g of *E. officinalis* extract and total reactivity is $6.23 \pm 0.15 \times 10^{-3}$ s^{-1} with diammonium salt method [6].

In the study of effect of *E. officinalis* extract on lipid profiles and oxidative stress in the aging process, the oral administration of ethyl acetate extract of *E. officinalis* for 100 days to aged rats partially prevented age-related increases in cholesterol and triglyceride levels in the serum and liver. Oral administration of *E. officinalis* significantly inhibited the serum and hepatic mitochondrial thiobarbituric acid-reactive substance levels in aged rats. These results indicate that *E. officinalis* may prevent age-related hyperlipidemia through attenuating oxidative stress in the aging process [7].

E. officinalis extracts on mature human osteoclasts suggest the possible use of this medicinal plant as therapeutic tools against different forms of arthritis and osteoporosis, improving the activity of already employed drugs [8]. *E. officinalis* have antidiabetic and hypotriglyceridemic activity, do not show any toxic effects, and improve liver function by normalizing the activity of liver-specific enzyme alanine transaminase [9].

E. officinalis can be used as an alternative/adjuvant drug in preventing and treating the extra pyramidal side effects of antipsychotic agents in clinical practice. It was shown that in addition to vitamin C, other polyphenols like tannins and gallic acid may contribute to its effectiveness to reduce oxidative stress and catalepsy [10].

Antibiotic activity against a wide variety of microorganisms—pathogenic and nonpathogenic gram-positive and gram-negative bacteria, yeast, and fungi—was also noted with fruits of *E. officinalis* [11].

The administration of ethyl acetate extract of *E. officinalis* reduced the elevated levels of serum creatinine and urea nitrogen in the aged rats. In addition, the tail arterial blood pressure was markedly elevated in aged control rats as compared with young rats, while the systolic blood pressure was significantly decreased and significantly reduced thiobarbituric acid-reactive substance levels of serum, renal homogenate, and mitochondria in aged rats. These results indicate that *E. officinalis* is an antioxidant for the prevention of age-related renal disease [12].

Role of *E. officinalis* in Prevention of Skin Disease

Mitochondrial activity of human skin fibroblasts was measured by 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (WST-8) assay for evaluation of cell proliferation. When fibroblasts were incubated with various concentrations (0–40 g/mL) of amla extract (prepared by solvent extraction) for 48 h, elevation in the mitochondrial activity in a concentration-dependent manner was observed. Amla extract when added to fibroblast culture media at varying concentrations (0–40 μg/mL) and subjected to incubation for 48 h or to time course test (0, 24 and 48 h) at 20 μg/mL, the result indicated concentration-dependent manner effect of amla extract on procollagen type I C-peptide production by human skin fibroblasts in culture. Also procollagen type I C-peptide significantly increased by 36% at 20 μg/mL and by 17% at 40 μg/mL, as compared with the non-treated control cells. In case of matrix metalloproteinases (MMPs), marked reduction was observed in MMP-1 production in a dose-dependant manner as compared with the non-treated control cells. On the other hand, MMP-2 levels did not change and hence amla extract has a number of potential cosmetic applications [13].

Different types of formulations of *E. officinalis* are used in different types of skin disorders treated as per survey Assamese people in India. The extracted juice of *E. officinalis* is mixed with sugar and the mixture is orally taken for scabies. The fruits are orally taken for dry skin. The paste is applied directly on the skin. The extracted juice is used for taking bath for wrinkled skin. The fruit and *Curcuma longa* are together crushed and the juice produced is orally taken for measles. The juice extracted from the fruit is mixed with citrus lemon juice and hot water. The obtained product is used for washing the hairs in pediculosis [14].

E. officinalis enhances the fibroblast proliferation in a concentration-dependent manner and also exhibits a highly significant photo-protective effect against UVB-induced cytotoxicity, thereby suggestive of strong skin protective ability. *E. officinalis* pretreatment significantly protects against this loss in cell viability in a concentration-dependent manner. *E. officinalis* possess the potential inhibitory effect on intracellular oxidative damage induced by UVB irradiation. The antioxidant activities were associated with the improved cell viability which is due to increased cellular levels of ROS lead to cellular damage and scavenging of reactive oxygen species (ROS) through the use of antioxidants, protecting the cells from such cellular damage has been a good strategy for development of photo-protective agents of cosmetic interests. *E. officinalis* has also shown strong skin photo-protective effects through its ability to quench ROS generated by UVB irradiation and thus preventing DNA damage which is due to antioxidant activity related to UV protection (antiphoto-aging). *E. officinalis* has strong anti-hyaluronidase activity which suggests the increased hyaluronic acid, and is highly beneficial for prevention of premature skin aging, i.e., wrinkle formation [15].

Toxicity

Acute oral toxicity with PartySmart (containing *E. officinalis*) revealed that the LD_{50} was greater than 2,000 mg/kg body weight. Repeated dose 90-day oral toxicity with PartySmart (1,000 mg/kg body weight) revealed no clinical signs and preterminal deaths were observed. Body weights of male and female rats in the PartySmart-treated groups were comparable with the control group. No change in food intake was observed. Hematological and biochemical parameters were within normal range in all the drug-treated groups. No gross abnormalities attributing to the drug toxicity were noticed in any of the treated groups. There was no significant difference in the organ weight profile of the animals in the treated groups as compared to control. Histopathological examination of all target organs showed no evidence of lesions attributing to drug toxicity. Repeated dose 90-day oral toxicity with PartySmart revealed no adverse effect on the parameters evaluated, thereby indicating that PartySmart is devoid of adverse effects with the doses employed [16].

In sub-acute toxicity study of Kalpaamruthaa (a modified indigenous formulation containing *E. officinalis*) for 30 days revealed no toxicity up to a dose level of 500 mg/kg, body weight did not cause any changes in biochemical and hematological changes, but transient rise in hemoglobin, leukocyte count, free fatty acid, plasma, and urine creatinine and significant decrease in blood glucose, triglyceride, and phospolipid level were observed [17].

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Chapter 41
Sphaeranthus indicus: Skin Disease Preventive Plant

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Key Points

- *Sphaeranthus indicus* Linn. is known as East Indian globe thistle.
- It is found to be useful in skin diseases like psoriasis and acne.
- It possesses good antimicrobial activity against a wide range of human pathogens.

Keywords *Sphaeranthus indicus* Linn. • Asteraceae • Skin disease • Antimicrobial

Introduction

Skin disease is now a common disorder in all over the world. It affects all age group people of society and causes harm in different ways. Skin is the vital and largest organ of human body. Skin serves many important functions such as protection, thermoregulation, percutaneaous absorption, secretary, and sensory. It has been estimated that skin diseases account for 34% of all occupational diseases [1].

Skin diseases are classified as noncontagious and contagious diseases, the primary of which are bacteria, fungi, viral, and parasitic diseases. These diseases occur all over the world, but are more prevalent in the rural and tropical regions due to lack of sanitation, portable water, and awareness of hygienic food habits [2]. The development of a new drug against multidrug-resistant pathogens is a challenging task for researchers. One potential approach is to screen local medicinal plants in search of suitable chemotherapeutic antimicrobial substances [1].

Medicinal plants form the origin of traditional system of health used by most population of developing countries. Due to side effects of synthetic drugs most of the physicians prefer medicinal plant remedies mentioned in Ayurveda and other traditional systems [3]. *Sphaeranthus indicus* Linn. is a plant species belonging to the genus *Sphaeranthus*, family Asteraceae (Compositae). It is also known as East Indian globe thistle. In Ayurveda, different parts such as leaves, stem, flower, seeds, and even whole plant of *S. indicus* Linn. have been used to cure various diseases. The plant is a spreading aromatic annual herb [4].

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Botanical Description

Taxonomic Classification

Kingdom: Plantae
Subkingdom: Viridaeplantae
Phyllum: Tracheophyta
Subphyllum: Euphyllophytina
Infraphyllum: Radiatopses
Class: Magnoliopsida
Subclass: Asteridae
Superorder: Asteranae
Order: Asterales
Family: Asteraceae (compositae)
Genus: *Sphaeranthus*
Species: *indicus*

Common Names

Sanskrit: *Mahamundi*, *shravani*, *tapasvini*, *mundi*, *hapus*
Hindi: *Gorakhmundi*, *mundi*
Bengali: *Chagulnadi*, *ghorkmundi*
Marathi: *Barasavodi*, *gorakhmundi*
Gujarati: *Bodiokalara*, *mundi*, *dorakhmundi*
Telugu: *Boddatarupa*, *boddasoram*
Tamil: *Kottakaranthai*
Urdu: *Kamdaryus*
Malayalam: *Adakkamanian*, *attakkamanni*, *mirangani*
Santal: *Belaunja*
Undari: *Mundi*
Riya: *Murisa*, *bokashungi*
Panjabi: *Ghundi*, *khamadrus*, *mundibuti*
English: *East Indian globe thistle*

Ayurvedic Properties

Rasa: Madhura, tikta
Guna: Lakhu
Virya: Ushna
Vipaka: Madhura [4]

Morphology

S. indicus Linn. is found abundantly in damp situations in the plains, ascending to an altitude of 1,500 m in the hills, especially weeds in the rice field. The herb is distributed throughout India, Ceylon-Malay, China, Africa, Sri Lanka, Burma, Malaysia, and Australia [5].

Fig. 41.1 *Sphaeranthus indicus* herb. (Source: Photograph taken by ourselves)

Plant: A spreading aromatic annual herb, about 30 cm high (Fig. 41.1).

Stems: Glandular hairy, cylindrical, toothed wings, much branched, branches cylindrical with toothed wings, more or less glandular hairy.

Leaves: Sessile, decurrent alternate, 2–7 cm long, 1–1.5 cm wide, oblong-spatulate, lancerate or dentate, base decurrent, and greenish-brown in color.

Flowers: In close terminal, pink to purple, globose or ovoid heads, bisexual.

Fruits: Oblong and have compressed achenes in which pappus is absent [4, 5].

S. indicus Linn. in Skin Diseases

The standardized extract of *S. indicus* Linn. (1.4 and 2.8 g/day) in dosage form was given orally to subjects (74 subjects with PASI score 10) with moderate to severe chronic plaque psoriasis over a 3-month period. In psoriasis cytokine, especially tumor necrosis factor alfa (TNF-α), has been shown to play an important role. Extract of *S. indicus* Linn. has been found to inhibit the release of TNF-α and IL-12/IL-23. Histopathology and gene expression analysis supported improvement in PASI score [6].

Wound healing activity of ethanolic extract of aerial part of *S. indicus* Linn. was assessed in guinea pigs. The cream containing the 10% extract was applied in vivo on the paravertebral area of six excised wounded models once a day for 15 days. The rate of wound contraction as well as the period of epithelialization in cream-treated animals was enhanced as compared to standard group treated with neomycin [7].

In another study, ointment was prepared using ethanolic extract of flower head of *S. indicus* in various proportions and screened in albino rats for the evaluation of wound healing activity. The formulation that contains 2% (w/w) alcoholic extract of flower head of *S. indicus* was found superior than control and standard formulation for the wound healing activity. Further, hydroxyproline content was also found higher in healed wounds as compared to control and standard formulation [8].

In acne pathogenesis, reactive oxygen species and pro-inflammatory cytokines are two important inflammatory mediators that were induced by *Propionibacterium acnes.* Suppression of reactive oxygen species was observed when the herb *S. indicus* (5 and 50 μg/mL) was treated on polymorphonuclear leukocytes and monocytes with culture supernatant of *P. acnes.* Further, the aqueous extract obtained from the root of *S. indicus* was found to be moderately active in suppressing *P. acnes*-induced TNF-α and IL-8 production [9].

Antibacterial and Antifungal Activities

S. indicus Linn. was reported for antimicrobial activity against a wide range of human pathogens responsible for skin diseases. The essential oil obtained from the leaves of *S. indicus* exhibited higher antifungal activity than 2% resorcinol against *Trichoderma viride*, *Rhizopus nodosus*, *Aspergillus niger, Trichophyton rubrum*, and *Curvularia parasadii* [10]. Further, it was found to have antibacterial activity against *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Salmonella paratyphi C*, *Schigella flexneri*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Shigella sonnei*, and *Vibrio cholera*. Aerial parts of *S. indicus* were shown to have antibacterial activity against *Bacillus cereus* var. *mycoides*, *Bacillus pumilus*, *Bacillus subtilis*, *Bordetella bronchiseptica*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Streptococcus faecalis* [11].

The alcoholic extract of flowers has been found to have antibacterial activity against 18 different species of gram-negative and gram-positive bacteria. This activity was present in alkaloidal as well as non-alkaloidal fractions [12]. A bicyclic sesquiterpene lactone has been isolated from petroleum ether extract of the aerial part of *S. indicus*. The compound demonstrated strong antimicrobial activity against *S. aureus*, *Staphylococcus albus*, *Escherichia coli*, *Fuscrium* sp., *Helminthosporium* sp., and other microorganisms [13]. Alcoholic as well as aqueous extract of plant were found to be highly effective against *Alternaria solani*, *Fusacrium oxysporum*, and *Penicillium pinophilum* by preventing their growth to greater extent [14].

In another study, the fruits of *S. indicus* exhibited good antibacterial activity against gram-positive as well as gram-negative bacteria [15]. The *n*-hexane, benzene, chloroform, ethyl acetate, and acetone extracts of aerial parts and flowers of *S. indicus* were tested for antibacterial and antifungal activities. The *n*-hexane extract of flowers showed significant higher activity against *S. aureus* and *Candida albicans* [16].

Ethanol, chloroform, and petroleum ether extracts of the *S. indicus* were screened for antimicrobial activity against the selected human pathogens *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Salmonella typhi*, *C. albicans*, and *Cryptococcus neoforman*. The chloroform extract of *S. indicus* was shown to have significantly more antimicrobial activity against the selected pathogens than ethanol and petroleum extract. Further, the molecule was identified as 7-hydroxyfrullanolide from active fraction, separated by using thin-layer chromatography and with the help of GCMS (Sangeetha et al., 17).

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Chapter 42
Mangosteen (*Garcinia mangostana* Linn.): Role in Prevention of Skin Disorders

Aman B. Upaganlawar and Sachin L. Badole

Key Points

- Mangosteen are the most valued part of the plant *Garcinia mangostana* (family: Guttiferae) and is famous for the remarkably pleasant flavor.
- Mangosteen is the rich source of xanthones which are found in abundance in the pericarp or rind of the fruit.
- The leaves and bark of mangosteen are recognized to have strong anti-inflammatory and antioxidant properties; an ointment derived from them is used for treating eczema, hyperkeratosis, and other skin disorders such as psoriasis and wounds.

Keywords Mangosteen • Xanthones • Acne • Aging • Allergy • Antibacterial

Introduction

Garcinia mangostana is thought to be originated in Southeast Asia. Traditionally it has been used for many years in Chinese, Ayurvedic, and folk medicine in Asia but was also grown and used for medical purposes in many countries. Today the fruit of mangosteen is cultivated in the tropical regions of both hemispheres with commercial plantations in Thailand, India, Malaysia, Vietnam, and the Philippines. Mangosteen requires warm, very humid, equatorial climate. The tree only can grow well in tropical areas and requires abundant moisture. Thailand and Burma are the original countries where people found mangosteen. Recently tropical Australia has been found to be an excellent area for mangosteen production. Mangosteen is a small and very slow-growing tropical evergreen tree. The height of the tree attains 20–82 ft. The length of its leaves is up to 10 in. The width of its flowers is 1½ to 2 in. And maybe male or hermaphrodite is on the same tree [1].

Mangosteen is the rich source of xanthones which are found in abundance in the pericarp or rind of the mangosteen fruit. The smooth, purple/dark red covering that was traditionally ground with ancient mortars and used to heal infection turns out to be very high in beneficial xanthones and potent

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antioxidants. Xanthones may provide beneficial effects on cardiovascular diseases, including ischemic heart disease, atherosclerosis, hypertension, and thrombosis. Mangosteen has reported to have an inhibitory action against *Mycobacterium tuberculosis* and *Staphylococcus aureus*. Mangosteen is also proving to be highly effective in supporting and strengthening a weak immune system. There are several important compounds found in the mangosteen that would appear to make this fruit an active and important nutritional supplement for the human body. Scientific research indicates activity against several cancer cell lines, including breast cancer, liver cancer, and leukemia. In addition, mangosteen also exhibits antihistamine and anti-inflammatory properties. Traditionally, mangosteen has been used for many years as a medicinal treatment for diarrhea, skin infection, and wounds [1, 2].

Scientific Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Malpighiales
Family: Clusiaceae
Genus: Garcinia
Species: *G. mangostana*
Binomial name: *Garcinia mangostana L.*

Common Name

English: Mangosteen
Portuguese: Mangostao
Sinhalese: Mangus
Tamil: Sulambali
Hindi: Mangustan
Bengali: Mangustan
German: Maogostane
Chinese: Dao nian zi
Gujarat and Maharashtra: Mangostin, mengut, mangastin, mangustan [3].

Botanical Description

Mangosteen is believed to be native of the Singapore. Escape to British Burma, Malayan Peninsular and Madras presidency [4]. *G. mangostana* is slow growing, cultivated in humid climate, and attains 7–25 m height with dense heavy profusely branched pyramidal crown.

Leaves. The leaves are fibrous, opposite, short stalked, thick, dark green and glossy above and yellowish-green beneath, 9–25 cm long, and 4.5–11 cm wide. The leaves have a cuneate base, acute apex, and entire margin, and contain numerous veins that are joined together by a vein running parallel to a midrib.

Flowers. Flowers are 5 cm in diameter, bisexual 4-parted, and borne at the ends of the branchlet. The flowers carry a lot of stamens although they bear no pollen as they have no anthers.

Fig. 42.1 Tree and fruits of mangosteen (*G. mangostana*). (*Source*: Structure taken from Google image)

Fruit. The mangosteen fruit is composed of pericarp (rind), pulp (fruit), and seeds. The pulp is composed of four to eight triangular segments of snow-white, juicy, soft flesh possessing the consistency of the pulp. A mature fruit may attain 6–7 cm in diameter and contains five to seven seeds surrounded by a white, sweet, and succulent flesh.

Seeds. The seeds are large, flattened, and embedded in snowy white or pinkish delicious pulp, which is botanically called the aril. The rind contains bitter yellow latex and a purple, staining juice [5, 6]. A large percentage of those who have tested the mangosteen fruit claimed that it is the most delicious of all fruits (Fig. 42.1). As a result of mangosteen fruit's exquisite flavor, it has been given the nickname "Queen of tropical fruits" and in French Caribbean the "Food of the Gods."

Chemical Constituents

Mangosteen is obtained by boiling the rind in water, tannin is removed by exhausting it by boiling in alcohol and evaporating, and the resulting product is mangosteen and resin. Resin is precipitated by redissolving in alcohol and water, and evaporating the water. It occurs in small yellow scales, tasteless neutral, insoluble in water, but readily soluble in alcohol and ether [3].

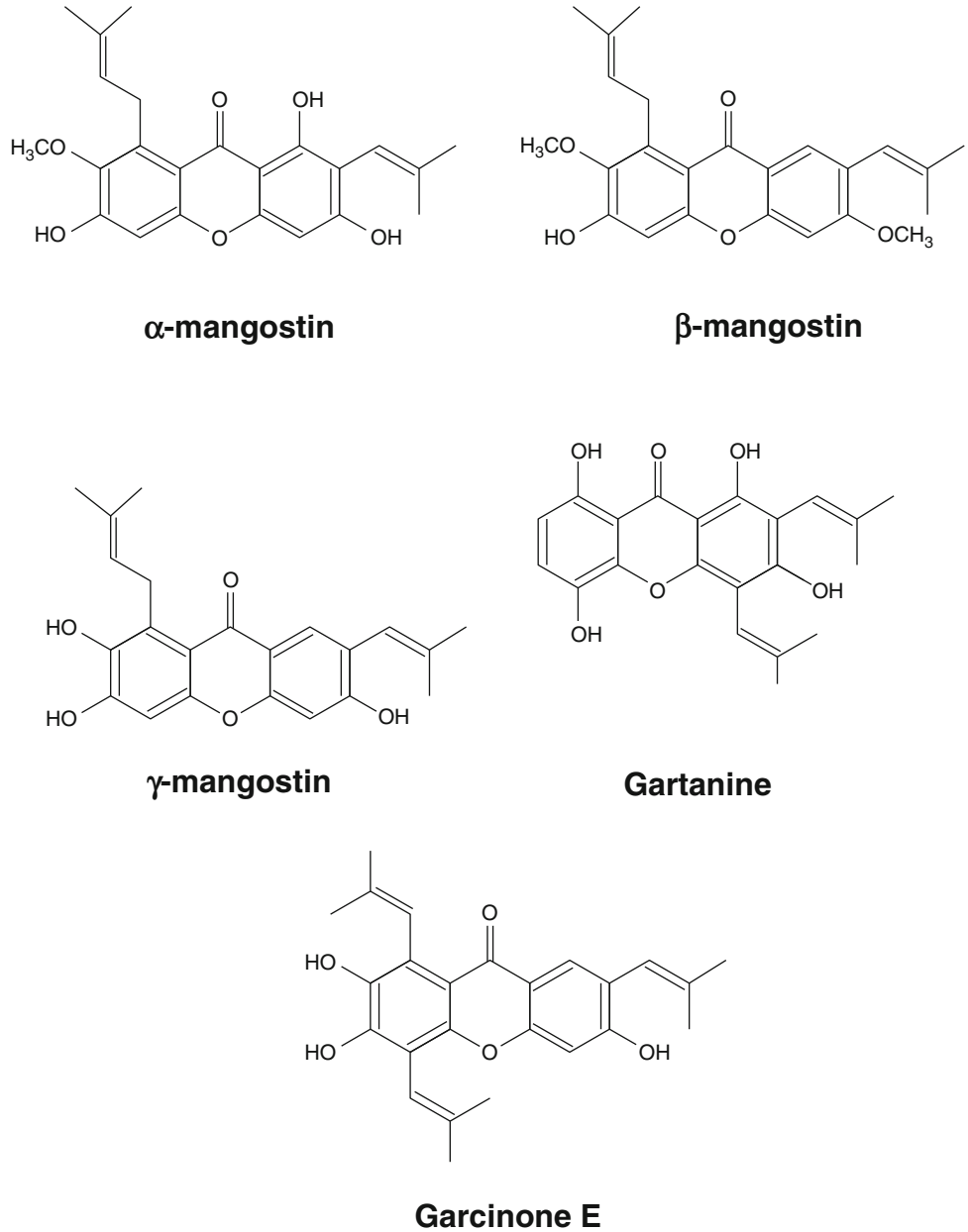

Fig. 42.2 Structure of important xanthones present in *G. mangostana.* (*Source*: Structure drawn by ourselves)

The fruit shell of mangosteen contains 7–13% tannin and the seeds contain 3% oil, a resin, and a yellow crystalline bitter principle, mangostin or mangosim isolated from the rind (Fig. 42.2). Xanthone derivatives are the major bioactive secondary metabolites of mangosteen. Various xanthones from pericarp, whole fruit, stem, arils, seed, and heartwood were isolated. The major active constituents from xanthone fraction of *G. mangostana* were found to be α-mangostin, β-mangostin, γ-mangostin, isomangostin, gartanin, and 8-desoxygartanin and were extracted from the fruit rind of mangosteen, and identified and quantitatively determined used high-performance liquid chromatography [7].

A new xanthone, named mangostinone, and seven known xanthones (α-, β-, γ-mangostins, gartanin, garcinone E, 1,5-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone, and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone) were isolated from pericarps of *G. mangostana*. A new polyoxygenated xanthone, mangostanol, was isolated from fruit hulls of *G. mangostana*, along with α-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, 5,9-dihydroxy-2,2-dimethyl-8-methoxy-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-b]xanthen-6-one, garcinone E, 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone, and epicatechin [8]. Pharmacological activities of xanthones have aroused great interest to this class of substances.

Role of Mangosteen in Skin Disorders

Different parts of *G. mangostana*, mostly fruit hull, bark, and roots, have been used for hundreds of years in Southeast Asia as a medicine for a great variety of medical conditions. Traditionally mangosteen leaves and bark are recognized to have strong anti-inflammatory properties and therefore an ointment derived from them is used for treating eczema, hyperkeratosis, and other skin disorders such as psoriasis [9, 10]. In Thai folk medicine the fruit hulls of *G. mangostana* have been in use for the treatment of skin infections, wounds, and for the relief of diarrhea [7]. In Venezuela parasitic skin infections are treated with poultices of the fruit rind [8]. The pericarp of mangosteen fruit has been used as a medicinal agent by Southeast Asians for centuries in the treatment of skin infections and wounds [2, 11].

Antiacne Activity

Propionibacterium acnes have been recognized as an obligate anaerobic organism which is usually found as a normal skin. This organism has been implicated over other cutaneous microflora in contributing to the inflammatory response of acne. It acts as an immunostimulator which can produce a variety of enzymes and biologically active molecules including lipases, proteases, hyaluronidases, and chemotactic factors, which are involved in the development of inflammatory acnes. It has been reported that *P. acnes* can stimulate the production of the IL-1, IL-8, and tumor necrosis factor-alpha (TNF-α) by human monocytic cell lines and freshly isolated peripheral blood mononuclear cells from acne patients. Previous findings suggest that *P. acnes* have a major role in the inflammation of acne vulgaris and can evoke mild local inflammation by producing neutrophil chemotactic factors. As a consequence, neutrophils which are attracted to the acne lesion constantly release inflammatory mediators such as reactive oxygen species (ROS) [12, 13].

Ethanolic extracts of pericarp part of *G. mangostana* show anti-inflammatory activity against in vitro models such as inhibition of proinflammatory cytokines (TNF-α) produced by human peripheral blood nuclear cells. Also the ethanol extract showed good antioxidant activity tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitroblue tetrazolium dye (NBT) reduction test, and superoxide radical scavenging assay [14].

