Chapter 17
Biological Cell Protection by Natural Compounds, a Second Line