Fruit rind extracts of mangosteen were prepared using different solvents including hexane, dichloromethane, ethanol, and water. Dichloromethane extract showed high antiacne activity against *P. acnes* and *Staphylococcus epidermidis* by thin-layer chromatography (TLC). α-Mangostin was separated from the dichloromethane extract of *G. mangostana* fruit rind by column chromatography. The dichloromethane extract exhibited the strongest antibacterial effect with minimum inhibitory concentration values for both bacterial species at 3.91 μg/ml, while the minimum bactericidal concentration

values against *P. acnes* and *S. epidermidis* were 3.91 μg/ml and 15.63 μg/ml, respectively. α-Mangostin was found to be the major active component against both *P. acnes* and *S. epidermidis* [15].

The ethanol extracts of mangosteen fruit rinds prepared by several extraction methods were examined for their contents of bioactive compounds, DPPH-scavenging activity, and antiacne-producing bacteria against *P. acnes* and *S. epidermidis*. The dried powder of the fruit rind was extracted with 95% ethanol by maceration, percolation, Soxhlet extraction, ultrasonic extraction, and extraction using a magnetic stirrer. Soxhlet extraction promoted the maximum contents of crude extract (26.60% dry weight) and α-mangostin and also gave the highest antiacne activity with MIC 7.81 and 15.63 μg/ml and MBC 15.53 and 31.25 μg/ml against *P. acnes* and *S. epidermidis*, respectively [16].

Bhaskar et al., 2009, have prepared a topical poly herbal gel for the treatment of mild acne vulgaris. He has formulated aqueous extracts of *G. mangostana* and *Aloe vera* in an aqueous-based carbopol-934 (1% w/w) gel system. Six formulations of the topical gel were prepared by varying the concentration of polymers and evaluated against *P. acne* and *S. epidermidis*. The microbial assay of all the formulations demonstrated better inhibitory activity against *P. acne* and *S. epidermidis* compared to the marketed clindamycin phosphate gel in equivalent amounts of application [17].

Antibacterial Activity

Prenylated xanthones present in *G. mangostana* were isolated by several authors. Prenylated xanthones 1, 2, 5, 6, 9–11, 14, and 15 were isolated from the green fruit hulls of *G. mangostana* and additionally γ-mangostin(3), garcinone D(4), mangostanin(8), and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone(12) were isolated from a larger quantity of the MeOH extract of the fresh green fruit hulls and compounds 7, 13, and more were obtained from the MeOH extract of the pulverized fresh arils and seeds. This xanthones showed good antimycobacterial activity against *M. tuberculosis* H_{37}Ra strain [18].

Pericarp extract powder of *G. mangostana* at different concentrations showed good antimicrobial activity against *S. aureus*, *Staphylococcus albus*, and *Micrococcus lutus* [19]. Due to the fruit's natural anti-inflammatory and antibacterial properties it will help soothe the skin irritation and prevent further flare-ups and infection. α-Mangostin showed antibacterial activity against five strains of vancomycin-resistant *Enterococci* and nine strains of methicillin resistance *S. aureus* [10].

Antiaging Activity

Oxidative damage is a major cause of aging skin. This damage causes cell renewal to slow down. The breakdown of the skin tissue and collagen protein will result in loss of radiance and vitality in the skin, wrinkles, and lines. Consuming antioxidant vitamins helps to capture and neutralize free radicals, reducing cellular damage and speeding up repairs. Mangosteen also contains colorful antioxidants such as flavones and flavonoids that help boost and assist other antioxidants such as vitamin C and E. Working together and strengthening the immune system, this partnership helps protect the body and assist in circulation to a more protective level.

Several authors have reported the powerful antioxidant activity of mangosteen and its active constituents. Different extracts and xanthones from *G. mangostana* possess antioxidant activity against various in vitro methods such as DPPH radical scavenging activity, NBT reduction test, superoxide radical scavenging assay [14], ferric thiocyanate method, and the 2,20-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay [20, 21]. The strong antioxidants in xanthones will help in skin cell renewal, ridding the skin of any accumulation of dead skin cells, clogged pores, and excess sebum.

Anti-allergic Activity

Methanolic extracts of α and γ mangostins were reported to show histaminergic and serotonergic receptor blocking action. α-Mangostin inhibited histamine-induced contractions in a dose-dependent manner with or without cimetidine, an antagonist of the H2-histamine receptor. Also, α-mangostin inhibits contractions mediated by the histamine H1 receptor [8]. Nakatani [22] showed that 40% ethanol extract (100 and 300 lg/mL) of mangostine fruit inhibits the histamine release induced by IgE in RBL-2H3 cells. This antihistaminic activity of mangosteen might be helpful for allergic reaction to skin.

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Chapter 43
Exploring Neem (*Azadirachta indica*) for Antidermatophytic Activity

Bhushan P. Pimple, Sachin L. Badole, and Farid Menaa

Key Points

- *Azadirachta indica* is a well-known medicinal plant in the Indian system of medicine for treatment of various skin diseases.
- The therapeutic action is due to the presence of several antimicrobial phytoconstituents such as azadirachtin, nimbin, nimbolide, gedunin and mahmoodin.
- *A. indica* is found to be active in prevention and treatment of dandruff.
- Neem does not have any negative health effects for humans because it is gentle.

Keywords *Azadirachta indica* • Antidermatophytic • Antifungal • Antidandruff

Abbreviations

MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
SD	Sabouraud dextrose

Introduction

Azadiracchta indica A. Juss. (Neem) is a large evergreen tree that usually attains a height of 18–20 m. The plant belongs to mahogany family Meliaceae. Neem is indigenous to Indian subcontinent. The plant flourishes in tropical or subtropical regions with semiarid to humid climate. The plant is widely spread in Pakistan, Malaysia, Australia and Africa. Neem plant was used in ancient Indian medicinal

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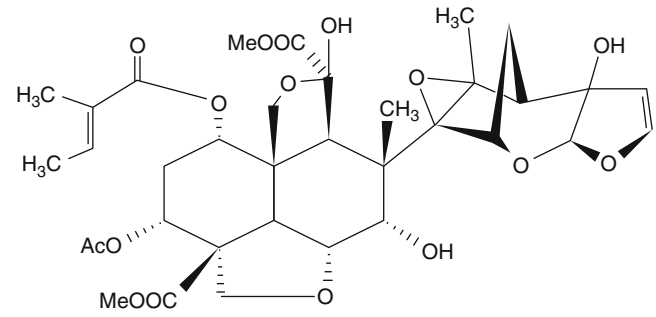
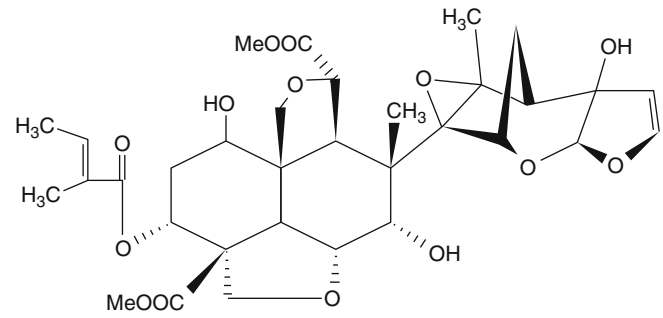
Fig. 43.1 Azadirachtin A



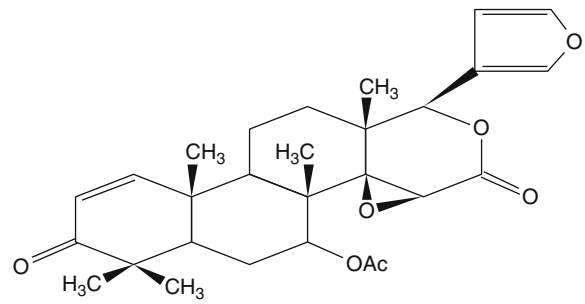
Azadirachtin A

Fig. 43.2 Azadirachtin B



Azadirachtin B

Fig. 43.3 Gedunin



Gedunin

practices for treatment of various skin ailments. Azadirachtin A (Fig. 43.1), azadirachtin B (Fig. 43.2), gedunin (Fig. 43.3), gallic acid (Fig. 43.4), isomargolonone (Fig. 43.5), mahmoodin (Fig. 43.6), margolone (Fig. 43.7), margolonone (Fig. 43.8), nimbin (Fig. 43.9), nimbolide (Fig. 43.10) and sodium nimbidate are the phytoconstituents responsible for antifungal, antiviral, antibacterial and antiseptic properties of the plant [1]. Hair care and treatment of conjunctivitis, psoriasis, pruritis, urticaria, chicken pox and measles are the major benefits of neem plant [2].

Fig. 43.4 Gallic acid

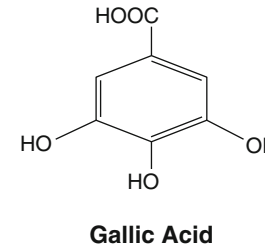


Fig. 43.5 Isomargolonone

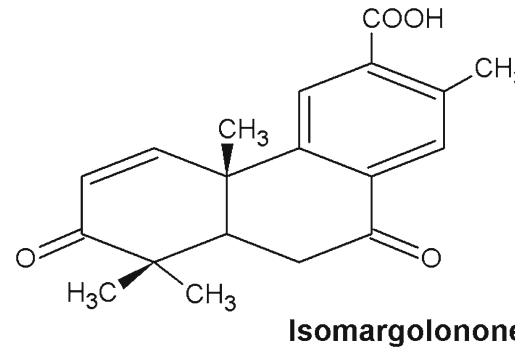


Fig. 43.6 Mahmoodin

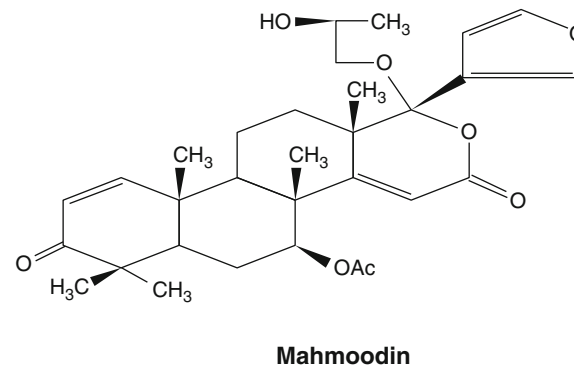


Fig. 43.7 Margolone

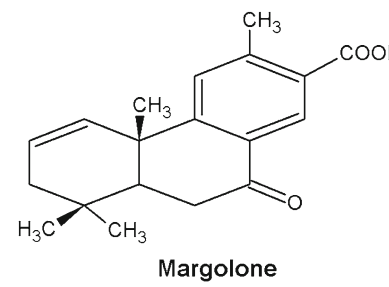
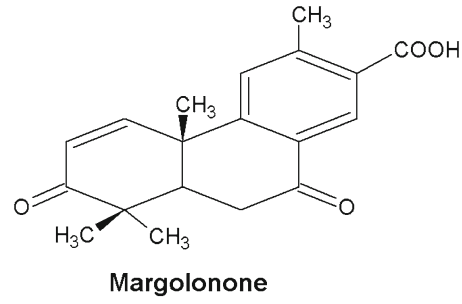
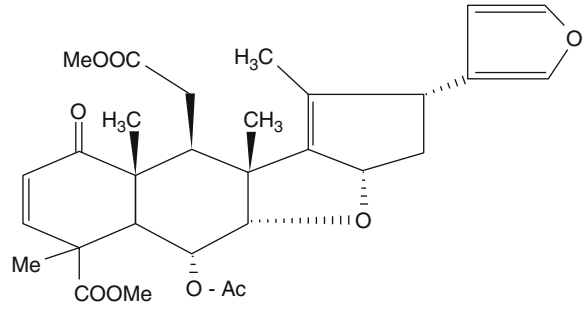


Fig. 43.8 Margolonone



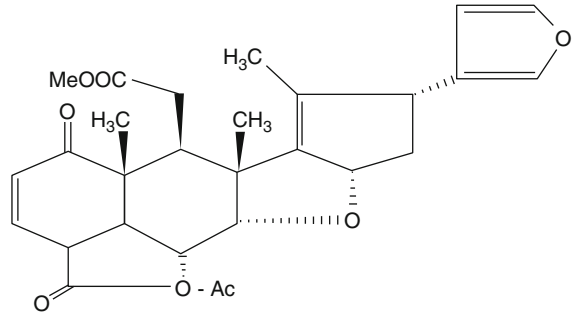
Margolonone

Fig. 43.9 Nimbin



Nimbin

Fig. 43.10 Nimbolide



Nimbolide

Scientific Classification of *A. indica* A. Juss

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Mangnoliophyta
Class: Mangnoliopsida
Subclass: Rosidae
Order: Sapindales
Family: Meliaceae

Genus: *Azadirachta*
Species: *Azadirachta indica* A. Juss.
Source: [1]

- Synonym: *Melia indica* (A. Juss.) Brand., *Melia parviflora* Moon
- Common name: Neem, margosa, Indian lilac [3]
- Vernacular names:
  - Sanskrit—Nimbah, prabhadrah
  - Hindi—Nim
  - Nepali and Urdu—Neem
  - Persian—Azad dirakt
  - Nigerian—Dongoyaro
  - Arabic—Margosa, neeb
  - Spanish—Paraiso
  - English—Indian liliac

Botanical Description

The genus Azadirachta (family Meliaceae) comprises two species: *A. indica* A. Juss syn. *Melia azadirachta* Linn. and *Azadirachta excelsa* (Jack) Jacobs syn. *Azadirachta integrifolia* Mers.

Plant. The tree is a hardy medium to large, mostly evergreen attaining 20 m height and 2.5 m girth. The branches are wide spreading and with glabrous twigs forming a round to oval crown. Neem is a fast-growing tree and can reach a height of 15–20 m, rarely to 35–40 m. It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are widespread. The fairly dense crown is roundish or oval and may reach the diameter of 15–20 m in old, free-standing specimens.

Bark. The bark is thick and woody. Externally it is dark-gray with numerous longitudinal furrows and transverse cracks. Internally the bark is reddish brown with few furrows. The bark is normally colonised by mites and insects (Fig. 43.11).

Fig. 43.11 Neem leaves

Leaves. Leaves are imparipinnately compound, alternate, exstipulate and 20–38 cm long. The leaves 20–31 medium to dark green leaflets about 3–8 cm (1–3 in.) long. The terminal leaflet is often missing. The petioles are short (Fig. 43.11).

Flowers. Flowers are white or pale yellow, small, bisexual, pentamerous and bracteate. Inflorescence is long, slender, axillary, or terminal panicle. Stamens ten; filaments unite to form a moniliform tube. Gynoecium is tricarpellary and syncarpous, ovary superior, trilocular. Each carpel bears two collateral ovules on parietal placentation.

Fruits. Fruit is one-seeded drupe with thin exocarp, bitter sweet pulpy mesocarp and woody endocarp. The ripe fruits are greenish yellow and are 1.4–2.8 × 1.0–1.5 cm in dimensions.

Seeds. Seed is ellipsoid, cotyledons thick, fleshy and oily. Neem has chromosome number $2n = 28$. Neem trees tend to become deciduous for a brief period in dry ecology. Ecotypes, exhibiting morphological variation in root growth, leaf size, contents, bole length, canopy, inflorescence, fruit bearing, seed size, shape and quality, exist in natural populations [3].

Chemical Compounds

Neem plant is chemically rich and has over 300 secondary compounds. Most of the active compounds are terpenoids, found in the fruit, seeds, twigs, stem and root bark. A tetranortriterpenoid azadirachtin (a liminoid) is the chief constituent in the seed kernels.

The seed oil is yellowish in color, malodorous and has an unpleasant taste due to the sulphur compounds. It chiefly comprises glycerides of oleic and stearic acids, at 50% and 20%, respectively. But, the azadirachtins are the most bioactive and popular commercially. In addition to the azadirachtins, other major liminoids are the salannins (Fig. 43.12). Three bitter compounds were extracted from neem oil, which were named nimbin, nimbinin and nimbidin [1]. Dry neem leaves contain nimbosterol (b-sitosterol), kaempferol and myricetin. Seed and oil contains desacetylnimbin, azadirachtin, nimbidol, meliantriol and tannic acid. Neem cake contains the highest sulphur content of 1.07% among all the oil cakes. Trunk bark contains nimbin 0.04%, nimbinin 0.001%, nimbidin 0.4%, nimbosterol 0.03%, essential oil 0.02%, tannins 6.0%, margosine and desacetylnimbin [3]. The antidermatophytic phytoconstituents are briefly summarised in Table 43.1.

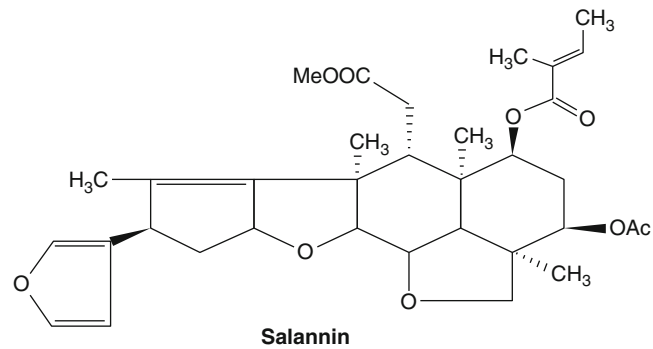


Fig. 43.12 Salannin

Table 43.1 Antidermatophytic neem compounds

Part	Major compound(s)	Medicinal activity
Leaf	Cyclic trisulphide (Fig. 43.13), Cyclic tetrasulphide (Fig. 43.14)	Antifungal
Seed	Azadirachtin, nimbin, nimbolide, gedunin, mahmoodin	Antifungal, antimalarial
Fruit	Nimbidin	Antifungal, antibacterial
Stem Bark	Gallic acid, margolone, margolonone, isomargolonone	Antibacterial
Roots	Azadirachtin	Antibacterial

Source: [1]

Pharmacological Activities

Antidermatophytic Activity of Neem: A. indica A. Juss

Traditional medicinal systems of India treat neem as "Panacea for all diseases." Products made from neem tree have been used in India for over two millennia for their medicinal properties. Neem has potent antifungal, antibacterial and antiviral property. It is considered a major component in ayurvedic and Unani medicine and is particularly prescribed for skin disease. Certain neem-based ayurvedic formulations prescribed for skin diseases are listed in Table 43.2.

Antifungal Activity

The DMSO extract of neem seeds and *n*-hexane, ethyl acetate and ethanol extract of the leaves were separately studied for their antifungal activity. The dermatophytic fungal cultures of *Trichophyton rubrum, Trichophyton mentagrophytes and Microsporum nanum* were used for the study. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were estimated by incorporating different concentrations of extracts in Sabouraud dextrose (SD) broth, and 20 µl of standard fungal inoculum was added to each tube and incubated at room temperature for 21 days. Suitable controls were also included. SD broth with 20 µl of inoculum served as positive control. SD broth alone served as negative control. The ethanol extracts of neem leaves showed MIC and MFC at 250 µg/ml concentrations for all the strains of *T. rubrum* and *M. nanum* tested. MIC and MFC recorded for stains of *T. mentagrophytes* was 125 µg/ml. The ethyl acetate extract of neem leaf showed MIC and MFC at 125 µg/ml for all the stains of *T. rubrum* and *T. mentagrophytes* and 250 µg/ml for *M. nanum*. Hexane extracts of neem leaf showed MIC and MFC at 500 µg/ml for all the strains of *T. rubrum*, *T. mentagrophytes* and *M. nanum*. The neem seed extract showed MIC and MFC at 31 µg/ml for all the dermatophytes tested.

The MIC and MFC of the neem seed extract were similar, which shows that MIC is sufficient for measuring fungicidal activity. Neem seed extract has high antidermatophytic properties. Neem seed extract at a concentration of 15 µg/ml (below MIC) was observed to distort the growth pattern of the *T. rubrum*, *T. mentagrophytes* and *M. nanum*. This finding supports the use of neem oil in the treatment of various skin infections by alternative systems of medicine [5].

Table 43.2 List of neem-based official ayurvedic formulations for skin disorders

Formulation	Part used	Indications	Ayurvedic formulary of India
Punarnavasava	Stem bark	Inflammation, dermaititis	[4]
Tiktaka Ghrta-A	Stem bark	Burning sensation, skin diseases, itching, cervical lymphadenitis, blisterous erupations, localised hyperpigmentation of skin, abscess	[4]
Tiktaka Ghrta-B	Stem bark	Burning sensation, skin diseases, itching, cervical lymphadenitis, localised hyperpigmentation of skin, abscess	[4]
Pancatikta Guggulu Ghrta	Stem bark	Leprosy, cervical lymphadenitis, abscess	[4]

Source: [4]

Antidandruff Property

Pityrosporum ovale is one of the fungi that cause dandruff. Ethanol extract of neem leaves was evaluated for the antifungal activity on *P. ovale*. Various concentrations of neem extract (25, 50, 75 and 100%) were used for the study. The inhibiting capacity of each level on the fungus was tested using agar cup method.

The results showed that 50% and above level of concentration had optimal level of inhibition on the dandruff growth. The higher the concentration, the higher was the inhibition on the growth of dandruff. The measurement of antifungal activity was done by calculating the zone of inhibition (diameter). The 100% extract of neem leaves produced the widest zone of inhibition (18 mm), which was found statistically highest than the other concentration levels. The sample treated with 75% neem extract showed the second widest zone of inhibition (11.33 mm) but was found at par with the 50% concentration, which produced about 9.33 mm zone of inhibition. The sample treated with the lowest concentration of 25% extract produced the smallest diameter of 6.67 mm. The study revealed that ethanol extract of neem leaves can inhibit the growth of *P. ovale* fungus, which is the main cause of dandruff [6].

Treatment of Scabies

A paste prepared by combination of neem leaves and *Curcuma longa* (turmeric) was used to treat scabies in 814 people. About 97% of them were cured within 3–15 days of application, and no adverse reactions were observed [7].

Antibacterial Activity

Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycobacterium tuberculosis* and streptomycin-resistant strains. A study on agar plates indicated that neem seed oil at concentration of 0.3 and 0.4% was active against *Staphylococcus aureus* and *Salmonella typhosa*, respectively. The seed oil was found

to be inactive against *Pseudomonas aeruginosa*, but was active against *E. coli* and *Proteus* species at a concentration of 3%, and active against *Klebsiella pneumoniae*, at a concentration of 6%.

In vitro, it inhibits *Vibrio cholerae*, *K. pneumoniae*, *M. tuberculosis* and *Streptococcus pyogenes*. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *Streptococcus faecalis*. NIM-76, a new vaginal contraceptive from neem oil, showed inhibitory effect on the growth of various pathogens, including bacteria, fungi and virus. Recently, the antibacterial activity of neem seed oil was assessed in vitro against 14 strains of pathogenic bacteria [1].

Antiviral Activity

Aqueous leaf extract offers antiviral activity against *vaccinia* virus, *chikungemya* and measles virus in vitro. The antiviral and virucidal effects of the methanolic extract of neem leaves (NCL-11) have recently been demonstrated against group-B coxsackie viruses. NCL-11 inhibits plaque formation in different antigenic types of coxsackie virus B at a concentration of 1 mg/ml at 96 h in vitro. Further studies indicated that NCL-11 is most effective in *coxsackie* virus B-4 as a virusidal agent, in addition to its interference at the early events of its replication [1].

Effect on Head Lice

A shampoo containing neem leaf extract (identified as Type AP30) has been proven to be highly effective against all stages of head lice for 66 children (4–15 years old) with significant head lice infestations, even after only 10 min of exposure time. The percentages of effectiveness ranged from 86 to 97% after a single application of the shampoo; only a second treatment was needed for most children to remain lice-free. No adverse effects were observed [8].

Mosquito Repellent Effect

Neem leaf paste is applied to the skin to treat acne, and in a similar vein is used for measles and chicken pox sufferers. Extract of neem leaves is thought to be helpful as malaria prophylaxis. Neem products are good mosquito repellents showing 90–100% protection against malaria vectors and about 70% against *Culex quinquefasciatus*. One controlled study evaluated the efficacy of a cream formulation containing 5% neem oil against *C. quinguefasciatus* and *Anopheles culicifacies*. Neem oil has been found to be an effective mosquito repellent. Neem oil cakes and karanja (*Pongamia glabra*) oil cake were used in combination against three mosquito species (*C. quinquefasciatus* (say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.)); the efficacy increased from four- to tenfold for the LC 50 and two- to sixfold for the LC 95 over individual applications.

About 4–5 g of the cream was applied to the exposed skin areas of human volunteers in Ghaziabad, India, in the summer months of May/June and the monsoon months of August/September. Neem cream was found to offer 82% protection against *Culex* bites and 100% protection against *Anopheles* bites, as compared to untreated controls [9].

Cosmetics

Neem oil is used for preparing cosmetics (toilet soaps, shampoos, sunscreen lotions, balms and creams), and is useful for skin care such as acne treatment, and maintaining the skin elasticity.

Safety and Toxicity

Neem does not have any negative health effects for humans because it is gentle. As with any bioactive compound, azadirachtin still needs to be treated with care because poisonings have been reported.

Neem seed oil can cause toxic encephalopathy, especially in infants and young children. The symptoms of poisoning are vomiting, drowsiness, tachypnea (abnormally fast breathing), recurrent generalised seizures (that may lead to coma or cardiopulmonary arrest), leucocytosis and metabolic acidosis. Treatment is primarily supportive and directed toward controlling convulsions. In addition, postmortem autopsies of children who died after ingesting margosa oil showed swelling of hepatocytes, fatty metamorphosis of liver, depletion of glycogen, mitochondrial pyknosis (cell degeneration), more peroxisomes and significantly more smooth endoplasmic reticulum (indicating increased detoxification activity). Neem oil is not approved by FDA for internal human use, but an estimated safe daily dose of azadirachtin is 15 mg/kg body weight [10] (Figs. 43.13 and 43.14).

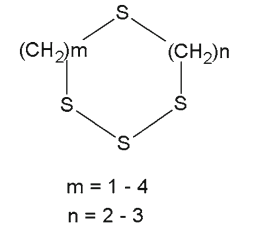


Fig. 43.13 Cyclic tetrasulphide

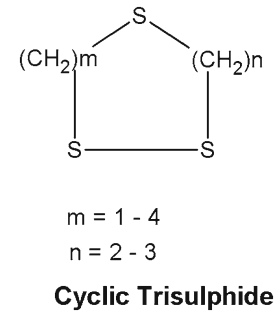


Fig. 43.14 Cyclic trisulphide

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Chapter 44
Pongamia pinnata (Linn.) Used in Skin Disease

Sachin L. Badole and Bhushan P. Pimple

Key Points

- *Pongamia pinnata* (Linn.) is a medium-sized glabrous tree. It is found throughout India.
- In Ayurveda, root and bark used in diseases of the eye, skin and vagina, itch, piles, splenomegaly, tumours, ulcers and wounds and the sprouts.
- The Karanja oil is known to have value in folk medicine for the treatment of rheumatism as well as human and animal skin diseases.
- Karanja oil is said to aid with skin aliments such as eczema, psoriasis and dandruff. *P. pinnata* plant possessed wound healing and anti-inflammatory activity.
- Pongamol and karanjin isolated from *P. pinnata* protective action throughout the broad ultraviolet region. It is enhanced and effectively contributes to the ultraviolet (UV) absorbing properties of the sunscreen.

Keywords *Pongamia pinnata* • Wound healing • Skin screen ointment • Anti-inflammatory

Introduction

Pongamia pinnata (Linn.) is a medium-sized glabrous tree, semi-evergreen and fast-growing tree which reaches 40 ft in height and spreads forming a broad, spreading canopy casting moderate shade. The plant comes up well in tropical areas with warm humid climate and well-distributed rainfall. It grows in distinguished types of soils; silty soils on river banks are most ideal [1].

It is an Indo-Malaysian species, found almost throughout India up to an altitude of 1,200 m and distributed further Eastwards, chiefly in the littoral regions of South-Eastern Asia, Sri Lanka, Burma, Malaya, Australia, Florida, Hawaii, Malaysia, Oceania, the Philippines, Polynesia and Seychelles. The plant is distributed throughout India from the central or eastern Himalaya to Kanyakumari. The tree is considered to be a native of Western Ghats and is chiefly found along the banks of streams and rivers or near sea coast in beach and tidal forests. It is well adapted to all soil types and climatic requirements and grows in dry places far in the interior, up to an elevation of 1,000 m. The tree is suitable for afforestation, especially in watershed areas and in drier parts of the country. Andhra Pradesh,

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Tamil Nadu and Karnataka provide the bulk of seeds of this tree. Large number of Karanja trees has been planted on roadside both on highways and in urban areas during the last two decades [1].

P. pinnata leaves are imparipinnate, alternate, shiny, young, pinkish red, mature leaves glossy and deep green. Flowers are lilac, white to pinkish, fragrant in paired along rachis in axillary, pendent, long racemes or panicles. Calyx cup shaped, truncate, short dentate, lowermost lobe sometimes longer; standard suborbicular broad, usually with two inflexed basal ears, thinly silky haired outside; wings oblique, long, somewhat adherent to the obtuse keel. Keel petals coherent at apex; stamens ten and monadelphous, vexillary stamen free at the base but joined with others into a close tube, ovary subsessile, stigma small, terminal. Pods are *c*ompressed, short, stalked, woody, indehiscent, yellowish grey when ripe varying in size and shape, elliptic to obliquely oblong, 4.0–7.5 cm long and 1.7–3.2 cm broad with a short curved beak. Seeds are usually one, rarely two, elliptical or reniform, 1.7–2.0 cm broad, wrinkled, with reddish brown leathery testa [2, 3].

Application of *P. pinnata* Against Skin Disease

In Ayurveda mentioned that, the root and bark used as alexipharmic, anthelmintic; useful in abdominal enlargement, ascites, biliousness, diseases of the eye, skin and vagina, itch, piles, splenomegaly, tumours, ulcers, wounds and the sprouts. The fruit and seed are used for keratitis, piles, urinary discharges and diseases of the brain, eye, head and skin, and the oil for biliousness, eye ailments, itch, leucoderma, rheumatism, skin diseases, worms and wounds. Unani system uses the ash to strengthen the teeth, the seed, carminative and depurative, for chest complaints, chronic fevers, earache, hydrocele and lumbago [4].

Karanja oil originated from India and is cold-pressed from seeds of the Pongam tree (*Pongamia glabra*). The oil is reddish-brown, rather viscous and non-edible. Karanja oil has a milder aroma than neem oil. Karanja oil's aroma blends well in products like soaps, hair oils and shampoos. The Karanja oil is known to have value in folk medicine for the treatment of rheumatism as well as human and animal skin diseases. It is effective in enhancing the pigmentation of skin affected by leucoderma or scabies. Traditionally the leaves of the plant were used externally in skin diseases and healing of wounds. The oil is extracted particularly from its seeds and it is not edible. When skin care required special treatment, these oils have a wonderful solution for the skin infections and ailments. Karanja oil has high content of omega-9-fatty acids in particular that is easily absorbed by the derma layer making the skin healthy and bright. Karanja oil is widely used in the manufacturing of beauty products, especially in facial lotions. Karanja has the capability of treating psoriasis, rheumatism, acne, scabies and skin ulcers. Karanja oil also plays a major role in the skin care treatment of pets. Mixing an ounce of Karanja oil with neem oil with the skin shampoo or lotion for pets can be effective in eradicating the ticks and mites holding over the skin of pets. Karanja oil—a "cousin" to neem oil—is used in hair care and skin products to promote healthy hair growth and address scalp issues. Karanja oil is prized for its insecticidal and antiseptic properties and has a "nutty" aroma. It is said to aid with skin aliments such as eczema, psoriasis and dandruff.

Anti-inflammatory effects of *P. pinnata* were observed against bradykinin- and PGE1-induced inflammation. Moreover, minimal effects were observed against histamine- and 5-HT-induced inflammation. The predominant action of extracts of *P. pinnata* appears to be a modulation of eicosanoid-events in inflammation [5].

The anti-inflammatory activity of 70 % ethanolic extract of *P. pinnata* leaves (PLE) in acute, subacute and chronic models of inflammation was assessed in rats. Administration of PLE (300, 1,000 mg/kg) exhibited significant anti-inflammatory activity in acute (carrageenin, histamine, 5-hydroxytryptamine and prostaglandin E2-induced hind paw oedema), subacute (kaolin-carrageenin and formaldehyde-induced hind paw oedema) and chronic (cotton pellet granuloma) models of inflammation. Both acute

as well as chronic administration of PLE (100, 300, and 1,000 mg/kg, p.o.) did not produce any gastric lesion in rats [6].

Oral administration of aqueous extract of *P. pinnata* stem bark (PPSB) (400, 800 mg/kg) exhibited significant anti-inflammatory activity in acute (carrageenin-induced hind paw oedema) and chronic (cotton pellet granuloma) models of inflammation [7].

Ointment prepared from methanolic leaf extract of *P. pinnata* has shown significant wound healing activity. On 16th day complete healing of wound was observed compared with standard marketed ointment. The studies on incision wound healing model revealed that the test group showed high breaking strength in wound area from 1st day to 10th day. Ointment prepared from methanolic leaf extract has shown significant wound healing activity. The rate of breaking strength is more compared to standard. On 10th day complete healing of wound was observed with standard marketed ointment, and ointment of methanolic leaf extract produced 485.17 g healing of wound as compared to control [8].

Pongamol and karanjin isolated from *P. pinnata* were found to be extremely good absorbents of the UV rays in the UVA and B regions. The seed of *P. pinnata* contains such photoabsorptive compounds which when put together in a single herbal formulation like ointments, lotions or creams can give rise to an extremely effective sunscreen preparation showing its protective action throughout the broad ultraviolet region. The plant oil extracts can be used along with other established standard drugs, as it enhance and effectively contribute to the UV absorbing properties of the sunscreen [6, 9].

Summary Points

- *P. pinnata* (Linn.) is a medium-sized glabrous tree found almost throughout India.
- The plant is used in various skin ailments.
- The Karanja oil is used in folk medicine for the treatment of rheumatism as well as human and animal skin diseases.
- *P. pinnata* produced wound healing and anti-inflammatory activity.
- Pongamol and karanjin isolated from *P. pinnata* showed photoabsorptive compounds.

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Chapter 45
Aloe vera: Use for Skin Disease

Sachin L. Badole, Pranita P. Bagul, and Farid Menaa

Key Points

- *Aloe vera* has been one of the most important plants used in folk medicine.
- It is traditionally used for wound healing, to relieve itching and swelling, as well as for its anti-inflammatory properties.
- *A. vera* is used in a variety of skin ailments such as mild cuts, insect stings, bruises, poison ivy, and eczema.
- *A. vera* possesses incredible moisturizing properties.

Keywords *Aloe vera* • Skin disease • Aloenin

Introduction

In nature, it may be damaged physically by ultraviolet (UV) irradiation or by insects.

Aloe vera (Fig. 45.1) has been one of the most important plants used in folk medicine. The Egyptians called aloe as the "*plant of immortality*" and included it among the funerary gifts buried with the *pharaohs*. It is indigenous to hot countries and has been used medicinally for over 5,000 years by Egyptian, Indian, Chinese, and European cultures [1]. *A. vera* (*Aloe barbadensis* Miller) is a perennial succulent belonging to the Liliaceae family. It is a cactus-like plant that grows in hot, dry climates. It is cultivated throughout India, wild on coasts of Maharashtra, Gujarat, and South India.

Botanical Description

Synonym: *A. barbadensis* Mill., *A. vera* Tourn. ex Linn., *Aleo indica* Royle, *Aleo littoralis* Koening.
Family: Liliaceae; Agavaceae.
English: Curacao aloe, Barbados aloe, Indian aloe, Jaffarabad aloe.

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Fig. 45.1 *Aloe vera* plant

Sanskrit: Ghrit kumari.
Ayurvedic: Kanyaasaara, eleyaka (dried juice of the leaves). kumaari, kumaarikaa, kanyaa, grihkanyaa, ghritkumaarika (plant).
Unani: Gheekwaar, sibr.
Siddha/Tamil: Sotru kattraazhai, kumaari. moosaambaram (dried juice).
Folk: Elwaa, musabbar (dried juice of leaves) [2].

A. vera is a stemless or very short-stemmed plant growing to 80–100 cm tall, spreading by offsets and root sprouts. The leaves are lanceolate, thick and fleshy, green to grey-green with a serrated margin. The flowers are produced on a spike up to 90 cm tall, each flower pendulous, with a yellow tubular corolla 2–3 cm long. The tissue in the center of the aloe leaf contains a gel which yields aloe gel or *A. vera* gel. *A. vera* contains acids, amino acids, enzymes, lectin, lipids, minerals, lactates and salicylates, phenolics, polysaccharides, urea, and vitamins [2].

Application in Skin Disease

A. vera is traditionally used for wound healing, to relieve itching and swelling, as well as for its anti-inflammatory and antibacterial properties. The carboxypeptidase and salicylate components of aloe gel can inhibit bradykinin, a pain-producing agent; C-glycosyl chromone appears to reduce topical inflammation. Aloe gel decreases or inhibits the synthesis of thromboxane, which may accelerate the healing of burns [2].

Aloenin is a major constituent of *Aloe arborescens* Miller which has been utilized in Japan as a folk remedy for burns, insect bites, and skin reaction. In the present study, the effects of aloenin on sebaceous gland size, hair growth, and damaged skin were investigated. Aloenin significantly promoted hair growth in depilated mice but did not affect sebaceous gland function in the hamster ear. Aloenin also had recuperative effects on tape-stripped human skin as determined from parameters such as the shape factor of corneocytes, thick abrasion, nuclear ghosts, and cellular arrangement of corneocytes. Since aloenin is effective in healing damaged skin, it may be useful for the treatment of dermatological conditions in the future [3].

Tolerability of topical *A. vera* extract 0.5% in a hydrophilic cream to cure patients with psoriasis vulgaris. Sixty patients (36 M/24 F) aged 18–50 years (mean 25.6) with slight to moderate chronic

plaque-type psoriasis and Psoriasis Area and Severity Index (PASI) scores between 4.8 and 16.7 (mean 9.3) were enrolled and randomized to two parallel groups. The mean duration of the disease prior to enrollment was 8.5 years (range 1–21). Patients were provided with a precoded 100 g tube, placebo or active (with 0.5% *A. vera* extract), and they self-administered trial medication topically (without occlusion) at home three times daily for 5 consecutive days per week (maximum 4 weeks active treatment). Patients were examined on a weekly basis and those showing a progressive reduction of lesions, desquamation followed by decreased erythema, infiltration, and lowered PASI score were considered healed. The study was scheduled for 16 weeks with 12 months of follow-up on a monthly basis. The treatment was well tolerated by all the patients, with no adverse drug-related symptoms and no dropouts. By the end of the study, the *A. vera* extract cream had cured 25/30 patients (83.3%) compared to the placebo cure rate of 2/30 (6.6%) ($P<0.001$) resulting in significant clearing of the psoriatic plaques (328/396 (82.8%) vs. placebo 28/366 (7.7%), $P<0.001$) and a decreased PASI score to a mean of 2.2. Topically applied *A. vera* extract 0.5% in a hydrophilic cream is more effective than placebo and has not shown toxic or any other objective side effects. Therefore, the regimen can be considered a safe and alternative treatment to cure patients suffering from psoriasis [4].

Anti-inflammation is the first step in the wound healing and this effect of two aloe preparations is believed to play a direct role in facilitating the fast healing. Topical administration of the whole leaf juice preparations, either *A. arborescens* Miller or *Aloe ferox* Miller, inhibits the growth of all bacterial strains tested and are fungitoxic to *Cryptococcus neoformans* only. The obtained inhibition zone might be attributed to the greater susceptibility of *C. neoformans* toward two whole-leaf juice preparations than to the control. *A. ferox* Miller might possibly be more potent in inhibiting *C. neoformans* growth than did *A. arborescens* Miller. Pande et al., 1998, reported that leaf extract of *A. vera* (50 and 100 mg/kg) showed radiomodifying effects on the testes of Swiss albino mice. This extract was nontoxic when injected up to 800 mg/kg and significantly enhanced survival time of the irradiated [5]. Kodym and Bujak revealed that aloin caused the development of stimulating contact dermatitis in skin-allergic patient who received topical application of *A. arborescens* Miller [6].

The moisturizing effects of cosmetic formulations containing different concentrations of lyophilized *A. vera* gel were studied, which showed that only formulations with higher concentrations (0.25% w/w and 0.5% w/w) increased the water content of the stratum corneum after a single application. When the formulations were applied twice daily for a period of 2 weeks, all the formulations (containing concentrations of 0.1% w/w, 0.25% w/w, and 0.5% w/w of *A. vera* gel powder) had the same effect. The transepidermal water loss was not changed by inclusion of the *A. vera* gel in the formulations compared to the vehicle used in the formulations. It was proposed that the *A. vera* gel-containing products improved skin hydration possibly by means of a humectants mechanism [7].

The dermal toxicity was one of the issues associated with the topical application of the whole-leaf juice for wound healing. By applying 2–3 ml of the prepared whole-leaf juice to the intact or damaged skin, no signs of irritant contact were observed. *A. ferox* Miller leaf extract (0.5 ml) was applied to the shaved skin of white New Zealand rabbits. Their results showed that a slight erythema was developed in the damaged tissue in one of the six rabbits and such symptom diminished after 3 days [8]. A case of the side effect associated with *A. ferox* leaf extract was reported, when it was instilled into the eye of white New Zealand rabbits, for the induction of minor changes in the eye. Such changes in the eyes became visible after instillation for 1 h and vanished after 1 day [8].

The anti-inflammatory activity of mannose 6-phosphate is believed to resemble the effects observed for acetylated mannan in aloe gel. Aloe gel reduces inflammation that is induced by agents via promotion of prostaglandin synthesis as well as increased infiltration of leucocytes, but is less effective against inflammation caused by agents that produce allergic reactions. Wound healing is a response to injured tissue that results in the restoration of tissue integrity. It was shown that aloe gel could improve wound healing after topical and systemic administration in several studies, while others claimed no effect or even a delay in wound healing. Conflicting results may be explained by stability of the active ingredients as it was shown that the time of treatment after harvesting was an important factor that

determined activity. Several mechanisms have been proposed for the wound healing effects of aloe gel, which include keeping the wound moist, increase epithelial cell migration, more rapid maturation of collagen, and reduction in inflammation. A 5.5 kDa glycoprotein that was isolated from *A. vera* showed an increase in cell migration and accelerated wound healing in a human keratinocyte monolayer [9].

A. vera leaves are succulent, broad at the base, and pointed at the tips, with spines along the edges. These fat leaves contain the clear healing gel that is 96% water. The healing effect of aloe results from its ability to prevent injury to epithelial tissues and promote healing of injured tissues. *A. vera* is used in a variety of skin ailments such as mild cuts, insect stings, bruises, poison ivy, and eczema. It also has antibacterial and antifungal qualities and increases blood flow to wounded areas. It stimulates fibroblasts, the skin cells responsible for wound healing and the manufacture of collagen, the protein that controls the aging process of the skin and wrinkling. The skin absorbs *A. vera* up to four times faster than water; it appears to help the pores of the skin open and receive the moisture and nutrients of the plant. Due to its soothing and cooling qualities, Maharishi Ayurveda recommended *A. vera* for a number of skin conditions. The leaf gel is applied several times a day for light burns and wounds; for mild sun burn apply the paste on affected areas and wash it off after 15 min. In addition to the skin, other epitheliums in our body include the lining of the gut, the bronchial tubes, and the genital tract, which also benefit from the healing effect of *A. vera*.

Three different solvents such as aqueous, ethanol, and acetone were used to extract the bioactive compounds from the leaves of *A. vera* to screen the antimicrobial activity selected human clinical pathogens by agar diffusion method. The maximum antibacterial activities were observed in acetone extracts, then aqueous extracts, and ethanol extract. Antifungal activity of *A. vera* was analyzed against *Aspergillus flavus* and *Aspergillus niger*. The maximum antifungal activity was observed in acetone extracts compared to the other extracts. *A. vera* plant extract with acetone can be used as antimicrobial agents [10].

A. vera has become so popular among consumers as it possesses incredible moisturizing properties. *A. vera* improves the skin's ability to hydrate itself, aids in the removal of dead skin cells, and has an effective penetrating ability that helps transport healthy substances through the skin. *A. vera* is an ideal ingredient in cosmetic and dermatological products. *A. vera* is best known for its soothing and healing effects on burns and other wounds. *A. vera* when applied to a wound increases both threat of wound closure and the tensile strength of the wound via the proliferation of cells including skin, liver, nerve, and blood cells. *A. vera* has been found to reverse degenerative skin changes by stimulating collagen and elastin synthesis, in essence turning back the clock on the effects aging has on skin. *A. vera* prevents suppression of the skin's immune system. Topical application of the *A. vera* can be made up to 24 h after exposure to ultraviolet light without reducing the degree of prevention regarding immune system suppression. *A. vera* is believed to reduce severe joint and muscle pain associated with arthritis as well as pain related to tendinitis and injuries. When applied directly to the area of pain, *A. vera* penetrates the skin to soothe the pain. *A. vera* promotes a variety of anti-inflammatory responses in the body, reducing swelling from injuries and promoting recovery from infections. Such anti-inflammatory responses not only aid in the relief of pain and discomfort but also enhance the overall wound process [11]. Application of a nonconventional *A. vera* and collagen-based dressing on an ischemic lesion in a patient with systemic arterial pressure and diabetes mellitus showed wound healing effect [12].

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Chapter 46
Tinospora cordifolia (Willd.) Miers. (Menispermaceae): Beneficial Effect on Skin Diseases

Sachin L. Badole, Swapnil M. Chaudhari, and Anand A. Zanwar

Key Points

- *Tinospora cordifolia (Willd.)* has been one of the most important plants used in folk medicine.
- *T. cordifolia* is used in various skin diseases and other ailments.
- *Gudduchi ghritha* and *gudduchi taila* have been used since time memorial in treatment of psoriasis.
- *T. cordifolia* is used in treatment of ringworm infections, skin neoplasms, leprosy, acne, chickenpox, and scabies.

Keywords *Tinospora cordifolia* • Skin diseases • Rasayna

Introduction

Tinospora cordifolia (Willd.) is well known as *guduchi* or heartleaf moonseed and belongs to family Menispermaceae. Its stem and roots are normally used for their medicinal properties. The plant grows well in tropical areas with warm humid climate and well-distributed rainfall. It grows in distinguished types of soils; silty soils on river banks are most ideal. *Guduchi* is an Indian medicinal plant and has been used in ayurvedic preparations for the treatment of various ailments throughout the centuries. Ancient Hindu physicians prescribed it for gonorrhea. *T. cordifolia* and similar species, i.e., *Tinospora crispa* and *Tinospora rumphii Boerl*, are used in ayurvedic herbal medicine as a hepatoprotective and as an immunostimulant [1].

Botanical Description

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida

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Order: Ranunculales
Family: Menispermaceae
Genus: *Tinospora*
Species: *Tinospora Cordifolia*
Synonym: *Cocculus cordifolius* Dec; *Menispermum cordifolium* Willd.; *Tinospora glabra* (N. Brum.) Merr.

Vernacular Names

English: Heartleaf moonseed
Sanskrit: *Guduchi, amrita, madhuparni*
Ayurvedic: *Guduuchi, guduuchikaa, guluuchi, amrita, amritaa, amritalataa, amritavalli*, *chinnaruuhaa, chinnodbhavaa, madhuparni, vatsaadani, tantrikaa, kundalin, guduuchi sattva* (starch)
Hindi: *Giloya, gurcha*
Arabic: *Gilo*
Unani: *Gilo, gulanchaa, sat-e-gilo* (starch)
Siddha: *Seenil, amrida-valli*
Folk: *Giloya* [2]

T. cordifolia is a glabrous, succulent, climbing shrub native to India. It is also found in Burma, China, and Sri Lanka. It thrives easily in the tropical region, often attains a great height, and climbs up the trunks of large neem trees. The stem of *T. Cordifolia* is rather succulent with long filiform fleshy aerial roots from the branches. The bark is gray with spiral and longitudinal deep clefts and the space between is spotted with large rosette-like lenticels. The wood is white, soft, and porous, and the freshly cut surface quickly assumes a yellow tint when exposed to air. The branches bear smooth heart-shaped leaves, unisexual greenish flowers in summer, and red berries in winter. The flowers are small and yellow or greenish yellow. In auxiliary and terminal racemes or racemose panicles, the male flowers are clustered and female are usually solitary. The drupes are ovoid, glossy, succulent, red, and pea sized. The seeds are curved. Fruits are fleshy and single seeded. Long threadlike aerial roots come up from the branches. The viscous sap is light yellow and has an odor and a nauseating bitter taste [1, 3].

Cultivation

It grows well in almost any type of soils under varying climatic conditions. The plant is cultivated by stem cutting in the months of May–June. It requires some support, preferably neem and mango trees. Periodical hoeing is done, both in the nursery and field as per requirement. The medicinal plants have to be grown without chemical fertilizers and use of pesticides. Organic manures like farm yard manure, vermi-compost, and green manure are used as per requirement of the species. To prevent diseases, biopesticides are used. The field after plantation should be irrigated periodically as and when required at weekly or fortnightly intervals. Mature plants are collected, cut into small pieces, and dried in shade. The yield obtained is approximately 8–10 quintal/ha [4].

Chemistry

A large number of compounds have been isolated from the aerial parts, stem, and roots of *T. Cordifolia*. In the early 1900s, giloin, gilenin, and gilosterol, as well as the bitter principles columbin, chasmanthin, and palmarin, were identified in the plant. A wide variety of sesquiterpenes and diterpenes have

been isolated from the stems of the plant. The major isolated compounds include the norditerpene furan glycosides cordiofolisides A, B, and C; the daucane-type sesquiterpenes tinocordifolin and tinocordifolioside; the furanoid diterpene glucosides palmatosides C and F, and amritosides; the clerodane diterpenoids cordioside, tinosponone, and tinocordioside; tinosporaside, a novel 18-norclerodane diterpene glucoside; and tinocordiside, a cadinane sesquiterpene glycoside. In addition, syringin, cordiol, cordioside, and phenylpropene disaccharides cordifoliosides A and B were identified as the active principles with anticomplement and immunomodulatory activities. The stems of the plant contain the alkaloid berberine. Cultures of the stem callus have the capability of synthesizing this compound. Ecdysterone, makisterone A, and 20 beta-hydroxyecdysone are phytoecdysones isolated from the aerial parts of the plant. Other constituents reported from *T. Cordifolia* include a phenolic lignan, octacosanol, nonacosan-15-one, heptacosanol, beta-sitosterol, tinosporidine, cordifol, cordifolone, magnoflorine, tembetarine, syringine and syringine apiosylglycoside, and a glucan polysaccharide. The roots of *T. Cordifolia* contain isocolumbin, palmatine, tetrahydropalmatine, magnoflorine, and jatrorrhizine [5].

Traditional Uses

According to *Nighantu*, *T. cordifolia* commonly known as *guduchi* is supposed to *amrita* (which means to rejuvenate the dead cells). The term refers to heavenly elixir, which was reputed to protect the celestial people from senescence and keep them eternally young. In Hindi, the plant is commonly known as *giloya*, which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. The plant is used in ayurvedic "*rasayanas*" to improve the immune system and the body resistance against infections [6]. *T. cordifolia* has been used to treat general weakness, fever, dyspepsia, dysentery, gonorrhea, secondary syphilis, urinary diseases, impotency, gout, viral hepatitis, skin diseases, and anemia. In compound formulations, *guduchi* is used clinically to treat jaundice, rheumatoid arthritis, and diabetes. The root is considered to be a strong emetic and is used for bowel obstruction [1].

Stem	Lung cancer, antimicrobial, jaundice, anti-inflammatory, antidiabetic, antituberculosis, antinociceptive, immunostimulant, antimalarial, typhoid, chronic sinusitis, antifungal, antiulcer, anticancer, fever, flatulence, hypertension, leucorrhoea, and diarrhea
Leaf	Antioxidant
Aerial root	Hepatoprotective, anemia, blood purifier, brain development, antiepileptic, anti-HIV, gynecological disorders, and spleen disorders
Entire plant	Antivenom, cardiotonic
Bark	Antispasmodic, antipyretic, anti-allergic, anti-inflammatory, and anti-leprotic properties [6, 7]

Application of *T. cordifolia* in Skin Diseases

T. cordifolia (*guduchi*) is one of the most highly valued and common herbs in ayurvedic medicine. It has a rich history in the Indian subcontinent where it has been used and written about for thousands of years. It is considered one of the best *rasayanas* (adaptogens) and is unusual in its potent versatility. In recent years, significant progress has been attained regarding its biological activity and medicinal applications. *Guduchi*, as it is most commonly called, has been described as "one which protects the body." The Sanskrit and Hindi name *amrita* is derived from ancient Hindu scriptures where *amrita* was used to bring the dead back to life and keep gods from growing ill and old. It is no wonder that it is also referred to as "nectar of immortality" and "heavenly elixir." Hence it finds an important place in treatment of various skin disorders, which are as follows:

Psoriasis

Psoriasis is a fairly common skin disease which is regarded as immunologically based disease which combines dermal inflammation with secondary epidermal hyperplasia. It is characterized by thick, silvery white scales surrounded by a red, inflamed border. *Gudduchi ghritha* and *gudduchi taila* have been used since time memorial in treatment of psoriasis. The method of preparation of *gudduchi ghritha* and *gudduchi taila* includes preparing hot infusion from stem pieces of *T. cordifolia* which is given for the treatment of psoriasis. In addition stem powder is applied over affected areas in morning and evening for 6–7 days [8].

Scabies

Scabies is a contagious skin infection that occurs among humans and other animals. It is caused by a tiny and usually not directly visible parasite, the mite *Sarcoptes scabiei*, which burrows under the host's skin, causing intense allergic itching. The infection in animals (caused by different but related mite species) is called sarcoptic mange. The disease may be transmitted from objects but is most often transmitted by direct skin-to-skin contact, with a higher risk with prolonged contact. Initial infections require 4–6 weeks to become symptomatic. Reinfection, however, may manifest symptoms within as little as 24 h. Because the symptoms are allergic, their delay in onset is often mirrored by a significant delay in relief after the parasites have been eradicated. Crusted scabies, formerly known as Norwegian scabies, is a more severe form of the infection often associated with immunosuppression. Paste prepared from *T. cordifolia* stem ash is applied over affected areas in scabies. Decoction of stem pieces of *T. cordifolia* is given to the patient suffering from scabies. Also stem powder is applied over affected areas in morning and evening for 6–7 days [8].

Ringworm

Ringworm or dermatophytosis is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, sheep, and cattle. The term "ringworm" is a misnomer, since the condition is caused by fungi of several different species and not by parasitic worms. The fungi that cause parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive on skin that is warm and moist, but may also survive directly on the outsides of hair shafts or in their interiors. In pets, the fungus responsible for the disease survives in skin and on the outer surface of hairs. The stem of *T. cordifolia* is crushed and the juice is applied externally to ringworm. A very potent activity is seen in treatment of ringworm infections [9].

Chicken Pox

Chicken pox is a highly contagious illness caused by primary infection with varicella zoster virus (VZV). It usually starts with vesicular skin rash mainly on the body and head rather than at the periphery and becomes itchy, raw pockmarks, which mostly heal without scarring. A person with chicken pox is infectious 1–2 days before the rash appears. The contagious period continues for 4–5 days after the appearance of the rash, or until all lesions have crusted over. Immunocompromised patients are probably contagious during the entire period and new lesions keep appearing. Crusted lesions are not contagious. It takes from 10 to 21 days after contact with an infected person for someone to develop

chicken pox. This disease is mainly characterized by immunosuppression of the body, but *T. cordifolia* is an immunostimulant property. So the stem extract of this plant helps in reducing the disease progression synergistically with other drugs. The dosing of the extract depends on the severity, age, and immunity of the diseased. *T. cordifolia* serves as a secondary line of treatment in chicken pox eradication [10].

Leprosy

Leprosy or Hansen's disease is a chronic disease caused by the bacteria *Mycobacterium leprae* and *Mycobacterium lepromatosis*. Named after the physician Gerhard Armauer Hansen, leprosy is primarily a granulomatous disease of the peripheral nerves and mucosa of the upper respiratory tract; skin lesions are the primary external sign. Left untreated, leprosy can be progressive, causing permanent damage to the skin, nerves, limbs, and eyes. In ayurveda whole plant of *T. cordifolia* has been used to treat the infectious leprosy disease. Every part of the plant *T. cordifolia* proves effective in treating the underlying causes of leprosy. *T. cordifolia*, though cannot cure the actual cause of the infection, proves beneficial in avoiding the secondary infections following the invasion of *M. leprae* [11].

Acne

Acne vulgaris (or cystic acne) is a common human skin disease, characterized by areas of skin with seborrhea, comedones papules (pinheads), pustules (pimples), nodules, and possibly scarring. Acne affects mostly skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back. Severe acne is inflammatory, but acne can also manifest in noninflammatory forms. The lesions are caused by changes in pilosebaceous units, skin structures consisting of a hair follicle and its associated sebaceous gland, and changes that require androgen stimulation. *Propionibacterium acnes* and *Staphylococcus epidermidis* are common pus-forming microbes responsible for the development of various forms of acne vulgaris [12].

T. cordifolia in treatment of acne vulgaris with new polyherbal formulations, where *T. cordifolia* is an active constituent. One hundred and five patients with active lesions of acne vulgaris were included in the open clinical trial. The grading of acne vulgaris was as follows: Grade I: Mild acne with only papules; Grade II: moderate acne with papules and comedones; Grade III: severe acne with papules and pustules; Grade IV: very severe acne with papules, pustules, and cysts. All the patients were administered Purim tablets (*Azadirachta indica*, *T. cordifolia*, *Embelia ribes*, *Eclipta alba*, *Andrographis paniculata*, *Curcuma longa*, *Cassia fistula*, and *Triphala*), at a dose of two tablets twice daily for 4 weeks. Simultaneously, they were instructed to apply Clarina cream twice daily on the affected area of acne lesion twice daily for 4 weeks. The response to treatment was excellent in Grades I and II after 4 weeks of treatment. In Grade III acne with large papules and pustules, the response was also significantly good, in healing the papules and pustules. The Grade IV acne required other adjuvant treatment. There were no local or systemic side effects seen in all these patients. Thus, Clarina cream along with Purim tablets was useful in treating patients with various degrees of acne [13].

Skin Carcinogenesis

Skin neoplasms (also known as "skin cancer") are skin growths with differing causes and varying degrees of malignancy. The three most common malignant skin cancers are basal cell cancer, squamous

cell cancer, and melanoma, each of which is named after the type of skin cell from which it arises. Skin cancer generally develops in the epidermis (the outermost layer of skin), so a tumor can usually be seen. This means that it is often possible to detect skin cancers at an early stage.

T. cordifolia (guduchi) was used to explore antitumor promoting activity in a two-stage skin carcinogenesis model. For this purpose, mice were treated by single application of 7,12-dimethylbenz(a) anthracene (DMBA) (100 μg/100 μl of acetone) and 2 weeks later promoted by croton oil (1% in acetone three times a week) until the end of the experiment (i.e., 16 weeks). Oral administration of *T. cordifolia* extract at the preinitiational stage (i.e., 7 days before and 7 days after DMBA application; group IV), promotional stage (i.e., from the time of croton oil application; group V), and both pre- and postinitiational stage (i.e., from the time of DMBA application and continued until the end of the experiment; group VI; on the shaven backs of the mice at the dose of 100 mg/kg body weight/day for 16 weeks) recorded significant reduction in tumor weight and tumor incidence in comparison to control (i.e., mice treated with DMBA and croton oil; group III). Furthermore, cumulative number of papillomas, tumor yield, tumor burden, and tumor weight showed significant reduction along with significant elevation of phase II detoxifying enzymes, and inhibition of lipid peroxidation in liver and skin in the animals administered with *T. cordifolia* extract concomitant to carcinogen exposure. *T. cordifolia* extract has antitumor potential in a two-stage skin carcinogenesis mouse model [14].

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Chapter 47
Withania somnifera: Use for Skin Disease

Anand A. Zanwar, Sachin L. Badole, and Rashmi Saini

Key Points

- *Withania somnifera* is a herbal drug from the Indian system of medicine and is officially mentioned in Indian Pharmacopoeia.
- The major pharmacological activity of the plant is attributed to the presence of several alkaloids and withaniols.
- *W. somnifera* is found to be effective in prevention of skin cancer.
- *W. somnifera* is nontoxic in subacute and chronic toxicity studies.

Keywords Ashwagandha • Carcinogenesis • *Withania somnifera* • Skin cancer

Introduction

Withania somnifera (*W. somnifera*) is popularly known as Ashwagandha or Winter Cherry. It is a green shrub. It is a herbal drug from the Indian system of medicine, the ancient Hindu system of medicine, and has been in use for more than 2,500 years. It consists of the dried roots of *W. somnifera*. Leaves and stem bases are also included in such preparations. It is commonly referred to as Indian ginseng [1]. *W. somnifera* is believed to have oriental origin and found throughout the drier parts of India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa, Egypt, Morocco and Jordan [2]. It is found in Mandsaur and Bastar in Madhya Pradesh, the foothills of Punjab, Himachal Pradesh, Uttar Pradesh and western Himalayas in India. The areas receiving 600–750 mm rainfall are best suited to this crop. It is also found wild in the Mediterranean region in North America. In India it is cultivated in Madhya Pradesh, Rajasthan and other drier parts of the country [3].

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W. somnifera is an official drug and is mentioned in the Indian Pharmacopoeia. The root is regarded as a tonic, aphrodisiac and is used in consumption, emaciation, debility, dyspepsia and rheumatism. The plant is used in treating syphilis and a decoction of the root bark is administered for asthma. Of all parts of this plant, the root has been considered to be the most active for therapeutic purposes [4].

Scientific Classification

Kingdom: Plantae
Order: Solanales
Family: Solanceae/nightshade
Genus: *Withania*
Species: *W. somnifera*
Binomial name: *Withania somnifera*
Synonym: *Physalis somnifera*

Common Name

Sanskrit: Aswagandha, varahakarni
Bengali: Ashvaganda
Hindi: Asgandh, punir
Malayalam: Amukkuram
Tamil: Amukkira
Telugu: Vajigandha
Marathi: Askandha, tilli
Punjab: Aksan
Gujarathi: Ghoda
Kannad: Viremaddinagaddi

Botanical Description

Plant: *W. somnifera* is a rigid grey under shrub of 60–120 cm high. *W. somnifera* is erect, evergreen, tomentose shrub, 30–75 cm in height. The cultivated plants have sizable differences from the wild forms in their morphological characters.

Leaves: Leaves are simple, ovate, glabrous and opposite.

Flower: Flowers are bisexual, inconspicuous, greenish or dull yellow in colour born on axillary umbellate cymes, comprising five sepals, petals and stamens each; the two celled ovary has a single style and a bilobed stigma. The petals are united and tubular. The stamens are attached to the corolla tube and bear erect anthers which form a close column or cone around the style. Pollen production is poor.

Fruit: Fruit is a small berry, globose, orange red when mature and is enclosed in persistent calyx.

Root: Roots are stout, fleshy, cylindrical, 1–2 cm in diameter and whitish brown in colour.

Seeds: Seeds are small, flat, yellow and reniform in shape and very light in weight. The chromosome number $2n = 48$ [3].

Traditional Uses of *W. somnifera*

It is considered as one of the best rejuvenating agents in Ayurveda. Its roots, leaves and seeds are used in Ayurvedic and Unani medicines to combat diseases ranging from tuberculosis to arthritis. It is well known and capable of imparting long life, youthful vigour and intellectual power. It is found to improve the physical strength and is commonly recommended in all cases of general debility. The major pharmacological activity of the plant is attributed to the presence of several alkaloids and withaniols. Roots are prescribed in medicines for hiccup, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammations and skin diseases. Its roots and paste of green leaves are used to relieve joint pains and inflammation. It is also an ingredient of medicaments prescribed for curing disability and sexual weakness in male. Leaves are used in eye diseases. Seeds are diuretic. It is a constituent of the herbal drug "*Lactare*" which is a galactagogue. Aswagandha was observed to increase cell-mediated immunity, prevent stress-induced changes in adrenal function and enhance protein synthesis. Milk fortified with it increases total proteins and body weight. *W. somnifera* powder (6–12 g) twice a day along with honey and ghee is advised for tuberculosis in Sushruta Samhita. It also provides sound sleep [3].

Different types of withanolides isolated from *W. somnifera* such as Withaferin A and 3-β-hydroxy-2,3 dihydro withanolide F show prominent antibacterial, antitumour, immunomodulating and anti-inflammatory properties [5].

Anti-inflammatory and lysosomal membrane stabilizing effect on adjuvant-induced arthritis of *W. somnifera* is proved. In case of immunomodulatory role of *W. somnifera* (root powder, in experimental induced inflammation), it has shown potent inhibitory activity towards the complement system, mitogen-induced lymphocyte proliferation and delayed-type hypersensitivity reaction. *W. somnifera* did not have a significant effect on humoral immune response in rats. Thus these results suggest the immunosuppressive effect of *W. somnifera* root powder [6].

Stimulation of haemopoetic system was observed when administration of *W. somnifera* was found to increase total WBC and bone marrow cells. Moreover there was an increased presence of α-esterase-positive bone marrow cells indicating that *W. somnifera* treatment could also enhance the differentiation of stem cells. *W. somnifera* extract was found to increase the circulating antibody titre and antibody-forming cells. *W. somnifera* extract was found to inhibit the delayed-type hypersensitivity reaction in mice (Mantoux test). *W. somnifera* extract also showed an enhancement in phagocytic activity of peritoneal macrophages (76.5 pigmented cells/200) when compared to control (31.5/200 cells) in mice, indicating the immunomodulatory activity [7].

In doxorubicin-induced cardiotoxicity, biochemical, endogenous antioxidant markers such as malondialdehyde, protein carbonyl levels, catalase activity, total antioxidant capacity and superoxide dismutase level in cardiac tissues followed by histopathological alterations were prominently reduced by pretreatment of *W. somnifera*. Hence biochemical alteration and histopathological results suggest a cardioprotective effect of *W. somnifera* in doxorubicin-induced cardiotoxicity. Further it might be a useful adjuvant therapy where doxorubicin is the cancer-treating drug [8].

Role of *W. somnifera* in Skin Disease

For development of malignancy due to exposure of UV B radiation (294 nm) for 20 days, pretreatment of the animals with 1-oxo-5-beta, 6-beta-epoxy-witha-2-enolide (20 mg/kg body weight), isolated from the roots of *W. somnifera*, prior to exposing the animals to UV B radiation, prevents the incidence of skin carcinoma. Malignancy in the cutaneous tissue was also prevented by administration of 1-oxo-5-beta, 6-beta-epoxy-witha-2-enolide, to the animals after exposing them to UV B radiation/UV B radiation and benzoyl peroxide. Further immunohistochemical staining of the cutaneous tissues of these rats showed the presence of p53 + foci (clusters of cells containing the mutated p53 protein),

whereas an absence of p53 + foci is observed in animals pretreated with 1-oxo-5-beta, 6-beta-epoxy-witha-2-enolide, indicating effective for prevention of skin carcinoma [9].

Two-stage skin carcinogenesis induced by dimethyl benzanthracene (DMBA) and croton oil reduced by *W. somnifera* 20 mg/dose/animal i.p. consecutively for 5 days prior to DMBA administration which was continued twice weekly for 10 weeks. After the 180th day of carcinogen administration, all of the animals developed papilloma in the control group whereas only 6 out of 12 animals developed papilloma in the treated group. A total of 11 papillomas were found in the control group while only six developed them in the *W. somnifera*-treated group. Further significant enrichment of endogenous antioxidant enzymes such as GSH, GST, glutathione peroxides and catalases of skin was found in *W. somnifera*-treated group compared with the control. On the other hand decrease in the level of lipid peroxide levels was found in *W. somnifera*-treated group [7].

In 7,12-dimethylbenz[a]anthracene-induced skin cancer, *W. somnifera* showed significant decreased in incidence and average number of skin lesions as compared to DMBA alone group, also endogenous antioxidant enzyme levels were near to normal in *W. somnifera* treated group as compared to DMBA-alone-treated rats, indicating protective effect of *W. somnifera* which was further confirmed in histopatholgical examination [10].

Carcinogen-induced forestomach and skin tumorigenesis in the Swiss albino mouse model, showed up to 60 and 92% inhibition in tumour incidence and multiplicity by *W. somnifera*. In DBMA-induced skin papillomagenesis, *W. somnifera* showed up to 45 and 71% inhibition in tumour incidence and multiplicity. This data suggests the chemopreventive role of *W. somnifera* [11].

Toxicity

W. somnifera is generally safe when taken in the prescribed dosage range. Subacute toxicity studies in rats did not reveal any toxicity. *W. somnifera* has not shown any toxicity on long-term administration. Also in 90-day subacute toxicity study in rats, the oral administration revealed the significant increase in body weight, food consumption and liver weight and improved hematopoiesis. Histopathological examination of brain, heart, lung, liver, spleen, kidneys, stomach, testis and ovaries was found to be normal. Hence *W. somnifera* is considered to be a safe herbal drug [12].

However there are anecdotal reports that *W. somnifera* may potentiate the effects of barbiturates; therefore, caution should be used if taking this combination. Chronic treatment with the root extract of *W. somnifera* attenuated the development of tolerance and also the development of dependence to morphine in mice [13].

As of today no herb–herb or herb–drug interactions have been reported in the literature with *W. somnifera*. Higher doses have shown to cause gastrointestinal upset, diarrhoea and vomiting. Large doses of *W. somnifera* may possess abortifacient properties; therefore, it should be avoided during pregnancy. Since *W. somnifera* acts as a mild central nervous system depressant, patients should avoid alcohol, sedatives and other anxiolytics while taking *W. somnifera* [14]. Hence these toxicological studies confirm that the plant is nontoxic in a wide range of reasonable doses and it can be assumed that the doses in which its preparations are indicated in humans can be very safe.

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Chapter 48
The Role of Probiotics in Atopic Dermatitis (Eczema) and Skin Allergy Reactions: Prevention and Therapy

Öner Özdemir and Anand A. Zanwar

Key Points

- Since conclusions on probiotics are limited to specific strains and models, they should not be generalized [1, 2].
- Probiotics should not be considered as completely harmless, particularly in the immunodeficient host, and more safety studies are needed [3–15].
- Physiological use (normal route, normal dose, normal growth phase, specific strain or substrain/species) is studied in all cases, so as not to overwhelm (high dose) or circumvent natural immune processing [3–13].
- Do probiotics really induce/exacerbate Th1 and/or Th2-mediated diseases? Such as being reported an increased rate of recurrent wheezing episodes, an augmented rate of atopic disorders, and increased sensitization to allergens as well as autoimmune disorders. Lactobacilli and Bifidobacteria have specific dose- and duration-dependent immunomodulatory effects on the proliferation of B-/T-lymphocytes [11–13].
- The researchers ought to look for more appropriate and safe combinations of probiotic species (as with VSL#3 or Lacto-mix) or modified probiotics with/without prebiotic and test them in human/experimental atopic dermatitis models [16].
- Research activities are currently focusing on identification of specific probiotic strains with immunomodulatory potential and on how dietary content interacts with the most efficacious probiotic strains. Further studies should be made for the identification of receptors and pathways through which gut microbes influence development of the immune system [17, 18].

Keywords Atopy • Allergy • Probiotics • Eczema • Atopic dermatitis • Skin allergy • Contact dermatitis • Immunomodulation • Immunoregulation • Toll-like receptor

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Introduction

Development of the child's immune system tends to be directed toward a T-helper 2 (Th2) phenotype in infants, whereas postnatal maturation is associated with gradual inhibition of Th2 and increasing Th1 affinity [19]. Thus, immature Th2-dominant neonatal responses must undergo environment-driven maturation via microbial contact in the early postnatal period to prevent development of childhood allergic diseases. Nevertheless, nowadays the increased use of antimicrobial medication, the consumption of sterile food, and reduced family size that result in lower rates of infection during childhood also reduce early contact to microbes. Consequentially, at an early age the infant's immune system results in subsequent polarization toward a Th2 phenotype during postnatal maturation. Among several other phenomena, the present increase in allergic diseases seen in the industrialized countries has been attributed, to a relative lack of microbial stimulation of the infantile gut immune system and the exaggerated hygiene of the typical western lifestyle during early childhood. And this is known as the hygiene hypothesis [20].

The newborn is first colonized by microbes at birth. The colonization of the gut that begins promptly after birth is affected by mode of delivery, early feeding strategies, and the hygienic conditions around the child (the early environment). The colonizing bacteria originate mainly from the mother's gut and vaginal tract [21]. For instance, children born by cesarean section are colonized with Bifidobacteria and Lactobacilli later than vaginally delivered children, and are shown to have more frequent respiratory allergies [22]. After delivery, breastfeeding continues to enhance the original inoculum by the introduction of specific lactic acid bacteria (LAB), Bifidobacteria, and bacteria from the mother's skin, all of which enable the infant gut microbiota that is dominated by Bifidobacteria. Breast milk also contains plentiful indigestible oligosaccharides, which pass through the whole intestine and promote the growth and activity of commensal bacteria; composed mainly of Bifidobacteria [23]. These bacteria set the basis for gut microbiota development and modulation, along with environmental exposures such as antibiotic administration.

The greatest differences between breast-fed and formula-fed infants appear to be in LAB and Bifidobacteria colonization. Usually, Bifidobacteria appear after birth and, within a week, are reported as the dominant bacterial group, with *Bifidobacterium* (*Bfdbm*) *infantis*/*longum*/*breve* being the most common species in breast-fed infants [24]. In addition, *Lactobacillus* (*Lctbs*) *acidophilus* is the most common Lctbs in the feces of breast-fed infants. Formula-fed infants, on the other hand, tend to have a flora that is more complex, consisting mostly of Coliforms and Bacteroides, with significantly lower the prevalence of Bifidobacteria [25]. After weaning, the microflora of children begins to resemble that of adults, with increased Bacteroides, Veillonella, and Fusobacterium [26].

Epidemiologic data showed that atopic children have a different intestinal flora from that of healthy ones, with higher levels of Clostridia and lower levels of Bifidobacteria. Furthermore, other studies have also shown that early colonization with potentially more pathogenic bacteria such as *Clostridium difficile* and *Staphylococcus aureus* is more likely to occur in children who go on to develop allergy. In contrast, LAB and Bifidobacteria are found more commonly in the composition of the intestinal flora of nonallergic children. The enhanced presence of these probiotic bacteria in the intestinal microbiota seems to correlate with protection against atopy [27, 28]. Based on these data, "harmless" microbial agents that are probiotics have been presently tested for their efficacy in the prevention and therapy of allergy in infants [29–32].

The interest in probiotic therapeutic potential in allergic disorders stemmed from the fact that they have been shown to improve intestinal permeability and reduce inflammatory cytokines. Such effects would be desirable in treating allergic disorders including atopic dermatitis (AD). Therefore, several studies have been designed to examine the efficacy of probiotics in many allergic conditions, such as eczema and food allergies [31, 32]. Including the first publication in 1997, over 30 randomized, double-blind, placebo-controlled clinical trials have been conducted to study the effects of various probiotics on treatment and prevention of allergic diseases. In total, almost 3,000 individuals (including those in placebo groups) have participated in these studies so far. In the first-time study done by Majamaa

and Isolauri in 1997, the administration of LGG to highly selected patients (age <2 years, challenge-proven cow's milk allergy, and mild-to-moderate eczema) significantly improved the total SCORAD score [33]. Later the Finnish study of Kalliomaki was the first report to describe that the frequency of AD in neonates treated with *Lctbs rhamnosus* GG (LGG) was half that of the placebo [34]. However, these results recently have been questioned by other trials, which reported no difference in the development and therapy of AD in neonates supplemented with LGG or other probiotics. Therefore, an allergen-preventive or therapeutic effect of probiotics in AD could not be consistently established. The aims of this chapter are to comprehensively define probiotic properties and to characterize current knowledge of probiotics, including the key mechanisms of probiotic effects as well as their preventative/therapeutic role in AD at last.

What Are Probiotics?

The year 2011 marks the 104th year since Eli Metchnikoff suggested that the consumption of LAB may benefit the human host's immune system [29]. However, not until the mid-1960 did the term probiotic become the trend. Probiotics means "for life" and is defined by the World Health Organization and the Food and Agriculture Organization of the United Nations as "live microorganisms which, when administered in adequate amounts as part of food, confer a beneficial health effect by producing gut microflora on the host." These probiotics are mainly represented by LAB [30]. Simply; probiotics are ingested live microbes that can modify intestinal microbial populations in a way that benefits the host.

Characteristics of Probiotics

There are several generally accepted characteristics that define probiotic bacteria. Probiotics:

- Are microbial organisms.
- Remain viable and stable after culture, manipulation, and storage before utilization.
- Survive gastric, biliary, and pancreatic digestion.
- Are able to induce a host response once they enter the intestinal microbial ecosystem.
- Yield a functional or clinical benefit to the host when consumed [28–34].

Atopic Dermatitis (Eczema) and Skin Allergy Reactions

The literature on probiotic use in skin allergy reactions mainly includes experiments in AD (human and animal), AD-like skin lesions and allergic contact dermatitis in animal experiments. And AD can be accepted as a prototypic disease for skin allergy reactions. Especially, the literature on the clinical probiotic use in other skin allergy reactions of human is very scarce. Therefore, this chapter mostly discusses the literature on the preventative and/or prophylactic role of probiotic use in AD.

AD is the most common chronic skin allergy reaction in children and adults, with a prevalence of 10–20% in population. Geographic location affects the prevalence of this disease, with the highest prevalence in the US and Europe [35]. Important factors in the susceptibility to develop AD include a genetic basis and environmental factors. Eczema refers to a chronic or relapsing itchy skin inflammation with typical lesions and locations. Eczema is called atopic if it is associated with IgE demonstrated either by positive skin prick tests or elevated antigen-specific IgE antibodies. The term atopy refers to a genetic predisposition to become sensitized and to mount an IgE response to allergens. AD has been linked to food hypersensitivity, especially milk and egg proteins. However, 40–60% of children with

AD may not develop IgE sensitization [35, 36]. The term eczema has been recently proposed, but for practical purposes, both AD and eczema are used in this chapter.

There have been several proposed methods for classifying the severity of AD in various research studies mentioned in this chapter, but only the Scoring of AD Severity Index (SCORAD), established by the European Task Force on AD, has been validated for reproducibility and accuracy in assessing therapeutic response [35, 36]. The SCORAD combines objective measures, such as extent and severity of skin lesions, and subjective criteria, such as pruritus and sleep loss. Children with AD can be further classified as having mild (≤25), moderate (25–50), or severe (≥50) disease based on their SCORAD score.

Concise Pathophysiology of AD (Eczema) and Skin Allergy Reactions

Allergic skin disease or reactions encompasses a broad range of dermatologic disorders related to the immune response to environmental triggers in genetically susceptible individuals. The pathogenesis of allergic skin diseases is complex, involving the interactions between immunologic and nonimmunologic factors [36].

Even the pathogenesis of AD, mostly known and prototype of skin allergy reactions, is not completely understood, although the complex interactions between susceptibility genes, genetically fixed immune abnormalities, epidermal barrier defects, pharmacological abnormalities, environmental factors and an intensive network of cytokines and chemokines have been the subject of research in recent years and shed light on the underlying mechanisms of AD.

Actually, AD has an immunological basis and is most probably mediated by bone marrow-derived cells. The resolution of the eczematoid skin lesions occurring after successful bone marrow transplantation to correct the immunologic defect in patients with Wiskott–Aldrich syndrome supports this opinion. Moreover, AD does not occur in the absence of T cells. And patients with primary T-cell immunodeficiency disorders frequently have raised concentrations of IgE in their sera.

The Genetic Background of AD

AD has a high level of genetic heterogeneity. This complex skin disease has a high familial occurrence, with a twofold increased risk for a child to develop AD when one of the parents is affected and a threefold increase in cases in which both of the parents are affected. Moreover, there is a high concordance rate of 77% in monozygotic twins and 15% in dizygotic twins. These observations in combination with the early age of onset suggest that AD may represent a genetically complex disease.

To date, two different approaches have been used to identify candidate genes: linkage gene analysis studies and the studies of candidate genes. Many associations of polymorphisms of a specific gene with the phenotype of AD have been found. For example, the arachidonate metabolite thromboxane A2 and thromboxane A2 receptor polymorphisms have been considered to be related to chronic inflammatory diseases, including AD, and might provide a major determinant for high serum IgE levels in AD. Associations of the AD phenotype with chromosomal regions were also investigated. For instance, a pivotal role of the IL-13 gene in AD, which is located at chromosome 5q31-33, has been found in linkage and association analysis studies [36].

Immunohistology of AD

Uninvolved skin in AD shows an abnormal histology because it contains a sparse perivascular T-cell infiltrate. In acute lesion and in nonlesional skin, antigen-presenting cells such as Langerhans cells exhibit surface-bound IgE. The significant perivenular lymphocytic infiltrate consists predominantly of activated

memory T cells suggesting prior encounter with antigen. Eosinophils, basophils, and neutrophils are rarely present in acute AD whereas mast cells are present in various stages of degranulation [37].

Impairment of the Epidermal Barrier

The genetically determined barrier deficiency is considered to increase the risk of sensitization to allergens and contribute to the exacerbation of allergic diseases. In this context, epidermal barrier dysfunction seems to be one of the most striking mechanisms involved in the pathophysiology of AD. The altered lipid composition of the stratum corneum is a basic defect of AD, which is responsible for the xerotic skin and results in a higher permeability to allergens and irritants. In patients with AD the activity of proteases and protease inhibitors in the cornified envelope undergo changes. Elevating the skin pH by using soap increases protease activity, and therefore, the breakdown of the cornified envelope occurs. A reduced content of ceramides, which serve as the major water-retaining molecules in the extracellular space of the cornified envelope, has been reported in both lesional and nonlesional skin in patients with AD.

One key protein in the epidermis that is crucial for the protective function of the skin barrier is filaggrin, which is responsible for the arrangement of the keratin intermediate filaments into bundles and is located at the cornified envelope. When filaggrin is proteolytically degraded, it serves as an osmolyte and accounts for the corneocyte hydration. Epidemiologic studies have shown that mutations in filaggrin pose an increased risk of early onset, severe, persistent AD and an increased risk of asthma. As a matter of fact, mutation of the filaggrin gene is a predictive marker in terms of AD predisposing and finally leading to asthma—the so-called atopic march [36, 37].

Microbial Components

Pityrosporum ovale is lipophilic yeast commonly found to colonize the skin and cause immediate and late-phase reactions in these patients. In more than 60% of patients with AD, *P. ovale* can be detected in the peripheral blood, supporting the allergy-triggering role of this organism.

Moreover, bacterial superinfections, in parts due to the impaired innate immunity, are thought to play a critical role in the clinical course of skin lesions. *S. aureus* is found in more than 90% of patients with chronic AD skin lesions. Scratching is an important factor, enhancing the binding of the bacteria by disturbing the skin barrier and exposing extracellular matrix molecules known to act as adhesins to *S. aureus.*

It is a well-known fact that patients with AD show also a higher risk of developing severe superinfections with different virus strains, like herpes (eczema herpeticum), molluscum (eczema molluscatum), or vaccinia virus (eczema vaccinatum).

Various factors such as cytokines and immunoglobulins, particularly IgE and several cell types such as T lymphocytes, Langerhans cells, eosinophils, and keratinocytes have been implicated in the pathogenesis of AD as well.

Neuroimmunological Factors

Stress is one of the most important factors that can lead to the exacerbation of AD. It is well known that stress can alter the levels of eosinophils and circulating lymphocyte subsets in patients with this disease. Although the exact mechanisms of the interaction of the skin immune system and the nervous system have not yet been fully understood, it is believed that this phenomenon might be mediated by neuroimmunologic factors, such as neuropeptides, which can be found in the blood and within the

epidermal nerve fibers in close association with epidermal Langerhans cells. One of the new topics of research in AD is neurotrophins, which have been regarded to be a link between the immune and the nervous system. These mediators play a role in vegetative reactions like vasodilatation, edema, itching sensations, pain and sweat excretion and also show an effect on T cells.

Immunoglobulins

Most patients with AD exhibit hyperproduction of IgE, particularly during disease onset or exacerbation, possibly the result of enhanced production of TH2-type cytokines during the acute phase of the disease. After crosslinking with an allergen, IgE plays a key role in type I hypersensitivity reactions by inducing mast cells and basophils to release various bioactive mediators, including histamine. Allergen-specific IgE activates mast cells and basophils by interacting with a high-affinity receptor for IgE (FcεRI) located on these cells. It has been suggested that the pruritus and erythema occurring after exposure to allergens may be related to substances released by mast cells bearing allergen-specific IgE.

Omega-3 Fatty Acids

A deficient conversion of omega-6 fatty acids to prostaglandin E1 is a causative factor for immunologic and biochemical alterations in AD. There is evidence for a deficiency of essential long-chain omega-6 fatty acids and E-type prostaglandins, which are important for thymic T-cell maturation and thymus hormone action.

Keratinocytes

Keratinocytes are integral to the structure of the stratum corneum as a result of their differentiation into corneocytes and subsequent production of ceramides. They also appear to act as primary inducers and targets of immunological responses occurring in the skin. In AD, the activity of keratinocytes in both areas is altered. For example, the stratum corneum which is the permeability barrier between the body and the external environment is impaired resulting in increased transepidermal water loss and diminished water-binding capacity in AD. This is responsible from the symptoms of dryness and intense pruritus seen in AD. In addition, scratching causes trauma and induces keratinocytes to release a variety of proinflammatory cytokines. The defective stratum corneum barrier also allows entry of *S. aureus* and other microbes [36, 37].

Keratinocytes of patients with AD also manifest an intrinsically abnormal chemokine-cytokine production pattern that contributes to the recruitment of distinct leukocyte subsets to the inflammatory site and maintains the inflammatory immune response of AD skin. As a consequence of the altered cytokine synthesis, keratinocytes also release high amounts of the proinflammatory cytokines TNF-α and IL-1β.

Lymphocytes

According to a concept known as the “hygiene hypothesis,” exposure to infection during early childhood protects a child against atopic disease by stimulating maturation of type 1T-helper (TH1) cells, that is, CD4+ T-helper lymphocytes (TH0 cells) differentiate into TH1 cells rather than TH2 cells.

In individuals genetically predisposed to AD, this exposure may be insufficient to counter a general susceptibility to imbalance of TH2 vs. TH1 immune responses.

However, some researchers postulate that reduced numbers of infections during early childhood resulting from decreased exposure to infections, greater use of antibiotics, and other aspects of a modern lifestyle, may lead to enhanced TH2 allergic responses. This may account for the recent increase in prevalence of AD.

Epidermal Dendritic and Langerhans Cells

Langerhans cells are located in the epidermis and are the first line of a cellular immune defense in the skin. Here, they form a network of cells that sample antigens getting through the skin barrier. Langerhans cells are CD1a+ and contain characteristic Birbeck's granules. Skin lesions from patients with AD contain increased levels of CD1a+ epidermal Langerhans cells, which belong to the dendritic cell family of antigen presenting cells. In AD, Langerhans cells express the high-affinity IgE receptor FcεRI on their surface and probably play an important role in allergen presentation to TH1 and TH2.

Eosinophils

In patients with AD, eosinophilia and increased levels of eosinophil granule proteins in the sera and the urine of patients are well documented. These findings also correlate with the activity of this disease and they decrease in response to therapy. Skin infiltration by eosinophils, and the consequent production of IL-12, is likely to be important in the chronic inflammatory response associated with AD lesions. Delayed eosinophil apoptosis and dysregulated apoptosis of other cells, due to the enhanced viability caused by cytokine IL-5, may contribute to the emergence and persistence of lesions in those patients [36, 37].

Monocytes

Monocytes, the predominant cell type in chronic AD lesions, also exhibit reduced apoptosis resulting in increased survival. Increased production of GM-CSF by monocytes and IL-10 may play an important role in the regulation of monocyte survival. The expression of FcεRI and FcεRII on monocytes in the peripheral blood is enhanced in atopic people, as well.

Experimental and Clinical Essentials of Preventative and Therapeutic Probiotic Use in Eczema and Skin Allergy Reactions

As briefly mentioned above, there is a good experimental and clinical theoretical basis for using probiotics in the prevention and therapy of AD. Germ-free animal models demonstrate that bacterial gut colonization is essential for maturation of immune function and induction of oral tolerance. It has been proposed that a similar but more subtle process may be occurring in human beings with progressively cleaner environments. Probiotic intestinal flora is arguably the most abundant source of early immune stimulation and contributes significantly to microbial burden in early life. A number of studies have suggested differences in the early colonization patterns of infants who go on to develop allergic disease. These studies strongly suggest that the pattern of colonization in the first weeks of life may influence the patterns of immune development [1, 37]. These notions have been supported

by observations that gut flora can influence local and systemic immune responses. There has been speculation that intestinal flora may influence the maturing precursor cells that circulate through the gut before they home to other tissues. This may explain how probiotic species can influence systemic immune responses and IgA production in distal sites, such as the respiratory tract. Together with reported clinical effects in early allergic disease, this has logically led to a growing interest in the role of probiotics in allergy prevention [31, 32].

The gastrointestinal tract of the newborn baby is sterile. Soon after birth, however, it is colonized by many different microorganisms. Colonization is complete after around 1 week, but the numbers and species of intestinal bacteria fluctuate markedly during the first several months of life. The composition of the gut microbiota differs between healthy and allergic infants and even in countries with a high and low prevalence of allergies [38]. Mode of delivery, either vaginal or through cesarean section, also has a major impact on early colonization patterns of the infant gut [22]. In the case of allergy, the rationale for modulating the intestinal microbiota is supported by observations that allergic children have a different microbiota composition than healthy infants. The main changes associated with allergic trait are less frequent colonization with Lactobacilli and lower counts of Bifidobacteria [27, 39]. In addition to these quantitative differences in the *Bfdbm microbiota*, qualitative differences have also been observed. Infants with AD have been found to have a more adult type *Bfdbm microbiota* with high prevalence of *Bfdbm adolescentis*. Healthy infants, on the other hand, were found to be colonized mainly by *Bfdbm bifidum*, typical for breast-fed infants [25, 26]. The Bifidobacteria from infants with AD were found to induce a higher secretion of proinflammatory cytokines in vitro, whereas the Bifidobacteria from healthy infants induced the secretion of more anti-inflammatory cytokines. Also, Bifidobacteria of dairy origin stimulated more anti-inflammatory and less inflammatory cytokines than Bifidobacteria from allergic infants. In addition to differing in their induction of cytokines, Bifidobacteria from allergic and healthy infants also exhibited different in vitro adhesion to Caco-2 tissue culture cells and intestinal mucus. This difference in adhesion to the intestinal mucosa may result in a different or reduced stimulation of the immune system through the gut-associated lymphoid tissue [40]. Lower counts of Bifidobacteria have been reported in atopic vs. nonatopic children preceding allergen sensitization. Bifidobacteria are hypothesized to more effectively promote tolerance to nonbacterial antigens, primarily by inhibiting the development of a Th2-type (proallergic) response. In a recent study, a positive change in stool colonization in atopic infants supplemented with *Bfdbm lactis* has been shown with a decrease of Bacteroides and *Escherichia coli* in the stool. Most interestingly, serum IgE correlated with *E. coli* counts, and in highly sensitized infants, IgE correlated with Bacteroides counts [41]. Thus, certain probiotics seem to influence the gut's allergen-stimulated inflammatory response and provide a barrier effect against antigens that might otherwise ultimately lead to systemic allergic symptoms (such as eczema).

A recent prospective study from three European birth cohorts found, however, no differences in gut microbiota by culture-dependent analysis of fecal samples among infants developing or not developing atopic eczema and food allergy. On the contrary, a subgroup analysis of the cohort by cultivation independent techniques indicated a significantly lower diversity in the gut microbiota of 1-week-old neonates who later manifested atopic eczema than in neonates remaining healthy during the first 18 months of life, highlighting once more that classical microbiological plating techniques are inappropriate for extensive characterization of the gut microbiota [42]. Similarly, less diverse microbial communities were found among 5-year-old allergic children than among nonallergic children by using another culture-independent technique. The same study demonstrated that *Bfdbm catenulatum/pseudocatenulatum* prevail in nonallergic children [43]. On the contrary, this particular Bifidobacterial species was associated with atopic eczema in a nested case-control study conducted in a different age group, country, and disease population, highlighting the complexity of the situation. As the immune modulation properties of bacteria seem to be distinctly strain specific, it cannot be ruled out that the nature of the immune response induced by a specific strain plays a more important role than its classification.

Probiotic intestinal flora contributes to microbial antigen exposure in early life and is one of the most abundant sources of early immune stimulation. Because allergic immune responses manifest early in life, there has been obvious interest in the potential benefits of modifying the gastrointestinal flora by using probiotic supplementation. However, the value of probiotics for primary prevention is controversial [31, 32]. So far, there have been only several studies to address the role of probiotics in primary prevention, with a reported suspicious reduction in the incidence of eczema. The role of probiotics in allergy prevention has remained controversial, and there has been an urgent call for similar studies to address this further. This chapter tries to highlight the issues with probiotics in the therapy/prevention of AD and future of this therapy. Here, first newly described mechanisms of probiotic effects are defined. Later, under the light of recent literature probiotic use in AD therapy and prevention is being discussed in detail.

Experimental (Animal) and Clinical (Human) Studies Showing Mechanisms of Probiotics' Effects in AD and Skin Allergy Reactions

Over the several decades, animal models of AD and skin allergy reactions have received increasing attention. These models include NC/Nga mice, a hapten-induced mouse model, and transgenic and knockout mouse models. Although the pathogenesis of skin inflammation elicited in these models is not quite the same, it is pertinent to ask what these animal models really tell us about the pathogenesis and possible therapies for the disease. NC/Nga mice may yield information relevant to the dissection of the crucial components of the pathophysiology of skin allergy reactions and AD rather than the assessment of potentially therapeutic agents for its treatment. And this hapten-induced mouse model has been mostly used and created by repeated application of 2,4,6-trinitrochlorobenzene (TNCB) that is a simple and reproducible one. This model offers several advantages over others: by changing hapten and the mouse strain used, various types of chronic inflammation, probably reflecting heterogeneity in clinical presentation of skin allergy reactions and AD, can be induced. This model is also of enormous value in its high reproducibility as well as the ease of quantitative assessment by measuring ear thickness [44].

Although the beneficial effects of probiotics on wide variety of atopic diseases have been suggested, little is known about how probiotics modulate the immune system, atopic disease development and skin allergy reactions. Currently, only limited publications are available defining the effects of probiotics in murine or human models of AD and skin allergy reactions. Therefore, it is important to explore the effect of probiotics in various experimental and clinical atopic disease models. Here, some experimental (animal) studies showing mechanisms of probiotics' effects in skin allergy reactions and AD are being described.

Maturing Gut Barrier: Probiotic Regulation in Intestinal Epithelium and Upregulation of Host Immune Responses

Recent data indicate that commensal intestinal microbiota represents a major modulator of intestinal homeostasis. Dysregulation of the symbiotic interaction between intestinal microbiota and the mucosa may result in a pathological condition with potential clinical repercussions. For instance, it is shown that mice reared in germ-free conditions have an underdeveloped immune system and have no oral tolerance. In contrast, pathogen-free mice are capable of reconstituting the bacterial flora with Bifidobacteria and tolerance development [45].

In addition to providing maturational signals for the gut-associated lymphoid tissue, probiotics balance the generation of pro- and anti-inflammatory cytokines in the gut. Some components of

heat-treated LGG may have an ability to delay the onset and suppress the development of AD, probably through a strong induction of IL-10 in intestinal lymphoid organs and systemic levels [46]. After probiotic consumption, decrease in fecal α-1 antitrypsin, serum TNF-α, and changes in TGF-β and other cytokines point to down-regulation of inflammatory mediators [47]. For instance, after a challenge study in infants allergic to cow's milk, fecal IgA levels were detected to be higher and TNF-α levels were lower in the *Lctbs rhamnosus* GG (LGG) applied group compared to the placebo [48]. Similarly, another study by Kirjavainen et al. suggested that *Bfdbm lactis* Bb12 might modify gut microflora to alleviate early onset atopic eczema. And this modification was found to be compatible with reductions of serum TNF-α and fecal α-1-antitrypsin levels as well as an increase in fecal IgA level [40].

Moreover, probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the permeability barrier of the intestine, and by enhancing the degradation of enteral antigens and altering their immunogenicity [49]. This gut-stabilizing effect of probiotics could be explained by the improvement by probiotics of the immunological barrier of the intestine through intestinal IgA responses, specifically [50, 51]. Oral treatment with probiotic *Lctbs johnsonii* NCC533 (La1) for a specific part of the weaning period was also shown to prevent the development of AD in model mice, NC/Nga, by modulating or accelerating the gut immune response with increased intestinal secretory IgA [52]. Consistent with these explanations, in children with food allergies, probiotics are shown to reverse increased intestinal permeability and to enhance frequently defective IgA responses [53].

Immunomodulation: Th1/Th2 Balance, IgE Production and Cytokines

In addition to maturing gut barrier, certain strains of Lactobacilli and Bifidobacteria modulate the production of cytokines by monocytes and lymphocytes, and may divert the immune system in a regulatory or tolerant mode [45, 54]. Nonetheless, there are still some studies showing no significant effects of probiotics on either Th1 or Th2 cell responses to allergens. Although the cytokine stimulation profiles of different probiotic strains vary, the strains isolated from healthy infants mainly stimulate non-inflammatory cytokines [2]. Therefore, it seems that changes in cytokine profile induced by probiotics may be probiotic strain- or site-specific and dependent on the experimental system used. For instance, *Lctbs reuteri* induced proinflammatory and Th1 cytokines; and *Bfdbm bifidum*/*infantis* and *Lctbs lactis* reduced Th2 cytokines [55].

Several studies have shown the immunomodulatory effects of probiotic bacteria. In one study, *Bfdbm bifidum*/*infantis* and *Lctbs lactis* reduced Th2 cytokines and acted as potent inducers of IL-10 production in different peripheral blood mononuclear cell cultures [56]. In another study, eight common Lctbs strains were studied with respect to induction of cytokines by the murine gut mucosa in response to a parenterally administered antigen. *Lctbs reuteri* induced proinflammatory and Th1 cytokines; however, *Lctbs casei* tended to induce IL-10/IL-4 [57]. Yet on the contrary, in some children receiving probiotics, reduced IL-10 responsiveness to house dust mites allergens was observed [58]. In a study, the effects of feeding Lctbs F19 were evaluated during weaning on the incidence of eczema and Th1/Th2 balance. In a double-blind, placebo-controlled randomized intervention trial, infants were fed cereals with (*n*: 89) or without Lctbs F19 (*n*: 90) from 4 to 13 months of age. At 13 months of age, the IFN-γ/IL-4 mRNA ratio was significantly higher in the probiotic compared with the placebo group. The higher Th1/Th2 ratio in the probiotic compared with the placebo group suggests enhancing effects of Lctbs F19 on the T cell-mediated immune response. And probiotics also increased Th1 cytokines and inhibited allergen-induced IgE and Th2 cytokines in some atopic children [59, 60].

In a mouse model, effect of oral probiotics administration, including *Bfdbm lactis*/*bifidum* and *Lctbs acidophilus*, on mice with ovalbumin (OVA)-induced food allergy was studied. The mice treated

with probiotics suppressed production of the OVA-specific IgE, IgG1, and IgA. Additionally, the level of IL-4 was significantly lower, and the levels of INF-γ and IL-10 were significantly higher in the mice treated with probiotics than that in the nontreated mice [61]. Another murine model showed that oral administration of an immunostimulatory DNA sequence from *Bfdbm longum* suppressed Th2 immune responses in mice and inhibited IgE production in vitro [62]. Similarly; *Lctbs acidophilus* strain L-55 suppressed the development of AD-like skin lesions induced by repeated application of 2,4,6-trinitrochlorobenzene (TNCB) in sensitized NC/Nga mice via a decrease in the serum total IgE level [63]. A final study showed that the administration of either *Bfdbm lactis* Bb-12 or LGG suppressed antigen-specific IgE production too [64].

Oral administration of LAB isolated from traditional South Asian fermented milk "dahi" inhibits the development of AD in NC/Nga mice as well. Of the 41 strains tested from "dahi", *Lctbs delbrueckii* subsp. *lactis* R-037 exhibited the greatest IL-12 induction, suggesting that it is a potent Th1 inducer [65]. Also, the anti-allergic effects of one strain (T120) of LAB isolated from Mongolian fermented milk using AD model mice (NC/Nga mice) were investigated. Strain T120 has already been identified as *Enterococcus faecium* and suppressed total IgE production and induced IL-12 and IFN-γ production by splenocytes of NC/Nga mice. Further, this strain enhanced the production of IL-10 by splenocytes and activation of regulatory T cells by strain T120 may inhibit atopic disease. In in vivo studies, intraperitoneal injection of strain T120 inhibited serum IgE elevation and AD symptoms in NC/Nga mice [66]. In another study, *Lctbs plantarum* strains from kimchi were demonstrated to inhibit AD (house-dust mite-induced dermatitis) in NC/Nga mouse. The three strains, CJLP55, CJLP133, and CJLP136, suppressed AD-like skin lesions and epidermal thickening. These same three strains decreased TH2 cytokines production such as IL-4 and IL-5 in lymph node cell cultures. The latter two, CJLP133 and CJLP136, increased IFN-γ secretion, while CJLP55 enhanced IL-10 production. These findings suggest that Lactobacilli isolated from kimchi inhibit AD, probably by altering the balance of Th1/Th2 ratio or inducing IL-10 production [67].

AD-like skin lesions were induced by sensitization to and repeated challenges with picrylchloride in the Th2-skewed NC/Nga mouse strain. A new synbiotic, *Lctbs casei* subsp. *casei* together with dextran, reduces murine allergic reaction such as the development of AD-like skin lesions in NC/Nga mice. This synbiotic combination significantly decreased clinical skin severity scores induced by picryl chloride and total IgE levels in sera of NC/Nga mice [68]. Also, supplementation with KW3110 strain of LAB significantly attenuated the onset and exacerbation of AD-like skin lesions, accompanied by less mast cell infiltration and lower plasma IgE levels thru its effects on IL-12 and IL-4 production in vitro [69]. Furthermore, oral administration of heat-killed *Lctbs brevis* SBC8803 ameliorates the development of dermatitis in AD model of NC/Nga mice. Eight-week-old male NC/Nga mice were sensitized by the topical application of picryl chloride to foot pads and shaved abdomen. Oral administration of *Lctbs brevis* SBC8803 significantly inhibited IgE production and ear swelling, and suppressed the development of dermatitis in a dose-dependent manner. Immunosuppressive cytokines such as IL-10 and TGF-β production from Peyer's patch cells significantly increased in the treatment group, compared to the control group [70]. Consistently, oral supplementation with *Lctbs rhamnosus* CGMCC 1.3724 (LPR) in a study by Tanaka et al. has been demonstrated to prevent development of AD in NC/NgaTnd mice possibly by modulating local production of IFN-γ and plasma total IgE in skin biopsies, compared with untreated mice [71].

A decrease in the secretion of pro-inflammatory cytokines, IFN-γ, TNF-α, and IL-12 has been demonstrated. Consistently, in an experimental study, probiotic supplementation decreased the severity of allergic skin responses in allergen-sensitized pigs with a corresponding increase in IFN-γ expression [72]. However, the study by Rosenfeldt et al. demonstrated no significant changes in serum cytokines (IL-2, IL-4, IL-10, and IFN-γ) during 6 weeks of probiotic treatment [73]. Another study by Brouwer et al. showed no statistically significant effects of probiotic supplementation on cytokine production (IL-4, IL-5, and IFN-γ) as well [74]. These results differ from those of Pohjavuori et al., who were able to demonstrate an increase of IFN-γ production in peripheral blood mononuclear cell

in infants with AD treated with LGG instead of placebo [75]. Additionally, the improvement in AD severity of very young children with probiotic treatment was detected to be associated with significant increases in the capacity for Th1 IFN-γ responses and altered responses to skin and enteric flora. This effect was still evident 2 months after the supplementation was ceased [76].

Reduction in serum soluble CD4 as a marker of T-cell activation described by Isolauri et al. They also found significant changes in indirect markers of allergic inflammation, such as sCD4 in the serum of infants with AD supplemented with *Bfdbm lactis* and LGG [77].

In a randomized controlled trial by Boyle et al. showed that LGG treatment during pregnancy (prenatal) for the prevention of eczema was not associated with any change in cord blood immune markers such as TGF-β, IL-10, IL-12, IL-13, IFN-γ, and TNF-α as well as dendritic and T Regulatory (Treg) cell numbers [78].

Twelve human studies were included in a review and 67% showed a positive association with TGF-β1 or TGF-β2 demonstrating protection against allergy-related outcomes in infancy and early childhood. High variability in concentrations of TGF-β was noted between and within studies, some of it explained by maternal history of atopy or by consumption of probiotics. Human milk TGF-β appears to be essential in developing and maintaining appropriate immune responses in infants and may provide protection against adverse immunological outcomes, corroborating findings from experimental animal studies. In a study, aim was to evaluate the effect of probiotic supplementation on the immunological composition of breast milk and colostrum in relation to sensitization and eczema in the babies. Total IgA, secretory IgA, TGF-β1, TGF-β2, IL-10, TNF-α, and soluble CD14 were analyzed in colostrum and mature milk obtained from women treated with probiotics from gestational week 36 until delivery. The total IgA, secretory IgA, TGF-β1, TNF-α, and sCD14 in breast milk were not affected by the intake of probiotics. Supplementation of probiotics during pregnancy was associated with low levels of TGF-β2 and slightly increased levels of IL-10 in colostrum. Infants receiving breast milk with low levels of TGF-β2 were less likely to become sensitized and possibly less IgE-associated eczema in breast-fed infants during their first 2 years of life [79]. However, another trial by Boyle et al. showed that LGG treatment during pregnancy (prenatal) for the prevention of eczema was associated with decreased breast milk soluble CD14 and IgA levels, not TGF-β [78]. The difference between these studies looks probiotic species, which may affect the immunological composition of breast milk.

Anti-inflammatory Effects: Their Effects on Serum Inflammatory Parameters

The anti-inflammatory effect of probiotics has been attributed to increased production of IL-10 by immune cells in the lamina propria, Peyer's patches, and the spleen of treated animals [2, 55–57]. Oral administration of LGG resulted in elevated IL-10 concentrations in atopic children, indicating that specific probiotics may have anti-inflammatory effects in vivo and possible enhancing regulatory or tolerance-inducing mechanisms as well. A review of the evidence from randomized controlled trials by Betsi et al. about probiotics for the treatment or prevention of AD: the results of 13 relevant randomized (placebo)-controlled trials (RCTs) were reviewed: 10 of which evaluated probiotics as treatment and 3 for prevention of AD. In four of these six RCTs, clinical improvement was associated with a change in some inflammatory markers [80]. Another randomized, double-blind, placebo-controlled study conducted by Brouwer et al. showed no statistically significant effects of probiotic supplementation on inflammatory parameters [74].

Some probiotics have been reported to reduce proinflammatory cytokines through Th17 cells. Suppression of this newly discovered subset of T cells by probiotics might explain effects observed in different experimental models that all involve inflammatory responses. For instance, Lctb casei suppressed inflammation reducing proinflammatory cytokines released from Th17 cells [81]. Also, in a study administration of the probiotics mixture (*Lctbs acidophilus*, *Lctbs casei*, *Lctbs reuteri, Bfdbm bifidium*, and *Streptococcus thermophilus*) induced both T-cell and B-cell hyporesponsiveness and down-regulated Th1, Th2, and Th17 cytokines [82].

A study by Woo et al. evaluated the effect of *Lctbs sakei* supplementation in children with atopic eczema-dermatitis syndrome. In this study, compared with placebo, probiotic administration was associated with lower pretreatment-adjusted serum levels of chemokines such as CCL17 and CCL27, which were significantly correlated with SCORAD total score [83].

Probiotic-induced chronic low-grade inflammation characterized by elevation of CRP, IgE, IgA, and IL-10 was shown in some studies, the changes typically observed in helminth infection-associated induction of regulatory mechanisms. The association of increased CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the view that chronic, low-grade inflammation protects from eczema. The findings emphasize the role of chronic microbial exposure as an immune modulator protecting from allergy [84].

Primary administration of *Lctbs johnsonii* NCC533 (La1) in weaning period suppressed the elevation of proinflammatory cytokines and CD86 gene expressions in skin lesions of NC/Nga model mice. The suppression of proinflammatory cytokines such as IL-8/-12/-23 and CD86 expression by primary administration of La1 may significantly contribute to the inhibitory effect on the skin lesion like AD [85].

A study by Rosenfeldt et al., two probiotic Lctbs strains (lyophilized *Lctbs rhamnosus* 19070-2 and *Lctbs reuteri* DSM 122460) were given in combination for 6 weeks to 1- to 13-year-old children with AD. During active treatment, serum eosinophil cationic protein (ECP) levels significantly decreased. A combination of *Lctbs rhamnosus* and *Lctbs reuteri* was beneficial in the management of AD and the effect was more pronounced in atopic eczema patients [73]. Another study was conducted by Brouwer et al. showed, during Lctbs species supplementation, a moderate but significant reduction in soluble ECP levels was found. ECP, a cytotoxic protein released from activated eosinophils, has been used to monitor disease activity in AD. Thus, sECP might be a more sensitive marker in acute exacerbations of the eczema than a marker of disease activity per se [74]. Although this study by Brouwer et al. revealed no statistically significant effects of probiotic supplementation on eosinophil protein X (EPX) in urine, Isolauri et al. found significant changes in EPX in the urine of infants supplemented with *Bfdbm lactis* and LGG [33].

Development of Tolerogenic Dendritic Cells

Selected species of the Bfdbm genus were demonstrated to prime in vitro cultured neonatal dendritic cell (DC)s to polarize T cell responses and may therefore be used as candidates in primary prevention of allergic diseases. *Bfdbm bifidum* was found to be most potent polarizer in vitro-cultured DCs to drive Th1-cell responses involving increased IFN-γ producing T cells concomitant with reduction of IL-4-producing T-cells [86]. In addition, T-cells stimulated by *Bfdbm bifidum* matured DCs as producers of more IL-10 [87]. Moreover, *Lctbs rhamnosus*, member of another genus of probiotic bacteria, modulates DC function to induce a novel form of T-cell hyporesponsiveness [88]. *Lctbs reuteri*/*casei* have been also shown to prime monocyte-derived DCs through the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) to drive the development of Treg cells [89]. These Treg cells produce increased levels of IL-10 and are capable of inhibiting the proliferation of bystander T cells. This study suggests that the targeting of DC-SIGN by certain probiotic bacteria might explain their beneficial effect in the treatment of a number of inflammatory diseases, including AD.

Immunoregulation by T Regulatory Cells

As mentioned earlier, *Lctbs reuteri*/*casei* have been also shown to prime monocyte-derived DCs through the DC-SIGN to drive the development of Treg cells [89]. And the probiotic combinations are alleged to cause a paradoxic Th2 stimulation and to induce chronic low-grade inflammation, practically

the same as in chronic and balanced helminth infection, which is associated with activation of Treg cells suppressing allergic inflammation. Since the colonization is yet transient, the induction of Treg cells is not permanent. Thus, when these immunologic effects no longer operate, the clinical effect is simultaneously lost. For instance, when helminth infections are treated, the prevalence of allergic sensitization increases rapidly. This is a plausible explanation for the fading probiotic effect as well [84].

Recent studies also provided evidence that one effect of probiotics may involve induction of differentiation of IL-10-dependent, TGF-β-bearing Tregs. In a food allergy mouse model, oral administration of *Bfdbm bifidum* and *Lctbs acidophilus* suppressed OVA-specific IgE production, which was caused by inducing Treg-associated TGF-β production [90]. Another study demonstrated that neonatal application of probiotic bacteria inhibits subsequent allergic sensitization and airway disease in a murine model of asthma by induction of Treg cells and TGF-β production [91].

Generation of CD4+/Foxp3+ Treg cells by probiotics administration suppresses immune and allergic disorders. Recently, two studies reported that oral administration of a certain probiotic strain could increase Foxp3+ Tregs [82]. It is known that the lower percentage of epidermal or dermal Foxp3+ cells in eczematous dermatitis might contribute to their pathogenesis [92]. The strain T120 of LAB was shown to be able to inhibit atopic disease in NC/Nga mice thru enhanced production of IL-10 by splenocytes and activation of Teg cells [93]. In a recent study, a mixture of probiotics (*Lctbs acidophilus*, *Lctbs casei*, *Lctbs reuteri*, *Bfdbm bifidium*, and *S. thermophilus*) was identified that up-regulates CD4+/Foxp3+ Treg cells. Administration of the probiotics mixture induced both T-cell and B-cell hyporesponsiveness and down-regulated Th1, Th2, and Th17 cytokines [82, 94]. It also induced generation of CD4+/Foxp3+-Tregs from the CD4+/25-population and increased the suppressor activity of naturally occurring CD4+/25+-Tregs. Conversion of T cells into Foxp3+ Tregs is directly mediated by regulatory DCs that express high levels of IL-10 and TGF-β. In a murine AD model, treatment with this probiotic mixture significantly inhibited the clinical symptoms of AD progression by reducing IgE levels [total and specific IgE levels], infiltrated lymphocytes and granulocytes, and levels of AD-associated cytokines [82]. *Lctbs casei* treatment enhanced the frequency of FoxP3(+)-Treg in the skin and increased the production of IL-10 by CD4+/25+-Treg cells in skin draining lymph nodes of hapten-sensitized mice. These data demonstrate that orally administered *Lctbs casei* (DN-114 001) efficiently alleviate T cell-mediated skin inflammation without causing immune suppression, via mechanisms that include control of CD8+ effector T cells and involve regulatory CD4+-T cells. *Lctbs casei* may thus represent a probiotic of potential interest for immunomodulation of T cell-mediated allergic skin diseases in human [93]. However, another study showed that Foxp3 mRNA expression at 6 months of age is higher in infants having AD, but it is not affected by giving probiotics from birth [95].

In sensitized BALB/c mice, skin inflammation was induced by topical allergen application. *E. coli* Nissle 1917 was administered orally in a preventive manner. Oral EcN administration improved allergen-induced dermatitis dose-dependently. In parallel, a reduction of epidermal thickness and infiltrating immune cells together with an enhanced number of Foxp3(+) cells and a trend of increased IFN-γ, IL-10 and TGF-β expression was detected in eczematous skin. Our findings indicate that *E. coli* Nissle alters the local allergen-induced immune response by increase of Foxp3(+) cells and by favoring an immunoregulatory cytokine pattern [96].

Lymphocyte Subpopulations

Several studies reveal that the probiotics differently modulate peripheral blood immune parameters in healthy subjects and patients with AD.

Gerasimov et al. conducted a study to assess the clinical efficacy and impact of *Lctbs acidophilus* DDS-1, *Bfdbm lactis* UABLA-12 with fructo-oligosaccharide on peripheral blood lymphocyte

subsets in preschool children with moderate-to-severe AD. The percentage of CD4, and the percentage and absolute count of CD25 decreased, and the percentage and absolute count of CD8 increased in the probiotic group at week 8, compared with placebo (p: <0.007). They found a significant correlation between CD4 percentage, CD25 percentage, CD25 absolute count, and SCORAD values in the probiotic group at week 8. The administration of a probiotic mixture and fructo-oligosaccharide was correlated with significant clinical improvement in children with AD, with corresponding lymphocyte subpopulation changes in peripheral blood [97].

Also in other mice studies, contact hypersensitivity to the hapten 2,4-dinitrofluorobenzene, a model of allergic contact dermatitis mediated by CD8+-cytotoxic T lymphocytes and controlled by CD4+-Treg cells was studied. Daily oral administration of fermented milk containing *Lctbs casei* or *Lctbs casei* alone decreased skin inflammation by inhibiting the priming/expansion of hapten-specific IFN-γ-producing CD8+-effector T cells. This study provides the first evidence that oral administration of *Lctbs casei* can reduce antigen-specific skin inflammation by controlling the size of the CD8+-effector pool [98]. Nevertheless, oral treatment with the probiotic bacteria *Lctbs casei* (DN-114 001) alone alleviated antigen-specific skin inflammation mediated by either protein-specific CD4(+) T cells or hapten-specific CD8(+) T cells in hapten-sensitized mice. In the model of CD8(+) T cell-mediated skin inflammation, reproducing allergic contact dermatitis in human, inhibition of skin inflammation was due to decreased CD8(+) effector T-cells recruitment into the skin during the elicitation (i.e., symptomatic) phase of contact hypersensitivity [93].

However, in another study major lymphocyte subsets were not affected by the probiotic intervention. The expression of CD4+/25+ T cells was similar in healthy subjects and AD patients, whereas CD4+/54+ decreased significantly in patients with AD and remained uninfluenced in healthy subjects. The purpose of a study by Roessler et al. was to elucidate the influence of a probiotic drink containing a combination of the probiotics *Lctbs paracasei* Lpc-37, *Lctbs acidophilus* 74-2, and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 (*Bfdbm lactis* 420) in healthy volunteers and in patients with AD on clinical and immunological parameters and their detection in feces. In a double-blind, randomized crossover study was conducted in 15 healthy adults and 15 patients with AD. The probiotic product or placebo was given over 2 months. In AD patients, the SCORAD tended to decrease by 15.5% (p: 0.08). However, CD57+ increased significantly in healthy subjects after probiotic intake and was not changed in patients [99].

Toll-Like Receptor Stimulation

A number of experiments indicate that infectious agents can promote protection from ADs through mechanisms independent of their constitutive antigens, leading to stimulation of non-antigen specific receptors such as Toll-like receptor (TLRs). A family of pattern recognition receptors such as TLRs on gut lymphoid and epithelial cells mediates innate immune responses to bacterial molecular patterns and, thereby, orchestrates acquired immunity. The transient protection offered by probiotics against IgE-associated allergic diseases is based on stimulation of TLRs, which produce mediators such as IL-6; these further induce IgA differentiation from naive B cells. Both these events were shown to occur after probiotic administration to infants with eczema, as well as in infants who showed increased levels of serum CRP, IL-10, and IgE at age 6 months.

Similarly, TLR stimulation was also thought to happen after probiotic administration in infants with eczema who showed increased levels of serum CRP, IL-10, and IgE [84]. This probiotic-induced low-grade inflammation was characterized by elevation of CRP, IgE, IgA, and IL-10, the changes typically observed in helminth infection-associated induction of regulatory mechanisms. Moreover, the association of increased CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the view that chronic, low-grade inflammation protects from eczema. The findings emphasize the role of chronic microbial exposure as an immune modulator protecting from allergy

thru activation of Treg cells. Consistently, LAB species such as *Bfdbm bifidum/infantis* and *Lctbs salivarius* were shown to be capable of activating TLR-2 [100]. Oral administration of *Lctbs reuteri* attenuated major characteristics of an asthmatic response, including airway eosinophilia, local cytokine responses, and hyperresponsiveness to methacholine. This effect of *Lctbs reuteri* on the allergic airway response was found to be dependent on TLR-9 [101].

In summary, local influences of probiotics potentially include reduction of gut permeability and systemic penetration of antigens, increased local IgA production, and alteration of local inflammation or tolerance induction. Some possible systemic effects consist of anti-inflammatory effects mediated by TLRs, Th1 skewing of responses to allergens, and activation of tolerogenic DCs, in addition to Treg cell production. [The various effects of different probiotic strains in atopic diseases as well as in AD are summarized in Table 48.1].

Table 48.1 Various mechanisms for effects of probiotic strains in atopic disorders including eczema are shown from experimental (animal) and clinical (human) studies referred in the chapter text

References	Probiotic strain	Effect of probiotic	Outcome
		Maturing gut barrier	
Sudo et al. [44]	Bfdbm	Oral tolerance	↑
Isolauri et al. [33, 46, 66, 92]	LGG	Fecal IgA levels	↑
Isolauri et al. [33, 46, 66, 92]	LGG	Gut-stabilizing effect	↑
Malin et al. [48]	LGG	Gut defense	↑
Kaila et al. [50]	Lctbs	Intestinal permeability	↓
		Immunomodulation	
Niers et al. [54, 74, 98]	*Bfdbm bifidum/infantis*; *Lctbs lactis*	Th2 cytokines	↓
Niers et al. [54, 74, 98]	*Bfdbm bifidum/infantis*; *Lctbs lactis*	IL-10 production	↑
Maassen et al. [2, 121]	*Lctbs casei*	IL-10 production	↑
Maassen et al. [2, 121]	*Lctbs reuteri*	Th1 cytokines	↑
Maassen et al. [2, 121]	*Lctbs casei*	IL-4 production	↑
Kim et al. [58, 78, 99]	*Bfdbm lactis/bifidum*; *Lctbs acidophilus*	IL-10 production	↑
Kim et al. [58, 78, 99]	*Bfdbm lactis/bifidum*; *Lctbs acidophilus*	IL-4 production	↓
Kim et al. [58, 78, 99]	*Bfdbm lactis/bifidum*; *Lctbs acidophilus*	IFN-γ production	↑
Kim et al. [58, 78, 99]	*Bfdbm lactis/bifidum*; *Lctbs acidophilus*	IgE production	↓
Takahashi [59]	*Bfdbm longum*	Th2 cytokines	↓
Takahashi [59]	*Bfdbm longum*	IgE production	↓
Sistek et al. [57]	LGG	IL-10 production	↑
Kruisselbrink et al. [55]	*Lctbs plantarum*	IL-10 production	↓
Hart et al. [75]	*Bfdbm bifidum*	IL-10 production	↑
		Th1/Th2 balance	
Niers et al. [74]	*Bfdbm bifidum*	Most potent dendritic cell polarizer in vitro	↑
Hart et al. [75]	*Bfdbm bifidum*	Human dendritic cell phenotype modulator	↑
Braat et al. [76]	*Lctbs rhamnosus*	Dendritic cell function modulator	↑
Smits et al. [77]	*Lctbs reuteri/casei*	Prime monocyte-derived dendritic cell	↑
		Serum inflammatory parameters	
Maassen et al. [2, 121]	*Lctbs reuteri*	Immunomodulation	↑
Sistek et al. [57]	LGG	Immunomodulation	↓
		Development of tolerogenic dendritic cells	
Niers et al. [54, 74, 98]	Bfdbm	Prime neonatal dendritic cell	↑
Braat et al. [76]	*Lctbs rhamnosus*	Modulates dendritic cell function	↑

(continued)

Table 48.1 (continued)

References	Probiotic strain	Effect of probiotic	Outcome
Smits et al. [77]	*Lctbs reuteri/casei*	Prime monocyte-derived dendritic cell	↑
		Toll-like receptor stimulation	
Hoarau et al. [86]	*Bfdbm bifidum/infantis*; *Lctbs salivarius*	Activate TLR-2	↑
Forsythe et al. [87]	*Lctbs reuteri*	Activate TLR-9	↑
		T-regulatory cell production	
Smits et al. [77]	*Lctbs reuteri/casei*	Prime monocyte-derived dendritic cell	↑
Kim et al. [58, 78, 99]	*Bfdbm bifidum*; *Lctbs acidophilus*	TGF-β production	↑
Feleszko [79]	LGG, *Bfdbm lactis* (Bb-12)	TGF-β production	↑
		T-cell hyporesponsiveness	
Kruisselbrink et al. [55]	*Lctbs plantarum*	Inhibits specific T-cell responses	↑
Braat et al. [76]	*Lctbs rhamnosus*	Modulates dendritic cell function	↑

Abbreviations: *Lctbs* Lactobacillus, *Bfdbm* Bifidobacterium, *LGG* Lactobacillus rhamnosus GG, ↑ = increase in symptoms or negative effect, ↓ = decrease in symptoms or positive effect, ↔ = no change in symptoms or no effect

The Role of Probiotics in the Prevention and Treatment of Skin Allergy Reactions Including AD

The increased prevalence of atopic diseases is nowadays defined as an epidemic. AD is known as the earliest of these conditions, might act as an indicator for the development of IgE-mediated atopic manifestations. Thus, being aware of possible measures, such as probiotic use, to prevent and/or heal atopic disease is essential for the practicing allergist. Here, their role in the prevention and therapy of AD under the recent literature gathered from Medline and Pubmed are discussed. The various effects of different probiotic strains in AD are summarized in Table 48.2.

Experimental (Animal Model) Studies Showing the Role of Probiotics in the Prevention and Treatment of Skin Allergy Reactions Including AD

The various effects of different probiotic strains, referred in the chapter text, on atopic dermatitis, atopic dermatitis-like skin lesions and allergic contact dermatitis in experimental (animal) studies are shown in Table 48.3 as well.

Murine Models of AD Induced by Sensitization to House Dust Mite

Oral administration of *Lctbs delbrueckii* subsp. *lactis* R-037 isolated from traditional South Asian fermented milk "dahi" inhibited the development of AD in NC/Nga mice [65]. In addition, the anti-allergic effect of one strain (T120) of LAB isolated from Mongolian fermented milk using AD model mice (NC/Nga mice) was investigated. And in in vivo studies, intraperitoneal injection of strain T120 subdued AD symptoms in NC/Nga mice [66]. In another study, *Lctbs plantarum* strains from kimchi were investigated for their capacity to inhibit AD (house dust mite-induced dermatitis) in NC/Nga mouse. The three strains, CJLP55, CJLP133, and CJLP136, suppressed AD-like skin lesions and epidermal thickening [67].

Ingestion of heat-treated *Lctbs rhamnosus* GG was shown to prevent development of AD of NC/Nga mice in a study. Maternal and infant mice were fed with food containing or not containing

Table 48.2 The various effects of different probiotic strains, referred in the chapter text, in human atopic and non-atopic eczema are shown

References	Probiotic species	Type of atopic dermatitis	Outcome
		Atopic (IgE-associated) eczema	
Isolauri et al. [33, 46, 66, 92]	Bfdbm or Lctbs	Food (cow's milk) allergy	↓
Wickens et al. [97]	*Lctbs rhamnosus*	IgE-associated eczema	↓
Viljanen et al. [45, 88]	LGG	Atopic eczema/dermatitis syndrome	↓
Sistek et al. [57]	*Lctbs rhamnosus* + *Bfdbm lactis*	Eczema, food-sensitized atopy	↓
Kuitunen et al. [90]	Lctbs + Bfdbm + Propionibacteria	IgE-associated allergy	↓
Majamaa et al. [33]	LGG	Food-sensitized eczema	↓
Rosenfeldt et al. [65]	*Lctbs rhamnosus* + *Lctbs reuteri*	Atopic eczema	↓
Kukkonen et al. [89, 124]	Mix (LGG, *Lctbs rhamnosus* LC705, *Bfdbm breve*, Propionibacterium)	Atopic eczema	↓
Abrahamsson et al. [91]	*Lctbs reuteri* ATCC 55730	Atopic eczema	↓
		Non-atopic eczema	
Kalliomäki et al. [34]	LGG	Atopic dermatitis	↓
Woo et al. [72]	*Lctbs sakei*	Atopic dermatitis	↓
Weston et al. [94]	*Lctbs fermentum*	Atopic dermatitis	↓
Hoang et al. [95]	*Lctbs rhamnosus*	Atopic dermatitis	↓
Hattori et al. [96]	*Bfdbm breve*	Atopic dermatitis	↓
Wickens et al. [97]	*Lctbs rhamnosus*, *Bfdbm animalis* (Bb-12)	Atopic dermatitis	↓
Marschan et al. [73]	Mix (LGG, *Lctbs rhamnosus* LC705, *Bfdbm breve*, Propionibacterium)	Atopic dermatitis	↓
Niers et al. [54, 74, 98]	*Bfdbm bifidum*, *Bfdbm lactis*, *Lactococcus lactis*	Atopic dermatitis	↓
Kim et al. [58, 78, 99]	*Bfdbm bifidum*, *Bfdbm lactis*, *Lctbs acidophilus*	Atopic dermatitis	↓
Dotterud et al. [100]	LGG, *Lctbs acidophilus*, *Bfdbm animalis* (Bb-12)	Atopic dermatitis	↓
Böttcher et al. [68]	*Lctbs reuteri*	Atopic dermatitis (sensitization)	↓
West et al. [56]	*Lctbs casei* F19	Atopic dermatitis	↓
Lodinova-Zadnikova et al. [101]	*Escherichia coli*	Atopic dermatitis (IgE allergies)	↓
Gerasimov et al. [84]	*Lctbs acidophilus*, *Bfdbm lactis*	Atopic dermatitis	↓
		Eczema (atopic dermatitis)	
Kopp et al. [104]	LGG	Atopic dermatitis (wheezing)	↔, (↑)
Taylor et al. [103]	LGG or *Lctbs acidophilus*	Atopic dermatitis (cow's milk allergy)	↔, (↑)
Boyle RJ et al. [67]	LGG	Atopic dermatitis	↔
Gruber et al. [105]	LGG	Atopic dermatitis	↔
Soh et al. [107]	*Bfdbm longum* + *Lctbs rhamnosus*	Eczema and atopic sensitization	↔
Lee et al. [111]	Various	Atopic dermatitis	↔
Brouwer et al. [63]	*Lctbs rhamnosus*	Atopic dermatitis	↔
Kuitunen et al. [90]	Lctbs + Bfdbm + Propionibacteria	Atopic dermatitis	↔
Fölster-Holst et al. [106]	LGG	Atopic dermatitis	↔

Abbreviations: *Lctbs* Lactobacillus, *Bfdbm* Bifidobacterium, *LGG* Lactobacillus rhamnosus GG, ↑ = increase in symptoms or negative effect, ↓ = decrease in symptoms or positive effect, ↔ = no change in symptoms or no effect

heat-treated LGG during pregnancy and breastfeeding, and after weaning. Administration of food containing heat-treated LGG inhibited the onset and development of atopic skin lesions, accompanied by smaller numbers of mast cells and eosinophils in the affected skin sites [46]. Nevertheless, administration of *Lctbs rhamnosus* GG to puppies appeared to reduce immunologic indicators

Table 48.3 The various effects of different probiotic strains, referred in the chapter text, on atopic dermatitis, atopic dermatitis-like skin lesions, and allergic contact dermatitis in experimental (animal) studies are shown

References	Probiotic species	Type of atopic dermatitis in murine	Outcome
		Atopic dermatitis (AD)	
Watanabe et al. [65]	*Lctbs delbrueckii* subsp. *lactis* R-037	Atopic dermatitis	↓
Hayashi et al. [66]	Lactic acid bacteria-T120	Atopic dermatitis	↓
Won et al. [67]	*Lctbs plantarum*	House dust mite-induced AD	↓
Sawada et al. [46]	LGG	Atopic dermatitis	↓
Marsella et al. [102]	LGG	Atopic dermatitis	↓
Ogawa et al. [68]	*Lctbs casei* subsp. *casei*	Atopic dermatitis	↓
		AD-like lesions (trinitrochlorobenzene sensitization)	
Sunada et al. [63]	*Lctbs acidophilus* strain L-55	Atopic dermatitis-like lesions	↓
Inoue et al. [52]	*Lctbs johnsonii* NCC533 (La1)	Atopic dermatitis-like lesions	↓
Tanaka et al. [71]	*Lctbs rhamnosus* CGMCC 1.3724	Atopic dermatitis-like lesions	↓
		AD-like lesions (picrylchloride sensitization)	
Ogawa et al. [68]	*Lctbs casei* subsp. *casei*	Atopic dermatitis-like lesions	↓
Wakabayashi et al. [69]	Lactic acid bacteria KW3110	Atopic dermatitis-like lesions	↓
Segawa et al. [70]	*Lctbs brevis* SBC8803	Atopic dermatitis-like lesions	↓
		Allergic contact dermatitis (dinitrofluorobenzene sensitization)	
Chapat et al. [98]	*Lctbs casei*	Allergic contact dermatitis	↓
Hacini-Rachinel et al. [93]	*Lctbs casei* (DN-114 001)	Allergic contact dermatitis	↓
Weise et al. [96]	*Escherichia coli* Nissle 1917	Allergic contact dermatitis	↓
Park et al. [67]	*Lctbs sakei* probio-65	(1-Chloro-2,4-dinitrobenzene)-induced allergic dermatitis	↓

Abbreviations: Lctbs Lactobacillus, *Bfdbm* Bifidobacterium, *LGG* Lactobacillus rhamnosus GG, ↑ = increase in symptoms or negative effect, ↓ = decrease in symptoms or positive effect, ↔ = no change in symptoms or no effect

(allergen-specific IgE) of AD, although no significant decrease in clinical signs (dermatitis and pruritus) was detected. In this study, the efficacy of the probiotic LGG for the alleviation or prevention of clinical signs of AD in genetically predisposed dogs (2 adult Beagles with severe AD and 16 puppies) was evaluated. LGG was administered to the bitch during the second pregnancy and to the puppies of the second litter from 3 weeks to 6 months of age. Both litters were epicutaneously sensitized to *Dermatophagoides farinae* [102]. A new synbiotic, *Lctbs casei* subsp. *casei* together with dextran, reduced murine allergic reaction such as the development of AD-like skin lesions developed by *Dermatophagoides pteronyssinus* crude extract in NC/Nga mice. This combination significantly decreased clinical skin severity scores and total IgE levels in sera of NC/Nga mice [68].

Murine Models of AD-Like Skin Lesions Induced by Sensitization to Trinitrochlorobenzene

In a study, *Lctbs acidophilus* strain L-55 suppresses the development of AD-like skin lesions induced by repeated application of 2,4,6-trinitrochlorobenzene (TNCB) in sensitized NC/Nga mice. The increase of dermatitis score and ear swelling was also inhibited by strain L-55. Scratching behavior observed in the back and ear was inhibited by strain L-55 as well. Furthermore, strain L-55 also caused an inhibition of histological changes induced by repeated application of TNCB [63].

Oral treatment with probiotic *Lctbs johnsonii* NCC533 (La1) during the specific part of the weaning period prevented the development of AD in model mice, NC/Nga [52]. In another study, La1 was also administered orally to the La1 group from 20 to 22 days after birth. After the induction of skin lesions

in 6-week-old mice, the expression of genes supposedly involved in AD was evaluated. Gene expression of the proinflammatory cytokines [interleukin-8 (IL-8), IL-12 and IL-23] was significantly enhanced in the lesional skin of the control group by the induction of the lesion, whereas gene expression of those in the La1 group was not elevated. Moreover, the La1 group showed a significantly lower gene expression of CD86 in Peyer's patches and mesenteric lymph nodes than the control group. The suppression of proinflammatory cytokines and CD86 expression by primary administration of La1 may significantly contribute to the inhibitory effect on the skin lesion [85].

Oral supplementation with *Lctbs rhamnosus* CGMCC 1.3724 (LPR) prevented development of AD in NC/NgaTnd mice possibly by modulating local production of IFN-γ in a study. Pregnant NC/NgaTnd mice were orally treated with the probiotic strain LPR, which was followed by treatment of pups until 12 weeks of age. LPR-treated mice exhibited significant lower clinical symptoms of dermatitis, reduced scratching frequency, compared with untreated mice. The protective effect was also observed when mice started to be treated at weaning time (5 weeks of age) even with limited supplementation period of 2 weeks. However, treatment of mice with the probiotic starting 1 week after the onset of the disease (8 weeks of age) had limited effects [71].

Murine Models of AD-Like Skin Lesions Induced by Sensitization to Picrylchloride

AD-like skin lesions were induced by sensitization to and repeated challenges with picrylchloride in the Th2-skewed NC/Nga mouse strain. A new synbiotic, *Lctbs casei* subsp. *casei* together with dextran, reduces murine allergic reaction such as the development of AD-like skin lesions in NC/Nga mice. This synbiotic combination significantly decreased clinical skin severity scores induced by picryl chloride in NC/Nga mice [68]. Supplementation with KW3110 strain of LAB significantly attenuated the onset and exacerbation of AD-like skin lesions, accompanied by less mast cell infiltration [69].

Oral administration of heat-killed *Lctbs brevis* SBC8803 ameliorated the development of dermatitis in AD model NC/Nga mice. Eight-week-old male NC/Nga mice were sensitized by the topical application of picryl chloride to foot pads and shaved abdomen. These mice were boosted with picryl chloride by topical application onto the ears once a week for 9 weeks. The mice ($n = 10$ per group) were fed a diet containing 0%, 0.05% or 0.5% of heat-killed *Lctbs brevis* SBC8803 from 2 weeks before the first sensitization to the end of the study. Oral administration of *Lctbs brevis* SBC8803 significantly inhibited ear swelling, and suppressed the development of dermatitis in a dose-dependent manner [70].

Murine Models of Allergic Contact Dermatitis Induced by Sensitization to Dinitrofluorobenzene

The aim of a few studies was to examine whether *Lctbs casei* could affect antigen-specific CD8+-T cell-mediated skin inflammation. In a study by Chapat et al., contact hypersensitivity to the hapten 2,4-dinitrofluorobenzene, a model of allergic contact dermatitis mediated by CD8+-cytotoxic T-lymphocytes and controlled by CD4+-Treg cells, was used. This study provides the first evidence that oral administration of *Lctbs casei* can reduce antigen-specific skin inflammation by controlling the size of the CD8+-effector pool [98]. Similarly, oral treatment with the probiotic bacteria *Lctbs casei* (DN-114 001) alone alleviated antigen-specific skin inflammation mediated by either protein-specific CD4(+) T cells or hapten-specific CD8(+) T cells in hapten-sensitized mice. In the model of CD8(+) T cell-mediated skin inflammation, which reproduces allergic contact dermatitis in human, inhibition of skin inflammation by *Lctbs casei* was due to attenuation of the recruitment of CD8(+) effector T cells into the skin during the elicitation (i.e., symptomatic) phase of contact hypersensitivity.

These data demonstrate that orally administered *Lctbs casei* (DN-114 001) efficiently alleviate T cell-mediated skin inflammation without causing immune suppression [93].

In sensitized BALB/c mice, skin inflammation was induced by topical allergen application. *E. coli* Nissle 1917 was administered orally in a preventive manner and improved allergen-induced dermatitis dose-dependently, consistent with a reduction of epidermal thickness was detected in eczematous skin [96].

Lctbs sakei probio-65 was isolated from kimchi, a traditional Korean fermented food, was found to be effective in reducing allergic dermatitis in chemical allergen (1-chloro-2,4-dinitrobenzene)-induced mice as well [67].

Clinical (Human) Studies Showing Probiotics' Effects in Skin Allergy Reactions Including Eczema

Mostly reported clinical (human) studies showing probiotics' effects in skin allergy reactions have been related to atopic dermatitis (eczema). Here, probiotics' effects in human AD are being discussed according to the IgE-sensitized (atopic) vs. non-IgE-sensitized (non-atopic) eczema groups.

Any Difference for IgE-Sensitized (Atopic) vs. Non-IgE-Sensitized (Non-atopic) Eczema Groups?

A number of studies could only relate probiotic benefits to a certain subset of AD patients. In support of the efficacy of probiotics in IgE-sensitized children, some other studies also demonstrated comparable results as well. In brief: Treatment with *Lctbs rhamnosus* for the first 2 years of life was associated with a significant reduction in the prevalence of any IgE-associated eczema by about a half [34]. Another study demonstrated that LGG alleviated atopic eczema/dermatitis syndrome symptoms in IgE-sensitized infants [47]. In food-sensitized atopic children, the efficacy of the probiotics such as *Lctbs rhamnosus* and *Bfdbm lactis* was demonstrated too [59]. This effect was more pronounced in patients with a positive skin prick test and increased IgE levels.

Yet, some other studies failed to demonstrate that the severity and frequency of AD were decreased with the supplementation of probiotics, regardless of their IgE sensitization status. For instance; Boyle et al. and others could not show any effect even for LGG in infants with AD [78]. A few meta-analyses also could not confirm that IgE sensitization was indeed a factor in determining the efficacy of probiotics in atopic children. However, the heterogeneity between studies may be attributable to probiotic strain-specific effects and other factors as well, meaning that some probiotic strains may still have a therapeutic role in eczema [31, 32].

IgE-Sensitized (Atopic) Eczema Therapy and Prevention

Recently published one of the largest studies by Viljanen et al. to date compared LGG or a probiotic mix (LGG, *Lctbs rhamnosus* LC705, *Bfdbm breve* Bb99, and *Propionibacterium freudenreichii* ssp. *shermanii* JS) with placebo. In this study, 230 Finnish children with AD were treated for 4 weeks with LGG, a mixture of four probiotic strains or placebo. With supplementation with probiotics (LGG), Viljanen et al. found significant improvement on the SCORAD index only in "IgE-sensitized-cow's milk-allergic-infants" of the atopic eczema/dermatitis syndrome (AEDS). Only in the subgroup of IgE-sensitized children, did the LGG group show a greater reduction in SCORAD than the placebo group but this effect could have been due to a higher baseline score in this subgroup. There was no

difference between the groups at the end of 4-week therapy and 4 weeks after therapy was discontinued. Contrary to what would be expected, improvement was seen 4 weeks after discontinuation of therapy rather than during treatment [47, 103]. Rosenfeldt et al. from Denmark in a study, two lyophilized probiotic Lctbs strains (lyophilized *Lctbs rhamnosus* 19070-2 and *Lctbs reuteri* DSM 122460) were given in combination for 6 weeks to 1- to 13-year-old (mean age, 5.2 years) children with AD. This study used two different Lctbs species in older children. A combination of these was beneficial in the management of AD. Statistically significant improvement in SCORAD score was seen only in a subset of children with positive skin prick test results and elevated IgE levels [73]. Another study by Sistek et al. showed the efficacy of the probiotics *Lctbs rhamnosus* and *Bfdbm lactis* in food-sensitized children [60].

A study by Finnish group used the same probiotic mixture with prebiotic. Kukkonen et al. in a trial using probiotic mix (*Lctbs rhamnosus* GG, *Lctbs rhamnosus* LC705, *Bfdbm breve* Bb99; and *P. freudenreichii* ssp. *shermanii* JS) and prebiotic galacto-oligosaccharides demonstrated that the prevention of atopic eczema in high-risk Finnish infants is possible by modulating the infant's gut microbiota with probiotics and prebiotics. Probiotic treatment compared with placebo reduced IgE-associated (atopic) diseases. Probiotic treatment also reduced eczema and atopic eczema [104]. In 2009, in a study by Kuitunen et al. 1223 Finnish mothers were randomized with infants at high risk for allergy to receive the same probiotic mixture (two Lactobacilli, Bifidobacteria, and Propionibacteria) or placebo during the last month of pregnancy and their infants to receive it from birth until age 6 months. Infants also received a prebiotic galacto-oligosaccharide or placebo. At 5 years, the cumulative incidence of allergic diseases (eczema, food allergy, allergic rhinitis, and asthma) and IgE sensitization were evaluated. Frequencies of allergic and IgE-associated allergic disease and sensitization in the probiotic and placebo groups were similar. However, less IgE-associated allergic disease occurred in cesarean-delivered children receiving probiotics. No allergy-preventive effect that extended to age 5 years was achieved with perinatal supplementation of probiotic bacteria to high-risk mothers and children. It conferred protection only to cesarean-delivered children [105].

Similarly; Abrahamsson et al. could not confirm a preventive effect of probiotics (*Lctbs reuteri* ATCC 55730) on infant eczema in a recently published study. However, he observed that the treated infants had less IgE-associated eczema at 2 years. Moreover, skin prick test reactivity was also less common in the treated group than in the placebo group, but this difference reached significance only for infants with allergic Swedish mothers [106].

In conclusion; all of these studies taken together demonstrate that probiotics are might not be effective and/or therapeutic for all children with AD, but offer benefit in a subset of IgE-sensitized children.

Non-IgE-Sensitized (Non-atopic) Eczema Therapy and Prevention

Until now, several clinical studies have been published that have focused on the use of probiotics for therapy and primary prevention of atopic diseases. To date, the results of at least 15 prospective preventive studies with different Lctbs or Bfdbm strains (or mixture) in children at high risk for allergic diseases have been published.

The first study in the literature by Isolauri et al. analyzed a benefit of LGG in mild AD disease in 1997. They observed 27 exclusively breastfed infants (median age 4–6 months) with mild AD (median SCORAD score of 16), receiving extensively hydrolysed whey formula with (LGG or Bfdbm strain) or without probiotics (placebo) for 8 weeks. They showed a reduction in the SCORAD by 15 points (from 16 to 1) for the LGG and by 16 points (from 16 to 0) for the Bfdbm arm, as compared with a reduction of 2–6 points (from 16 to 13–4) in the placebo arm. However, 1 month after therapy, SCORAD scores were comparable with those of placebo. Therefore, the probiotic effect was limited to acceleration of improvement in infants with mild disease [33]. The same investigators subsequently published two additional studies. One of these studies compared LGG with *Bfdbm lactis* Bb-12, both

of which showed a significant improvement in SCORAD score over placebo. However, after 6 months, the median SCORAD score was zero in all groups, again suggesting that the effect is limited to rapid initiation of improvement [107]. The other study underlined the importance of viability for probiotic species. The use of heat inactivated LGG resulted in adverse gastrointestinal symptoms with diarrhea, and study recruitment was halted. They concluded that supplementation of infant formulas with viable but not heat-inactivated LGG was found to be a potential approach for the management of atopic eczema and cow's milk allergy [108].

In an earlier study by Viljanen et al. probiotics have been suggested to be useful in children with AEDS. In 2010, a study by Woo et al. was performed to assess the clinical effect of *Lctbs sakei* supplementation in children with AEDS. In this study, children aged 2–10 years with AEDS with a minimum SCORAD score of 25 were randomized to receive either daily *Lctbs sakei* KCTC 10755BP or daily placebo supplementation for 12 weeks. At week 12, SCORAD total scores adjusted by pretreatment values were lower after probiotic treatment than after placebo treatment. There was a 31% improvement in mean disease activity with probiotic use compared with a 13% improvement with placebo use. Therefore, significant differences in favor of probiotic treatment were also observed in proportions of patients achieving improvement of at least 30 and 50%. Interestingly, clinical improvement in this study was not just observed in the subgroup of IgE-sensitized children, contrary to Viljanen et al. study, and it was regardless of IgE sensitization [83]. Weston et al. from Australia published their experience with using *Lctbs fermentum* VRI-003 PCC for 8 weeks in 53 infants with AD. After 16 weeks the probiotic group had significant reduction of SCORAD scores while the placebo group did not. *Lctbs fermentum* caused a significant reduction in SCORAD scores. Although the change in SCORAD score from baseline in the probiotic group was significant, the difference between the probiotic and placebo groups did not reach significance by week 16 [109]. In a study by Hoang et al., they followed 14 cases of pediatric patients (ages of 8 months to 64 months) with a history of resistant eczema for a period of at least 6 months. All of these children received *Lctbs rhamnosus* cell lysate daily as an immunobiotic supplement. The results of this open label non-randomized clinical observation showed a substantial improvement in quality of life, skin symptoms and day- and nighttime irritation scores in children with the supplementation of *Lctbs rhamnosus* lysate. There were no intolerance or adverse reactions observed in these children. *Lctbs rhamnosus* cell lysate may thus be used as a safe and effective immunobiotic for the treatment and prevention of childhood eczema [110]. *Bfdbm breve* has been reported by Hattori et al. to improve cutaneous symptoms of AD patients. Fifteen children with AD who had Bfdbm-deficient microflora were selected for this study. Eight subjects in the Bifidobacteria-administered group were given oral administration of lyophilized *Bfdbm breve* M-16V strain. In the Bifidobacteria-administered group, the proportion of Bfdbm in the fecal microflora was increased and the proportion of aerobic bacteria was decreased after 1 month of administration. Furthermore, significant improvement of allergic symptoms (in cutaneous symptom and total allergic scores) was also observed in the Bifidobacteria-administered group. The tendency of allergic symptom improvement was remarkable compared with the control group; however, there was no correlation between changes in fecal microflora and allergic symptoms [111].

The Finnish study of Kalliomäki et al. was the first report to describe that the frequency of AD in the probiotic group was half that of the placebo. This hallmark study demonstrated that administration of LGG for 1 month before and 6 months after birth to their infants was associated with a significant reduction in the cumulative incidence of eczema during the first 7 year of life. The effect of probiotics on preventing AD has been demonstrated in infants of Finnish pregnant mothers with a strong family history of eczema, allergic rhinitis or asthma. The frequency of developing AD in the offspring was significantly reduced by 2, 4, and 7 years, by 50%, 44%, and 36%; respectively. But there were no preventive effects on atopic sensitization and onset of respiratory allergic diseases [34].

Wickens et al. studied a differential effect of two probiotics in the prevention of eczema and atopy. Infants receiving *Lctbs rhamnosus* had a significantly reduced risk of eczema, compared with placebo, but this was not the case for *Bfdbm. animalis* subsp. *lactis*. In a double-blind, randomized

placebo-controlled trial of infants at risk of allergic disease, pregnant women were randomized to take *Lctbs rhamnosus* HN001, *B. animalis* subsp. *lactis* strain HN019 or placebo daily from 35 weeks gestation until 6 months if breastfeeding, and their infants were randomized to receive the same treatment from birth to 2 years (*n*: 474). Infants receiving *Lctbs rhamnosus* had a significantly reduced risk of eczema compared with placebo, but this was not the case for *Bfdbm animalis* subsp. *lactis*. There was no significant effect of *Lctbs rhamnosus* or *Bfdbm animalis* subsp. *lactis* on atopy. *Lctbs rhamnosus* (71.5%) was more likely than *Bfdbm animalis* subsp. *lactis* (22.6%) to be present in the feces at 3 months, although detection rates were similar by 24 months. The authors found out that supplementation with *Lctbs rhamnosus*, but not *Bfdbm animalis* subsp. *lactis*, substantially reduced the cumulative prevalence of eczema, but not atopy, by 2 years [112].

In a randomized double-blind study by Marschan et al., probiotic bacteria (*Lctbs rhamnosus* GG (ATCC 53103), *Lctbs rhamnosus* LC705, *Bfdbm breve* Bb99, and Propionibacterium freudenreichii ssp. Shermanii JS) or placebo were given for 1 month before delivery to mothers and for 6 months to infants with a family history of allergy. Infants receiving probiotic bacteria had higher plasma levels of CRP, total IgA, total IgE, and IL-10 than infants in the placebo group. Increased plasma CRP level at age 6 months was associated with a decreased risk of eczema and with a decreased risk of allergic disease at age 2 years, when adjusted with probiotic use. The association of CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the view that chronic, low-grade inflammation protects from eczema. Probiotic-induced low-grade inflammation was characterized by elevation of IgE, IgA, and IL-10, the changes typically observed in helminth infection-associated induction of regulatory mechanisms [84] (please see the Sect. "Experimental (Animal) and Clinical (Human) Studies Showing Mechanisms of Probiotics' Effects in AD and Skin Allergy Reactions").

In the PandA study of Niers et al. administered a mixture of probiotic bacteria (*Bfdbm bifidum* W23, *Bfdbm lactis* W52, and *Lactococcus lactis* W58; Ecologic Panda) for 6 week prenatally to mothers of high-risk children and to their offspring for the first 12 months of life. Although cumulative incidence of atopic eczema and IgE levels were similar in both treated and placebo groups, the parental reported eczema was significantly lower during the first 3 months of life in infants receiving probiotics. This particular combination of probiotic bacteria showed a preventive effect on the incidence of eczema in high-risk children, which seems to be sustained during the first 2 years of life. In addition to previous studies, the preventive effect appeared to be established within the first 3 months of life in this study [113].

In a trial by Kim et al., 112 pregnant women with a family history of allergic diseases received a mixture of *Bfdbm bifidum* BGN4, *Bfdbm lactis* AD011, and *Lctbs acidophilus* AD031, starting at 4–8 weeks before delivery and continuing until 6 months after delivery. The cumulative incidence of eczema during the first 12 months was reduced significantly in probiotic group; however, there was no difference in serum total IgE level or the sensitization against food allergens between the two groups. Prenatal and postnatal supplementation with a mixture of probiotics is an effective approach in preventing the development of eczema in infants at high risk of allergy during the first year of life [114].

In a randomized, double-blind trial by Dotterud et al., probiotics was given in pregnant women to prevent allergic disease. In this study, children from a nonselected maternal population, women received probiotic milk or placebo from 36 weeks of gestation to 3 months postnatally during breastfeeding. The probiotic milk contained *Lctbs rhamnosus* GG, *Lctbs acidophilus* La-5 and *Bfdbm animalis* subsp. *lactis* Bb-12. At 2 years of age, all children were assessed for atopic sensitization, AD, asthma and allergic rhinoconjunctivitis. Probiotics given to nonselected mothers reduced the cumulative incidence of AD, but had no effect on asthma or atopic sensitization [115].

Böttcher et al.'s study demonstrated that *Lctbs reuteri* supplementation during pregnancy associated with reduced risk of sensitization during infancy. Swedish pregnant women were treated with *Lctbs reuteri* (*n*: 54) or placebo (*n*: 55) from gestational week 36 until delivery. The infants were followed prospectively for 2 years regarding development of eczema and sensitization as defined

by a positive skin prick test and/or circulating allergen-specific IgE antibodies at 6, 12, and 24 months of age [79].

Of note, another recently published Swedish study demonstrated that administration of *Lctbs casei* F19 during weaning significantly reduced the incidence of eczema, indicating that proper timing of the probiotic intervention is a critical factor. This study also supports the notion that there is more than a single window of opportunity to manage allergic diseases. This study evaluated the effects of feeding with Lctbs F19 during weaning period on the incidence of eczema and Th1/Th2 balance. In this intervention trial by West et al., infants were fed cereals with (*n*: 89) or without Lctbs F19 (*n*: 90) from 4 to 13 months of age. The cumulative incidence of eczema at 13 months was 11 and 22% in the probiotic and placebo groups, respectively (*p*: <0.05). At 13 months of age, the IFN-γ/IL-4 mRNA ratio was significantly higher in the probiotic compared with the placebo group. The higher Th1/Th2 ratio in the probiotic compared with the placebo group suggests enhancing effects of Lctbs F19 on the T cell-mediated immune response. In contrast, there were no differences between groups in serum IgE concentrations. As a result, feeding Lctbs F19 during weaning could be an effective tool in the prevention of early manifestation of allergy such as eczema [59].

Oral administration of probiotic *E. coli* after birth in the early postnatal period by Lodinova-Zadnikova et al. reduced frequency of serum specific IgE allergies later in life (after 10 and 20 years) [116].

Gerasimov et al. conducted a study to assess the clinical efficacy and impact of *Lctbs acidophilus* DDS-1, *Bfdbm lactis* UABLA-12 with fructo-oligosaccharide on peripheral blood lymphocyte subsets in preschool children with moderate-to-severe AD. In a randomized, double-blind, placebo-controlled, prospective trial of 90 children aged 1–3 years with moderate-to-severe AD who were treated with a mixture of probiotics with fructo-oligosaccharide for 8 weeks vs. placebo. At the final visit, the percentage significant decrease in SCORAD was 33.7% in the probiotic group compared with 19.4% in the placebo group. Children receiving probiotic showed a greater decrease in the mean SCORAD score than did children from the placebo group at week 8. The administration of a probiotic mixture and fructo-oligosaccharide was associated with significant clinical improvement in children with AD, with corresponding lymphocyte subset changes in peripheral blood [97].

In conclusion; here probiotics were more likely to be effective in treating moderately severe AD as well as mild atopic disease. Although not every study result above was significant, the effect of probiotics did not seem to be greater just in the IgE-sensitized group than in the non-IgE-sensitized group. Nevertheless, there have been several reports in the literature showing no effect of probiotics, which are being discussed the section below.

No Therapeutic or Preventive Effect of Probiotics in AD Regardless of IgE Sensitization

It is striking that the proportion of children with AD and allergic sensitization such as in the study of Taylor and Huurre et al. was significantly higher in the probiotic group [117]. In Taylor et al.'s trial probiotic supplementation postnatally failed to reduce the risk of AD and increased the risk of allergen sensitization in high-risk children. Newborns of women with allergy (*n*: 231) received either *Lctbs acidophilus* (LAVRI-A1) or placebo daily for the first 6 months of life. Children were assessed for AD and other symptoms at 6 and 12 months and had allergen skin prick tests at 12 months of age. At 6 and 12 months, AD rates were similar in the probiotic and placebo groups. At 12 months, the rate of sensitization was significantly higher in the probiotic group. The presence of culturable Lactobacilli or Bfdbm in stools in the first month of life was not associated with the risk of subsequent sensitization or disease; however, the presence of Lctbs at 6 months of age was associated with increased risk of subsequent cow's milk sensitization. Early probiotic supplementation with *Lctbs acidophilus* did not reduce the risk of AD in high-risk infants and was associated with increased allergen sensitization in infants receiving supplements. There were three major differences between Taylor's study and the

others. The type of probiotic product (*Lctbs acidophilus*), the supplementation period (1 year) as well as the timing of the introduction of the probiotic were different. Taylor et al. administered the probiotic supplement postnatally, while other studies administered probiotics before and after birth. Prenatal supplementation may prove to be crucial for the preventive benefit of probiotics in this disorder. The data from the Taylor et al.'s study point in the same direction regarding allergic sensitization, also suggesting that the use of probiotics for primary prevention must be exercised with caution [118].

Similarly, a randomized, double-blind, placebo-controlled prospective trial by Kopp et al. of probiotics for primary prevention did show no clinical effects of LGG supplementation. Hundred and five pregnant women from families with ≥1 member (mother, father, or child) with an atopic disease were randomly assigned to receive either the probiotic LGG or placebo. The supplementation period started 4–6 weeks before expected delivery, followed by a postnatal period of 6 months. The primary end point was the occurrence of AD at the age of 2 years. Secondary outcomes were severity of AD, recurrent episodes of wheezing bronchitis, and allergic sensitization at the age of 2 years. Notably, children with recurrent (≥5) episodes of wheezing bronchitis were more frequent in the LGG group (26%), as compared with the placebo group (9%). As a result, supplementation with LGG during pregnancy and early infancy neither reduced the incidence of AD nor altered the severity of AD in affected children but was associated with an increased rate of recurrent episodes of wheezing bronchitis. No difference was observed between both groups in total Ig E concentrations or numbers of specific sensitization to inhalant allergens [119].

Furthermore; prenatal probiotic LGG treatment during pregnancy was not associated with reduced risk of eczema or IgE-associated eczema in a randomized controlled trial by Boyle [78]. In a recent study, 250 pregnant women were recruited carrying infants at high risk of allergic disease to a randomized controlled trial of probiotic supplementation (LGG) from 36 weeks gestation until delivery. Gruber et al.'s study also did not show any effect for LGG in infants with AD regardless of their IgE sensitization status [120].

However, a study from the Netherlands by Brouwer et al. and another study from Germany by Folster-Holst et al. showed no effect of LGG in infants with AD regardless of their IgE sensitization status. In a study conducted by Brouwer et al., after 4–6 weeks of baseline and double-blind, placebo-controlled challenges for diagnosis of cow's milk allergy, infants less than 5 months old with AD received a hydrolysed whey-based formula as placebo (*n*: 17), or supplemented with either *Lctbs rhamnosus* (*n*: 17) or LGG (*n*: 16) for 3 months. No statistically significant effects of probiotic supplementation on SCORAD, sensitization, inflammatory parameters or cytokine production between groups were found. No clinical or immunological effect of the probiotic bacteria used in infants with AD [74]. A similar prospective study by Fölster-Holst et al. was performed to reassess the efficacy of orally administered LGG in infants with AD. In a randomized, double-blind, placebo-controlled study, 54 infants aged 1–55 months with moderate to severe AD were randomized to receive LGG or to placebo during an 8-week intervention phase. At the end of treatment there were no significant differences between the groups with respect to clinical symptoms (SCORAD, pruritus, and sleep loss), immunological parameters, or health-related quality of life of the parents [121]. Additionally; Soh et al. in a clinical trial involving 253 infants with a family history of allergic disease utilized probiotic supplementation (*Bfdbm longum*+*Lctbs rhamnosus*) in the first 6 months of life in Asian infants at risk and evaluated the effects on eczema and atopic sensitization at the age of 1 year. Early life administration of a cow's milk formula supplemented with probiotics showed no effect on prevention of eczema or allergen sensitization in the first year of life in Asian infants at risk of allergic disease [122].

In conclusion; LGG was mostly used probiotic species in these studies. Firstly used by Kalliomaki et al. [34] with a success however, other groups including Brouwer, Boyle, Kopp, Gruber, and Fölster-Holst et al. [74, 78, 118, 119, 121] could not demonstrate any benefit in AD. For instance: Kopp et al. have shown that the probiotic LGG has no preventive effect on the development or the severity of AD at the age of 2 years in a German population of infants at high risk. Instead, there was a significantly

higher risk of ≥5 episodes with wheezing bronchitis during the first 2 years in the LGG group, as compared with placebo. There were several methodological differences between these studies: Kopp et al. adapted the protocol of Kalliomäki et al. and continued to supplement LGG for 3 months after birth to the breastfeeding mothers and the following 3 months only to the neonates. This modification was made to achieve a more consistent probiotic delivery. Second, Finnish mothers received supplementation during the last 4 weeks of pregnancy, whereas pregnant women in this population commenced with LGG or placebo for 4–6 weeks. They extended the prenatal supplementation period, because a 4-week period is thought to possibly be too short for suspected in utero effects of LGG supplementation. Also, population in this study by Kopp et al. was being of higher risk compared with the Finnish population, which might account for the differing results. And more infants with older siblings were recruited compared with the Finnish study. Lastly, the Finnish and German populations are of different genetic background.

A randomized, double-blind, placebo-controlled study was conducted in 34 adult type AD subjects who were treated with conventional topical corticosteroid and tacrolimus. In these kinds of patients, heat-killed *Lctbs paracasei* K71 (LAB diet) may have shown to have some benefits as a complementary therapy for adult AD patients who are managed with the conventional treatment [123].

In a double-blind, placebo-controlled, crossover study, *Bfdbm animalis* subsp. *lactis* LKM512 yogurt was given for 4 weeks to ten adult AD patients (four males + six females; average age: 22 years) who were diagnosed with moderate AD. Scores of itch and burning tended to improve to a greater extent by LKM512 yogurt consumption than by placebo consumption. LKM512 yogurt consumption may be effective against intractable adult-type AD [124].

A prospective, double-blind, placebo-controlled clinical study with a cream containing a 5% lysate of the nonpathogenic bacteria *Vitreoscilla filiformis* was performed. Seventy-five volunteers with AD (6–70 years of age) were randomized to receive either *V. filiformis* cream 5% or vehicle cream daily for 30 days. Compared with placebo, *V. filiformis* lysate significantly decreased SCORAD levels and pruritus. Active cream significantly decreased loss of sleep from day 0 to day 29. *V. filiformis* lysate reduced *S. aureus* colonization of the skin. *V. filiformis* lysate significantly improved AD, which may be in part due to reduction of *S. aureus*, but seems to relate in most parts to a direct immunomodulatory effect on skin-associated immune responses [125].

Recent Meta-analyses and Reviews from Literature

A meta-analysis of the evidence from randomized controlled trials by Betsi et al. on probiotics for the treatment or prevention of AD: the results of 13 relevant randomized (placebo)-controlled trials were reviewed: 10 of which evaluated probiotics as treatment and 3 for prevention of AD. Four RCTs suggested that there was a statistically significant decrease in SCORAD after probiotic administration to infants or children with AD for 1 or 2 months compared with that after placebo. While in two RCTs SCORAD was significantly reduced after treatment with Lactobacilli only in children with IgE-associated AD. In three RCTs, the change in SCORAD was not statistically significant between probiotic- and placebo-treated children, although in one of these trials SCORAD was significantly lower after probiotic than with placebo treatment in food-sensitized children. As a result; probiotics, especially *Lctbs rhamnosus* GG, seem to be effective for the prevention of AD and they were also found to reduce the severity of AD in approximately half of the RCTs evaluated [80]. Likewise, Zhu et al. did a meta-analysis of LAB as probiotics for the primary prevention of infantile eczema. The data from this meta-analysis suggested that lactic acid probiotics combined with other probiotics play a role in the prevention of infantile eczema. Conversely, another recent meta-analysis did not show a therapeutic difference among children receiving probiotics. This analysis excluded six of the ten studies published, making the validity of the report questionable [126].

In a review article; atopic disease and/or food hypersensitivity outcomes were assessed by Osborn et al. in six studies enrolling 2,080 infants, but outcomes for only 1,549 infants were reported. Meta-analysis of five studies reporting the outcomes of 1,477 infants found a significant reduction in infantile eczema. When the analysis was restricted to studies reporting atopic eczema (confirmed by skin prick test or specific IgE), the findings were no longer significant. All studies reporting significant benefits used probiotic supplements containing *Lctbs rhamnosus* and enrolled infants at high risk of allergy [127]. Another recent meta-analysis suggested that probiotics may benefit children and infants with the disorder. The meta-analysis identified ten randomized, controlled trials. A significant overall benefit was demonstrated after the use of probiotics, resulting in a reduction of the SCORAD scores compared to placebo. LGG appeared to be more effective than other probiotic preparations and children with more severe disease were more likely to benefit from the use of probiotics [128]. This remarkable meta-analysis was done to determine whether probiotics are efficacious in treating AD and to explore whether type of probiotic used, duration of therapy, patient age, severity of disease, and IgE sensitization are factors in determining efficacy. For this meta-analysis of randomized controlled trials describing the efficacy of probiotics in AD, a comprehensive search was performed of databases through January 2008. Eleven studies were identified, and data from ten studies (n: ≥678) were available to analyze. There was an overall statistically significant difference favoring probiotics compared with placebo in reducing the SCORAD index. Children with moderately severe disease were more likely to benefit. Duration of probiotic administration, age, and type of probiotic used did not affect outcome. Data from this meta-analysis suggest a modest role for probiotics in pediatric AD and the effect is seen in moderately severe rather than mild disease [128]. Lee et al. meta-analyzed ten double-blind randomized controlled clinical trials. And they found out that current evidence was more convincing for probiotics' efficacy in prevention than treatment of pediatric AD [129].

However, in a Cochrane Review by Boyle et al. concluded that the evidence suggests that probiotics are not an effective treatment for eczema, and probiotic treatment carries a small risk of adverse events [130]. Another review of 13 studies of probiotics for treating established eczema by Williams et al. did not show convincing evidence of a clinically worthwhile benefit [131].

Taken together, most of these meta-analytic studies show a mild-moderate benefit over placebo for the treatment and/or preventionof AD. However, several of the studies still show no benefit. Some probiotics appeared to be more effective than other probiotic preparations and in children with more severe disease. It seems that duration of probiotic administration, age, and type of probiotic used did not affect outcome. Although there was a reduction in clinical eczema score in infants, this effect was not consistent between studies and caution is advised in view of methodological concerns regarding included studies.

Affecting Factors Coexisting in Various Studies and Why Inconsistent Results in Some Studies?

Most of the studies have been conducted in small numbers of patients and results have varied considerably, even with the same strain of probiotics. When compared to the hallmark Finnish Kalliomaki study, there were also a number of other key differences between these studies that could contribute to the disparity in clinical findings. *Firstly* (biological difference of probiotic strains), different probiotic species were used in various studies. *Lctbs rhamnosus* GG is the strain that has been most studied. Probiotic doses have also varied considerably between the studies. Although there are noted biological differences between strains, various strains have been observed to have different immunologic effects both in vivo and in vitro [2]. Thus, the effects of preparations differ markedly, and the concept itself might be misleading in these studies and could be changed: continuous change in the supplemented strain could lead to continued immunologic stimulus and sustained and stronger effects. As a result,

on the basis of these studies, immunologic effects expressed as chronic low-grade inflammation were more pronounced with LGG alone rather than with the mixture of four strains used in some prevention studies [84]. This might explain the more sustained preventive effect observed earlier. The results of various studies also demonstrate that findings from any probiotic bacteria cannot be extrapolated to other probiotic bacteria [1]. *Secondly* (use of probiotics in prenatal/intrauterine or postnatal or weaning period), Finnish mothers commenced supplementation during pregnancy, whereas some supplementation began in the first days of life. Prenatal/intrauterine use of probiotics would imply a direct immune effect in utero rather than any effect on postnatal colonization. However, it seems unlikely that supplementation for only a few weeks in the antenatal period alone would account for such significant differences in study outcomes, although this remains a possibility. *Thirdly* (intervention methods), in most studies all babies received the supplement directly, regardless of feeding method, whereas in the Finnish and other studies, the mother took the probiotics if babies were breast-fed. Therefore, the Finnish probiotic group and others included breast-fed infants who did not receive probiotics directly in addition to the bottle-fed infants who received probiotics for 6 months. *Fourthly* (postnatal assessment time), some researchers assessed clinical outcomes at 12 months of age, whereas the effects on AD in the Finnish or other studies were reported at 2, 4 and subsequently at 7 years of age. AD typically begins in the first year of life, and it is possible that more children could become affected in their second year of life. And in young infants, the immune system is still developing. There is still a possibility to direct it toward tolerance. In older children, the allergic phenotype is already established, and here one may only be able to relieve the symptoms. *Fifthly* (atopy risk and host factors of targeted group), some studies were performed in high-risk population all had maternal allergic disease confirmed by SPT, whereas the Finnish and other population included children with maternal, paternal, or sibling allergy. This may lead to some population being of slightly higher risk, compared with the Finnish and other population at the same age. Explanations for varied study results include host factors such as genetic susceptibility, environmental factors such as geographic region and diet [132]. Genotyping of study patients in relation to different genes predisposing to allergic diseases may help to find patients that might especially benefit from probiotic intervention. For example, two independent mutations in the gene encoding the epidermal protein filaggrin have been shown to be strong predisposing factors for childhood eczema [133]. Of note, these same mutations have recently been demonstrated to be associated not only with eczema-associated asthma susceptibility but also with asthma severity independent of eczema status. More generally, any means to better stratify or select defined subpopulations of subjects (e.g., patients with food allergy as a separate group) would help in clarifying the potency and limits of probiotic interventions against atopic diseases. *Sixthly* (other methodological setup), there may be still due to differences in the clinical and methodological setup (additional treatments such as topical treatment or feeding hydrolyzed infant formulae and importantly, different probiotic preparations or formulations or combinations). Other differences include age (6–18 months) of the studied children and an intervention period (16 weeks) of time. *Seventhly* (nomenclature of the disease), to date, randomized clinical trials of probiotics in allergic diseases have mostly focused on children with eczema and atopic eczema. The definitions of the disease have recently been revised by an international expert group [35–37]. In many of the studies published before the revision of the nomenclature, different definitions have been used, making direct comparisons between the studies difficult. The severity of AD at the start of an intervention may influence the outcome as well, as you imagine.

Safety

Probiotics available as food ingredients or dietary supplements that contain microorganisms have been used extensively in food processing for years, with a long history of safety and no adverse effects on metabolism. However, when considering the safety of probiotics, potential adverse effects include

systemic infections, altered metabolism, and gene transfer [3]. A recent report has identified *Lctbs septicemia* in two children with short bowel syndrome who were receiving LGG supplementation for control of bacterial overgrowth [4]. Land et al. recently reported LGG probiotic sepsis occurring in immunocompromised infants and children [5]. A medically fragile infant 6 weeks of age became septic with a strain of LGG that was being provided as a supplement. Molecular DNA fingerprinting confirmed that the LGG probiotic supplement was the bacterial isolate from the infant. Neonatal sepsis and meningitis that were apparently associated with the administration of a probiotic supplement were also reported [6, 7]. Children with abnormal immune function, premature infants, and those with indwelling central lines should use these products with caution, because many species such as Lactobacilli, Streptococci, and Enterococci are potential opportunistic pathogens. Owing to the theoretical risk of immunomodulation, especially in immunocompromised hosts or those with autoimmune disorders, few reports of probiotic-related disease have been reported [8].

Bifidobacteria have also been consumed in infant formulas for ≥15 years worldwide and have not been associated with any pathologic or adverse event. In particular, studies have documented safety and adequate growth with *Bfdbm lactis* in infants from birth and in vulnerable populations, including preterm infants, malnourished infants, and infants born to mothers with HIV disease. From the safety point of view, according to current available information, Bifidobacteria, particularly *Bfdbm lactis*, has a uniquely strong safety profile, making it a good probiotic candidate for newborns and young infants [9]. Lactobacilli, particularly *Lctbs rhamnosus* (LGG), also seem generally safe and may be a probiotic appropriate for older infants and children [10]. Until adequate data are available for each specific probiotic bacterium, use of probiotics in general cannot be recommended in immunocompromised populations. However, as safety is better documented for specific bacteria, we may be able to use them in certain populations (such as premature infants) that may stand to benefit the most from probiotic use.

Another consideration is that cow's milk protein allergy is one of the common food allergies in infants. Culture conditions used in growing several probiotic products may contain cow's milk protein. There have been reports of severe adverse reactions when pediatric patients with cow's milk protein allergy ingested probiotics. Therefore, caution should be used in prescribing such probiotic products in sensitized children to avoid significant reactions. There is also a study worth mentioning by Taylor et al. using *Lctbs acidophilus* daily for the first 6 months of life in newborns of women with allergy. The presence of Lctbs in the body at 6 months of age was associated with increased risk of subsequent cow's milk sensitization as well [118]. Nevertheless, other studies have examined the effect of probiotic consumption on sensitization to several allergens (e.g., peanut, hen's egg, soy, wheat, milk, cat, dog), as determined by specific IgE production or skin prick test. The authors could not find a difference before and after the treatment. Interesting another trial by Kopp et al. demonstrated that supplementation with LGG during pregnancy and early infancy in affected children it might be associated with an increased rate of recurrent episodes of wheezing bronchitis [119]. However, a recent study was done by Kukkonen et al. evaluating airway inflammation in probiotic-treated children at 5 years in 1,018 children. Early intervention with probiotics and prebiotics did not affect airway inflammation later in childhood [11].

Furthermore, similarly certain probiotics are known to stimulate Th1 immunity, which has been suggested as one of the mechanisms by which they can suppress Th2-mediated allergic diseases. However, this presumed excessive immunostimulation might aggravate or induce Th1-mediated immune responses and diseases such as type 1 diabetes, multiple sclerosis; and it might cause an additional safety issue [8]. Consequence of over-activation of the immune system by probiotics in hosts with immune dysfunctions, such as individuals genetically predisposed to autoimmunity, has raised some concerns too. With respect to the association between bacterial antigens and autoimmune responses and the adjuvant activity of LAB strains, the involvement of LAB in the pathogenesis of some models of autoimmunity in experimental animals and possibly in humans has been suggested [12]. Thus, from a safety point of view, the potential of probiotic bacteria (especially the immunostimulatory

strains), to induce destructive inflammation or autoimmunity needs to be investigated. For instance, it has been experimentally demonstrated that *Lctbs casei* cell wall components (given intraperitoneally) are able to induce cardioangitis (an autoimmunity-associated heart disease) in mice [13].

Conclusion

As mentioned above, there is a large amount of conflicting data on the preventive/therapeutic effects of probiotics in AD. Results from meta-analyses and systematic reviews that combine results of studies from different types of probiotics to examine the effects in any disease should be interpreted with caution. One may quickly recognize the degree of heterogeneity among the different probiotic studies. As mentioned, very few studies were similar in design. Several different probiotic strains with different dosing regimens were used, and many studies showed similarity in efficacy to placebo shortly after probiotic therapy was discontinued. Some probiotic studies suggest short-term statistically significant improvement in SCORAD scores and no sustained benefit from continued ingestion. Therefore, subgroup analysis became critical in understanding the outcomes of the studies. Not all children receiving the probiotic agent benefited, but subsets of these patients, mainly those with moderate disease activity and IgE-associated disease (atopic eczema), seemed to have benefited the most. There are also difficulties of recognizing etiology and pathogenesis of AD in which have many mechanisms involved. Similarly, with various strains, especially for example in Kopp and Taylor et al.'s study, development and/or stimulation of Th2-mediated immune responses have been described [118, 119]. Additionally, if probiotics are used in patients with ADs for any reason—therapy or prevention-cautionary approach ought to be taken. Thus, probiotics cannot be recommended generally for primary prevention of ADs. Any probiotics should not be used especially in immune-compromised children; even they have at risk for ADs. Finally, there is insufficient but fairly promising evidence to recommend the addition of probiotics to foods for prevention and treatment of AD [134].

Five-Year View and Future Expectations

Involvement of commensal enteric microflora and its components with strong immunoactivating properties in etiopathogenetic mechanism of multifactorial diseases, including atopic diseases has been recently suggested. Regulation of intestinal microflora composition (e.g., by probiotics) offers the possibility to influence the development of mucosal and systemic immunity as well as it can play a role also in prevention and treatment of AD. Progress has been made by the identification of receptors and pathways through which gut microbes influence development of the immune system. Such mechanistic data have moved a field that was once regarded as being on the scientific fringe to the mainstream, and support increased funding to advance this promising area of research in the hope that it might deliver the long awaited answer of how to safely prevent AD.

Better understanding of the effects of different probiotic strains and a deeper insight into the mechanisms of the heterogeneous manifestations of AD are needed for the validation of specific strains carrying anti-allergic potential. Therefore, research activities are currently focusing on identification of specific probiotic strains with immunomodulatory potential and on how dietary content interacts with the most efficacious probiotic strains. Moreover, the selection of the most beneficial probiotic strain, the dose, and the timing of supplementation still need to be determined. Further studies should also clarify if any susceptible groups of AD exist and how these groups benefit from supplementation with certain probiotic strains.

Some studies in the management of AD suggest that therapeutic benefit requires a combination of probiotic species (as with VSL#3 or Lacto-mix) or that the component(s) responsible for the anti-inflammatory effect in combination preparations have specific properties that monotherapy probiotics do not [16]. This concept also supports the use of prebiotics that increase concentrations of several commensal immunoregulatory bacteria. Prebiotic use was shown to be associated with a reduction in the fecal concentration of *Bacteroides fragilis*, but had no effect on Lactobacilli or Bifidobacteria. Genetically modified probiotics will be tested for their ability to attenuate AD thru secreting regulatory cytokines in experimental models as well [135]. In near future, the researchers will look for more appropriate combinations of probiotic species or modified probiotics with/without prebiotic and test them in human/experimental AD models.

Additionally, side effects are very low and they might not be nonexistent, as shown in a set of patients with different diseases. However, probiotics should not be considered as totally harmless, particularly in the immunodeficient host, and more safety studies are needed. As imagined, probiotics may have unpredictable behavior like all microorganisms, such as unanticipated gene expression in nonnative host environment, or acquired mutations occurring spontaneously via bacterial DNA-transfer mechanisms [136]. And certain probiotics are known to stimulate Th1 immunity, which has been suggested as one of the mechanisms by which they can suppress Th2-mediated allergic diseases [13].

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ERRATUM

Chapter 48
The Role of Probiotics in Atopic Dermatitis (Eczema) and Skin Allergy Reactions: Prevention and Therapy

Öner Özdemir and Anand A. Zanwar

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Anand A. Zanwar is incorrectly listed in the Table of Contents and on Page 493 as a contributing author to Chapter 48: "The Role of Probiotics in Atopic Dermatitis (Eczema) and Skin Allergy Reactions: Prevention and Therapy".

Öner Özdemir M.D. is the sole author of that chapter.

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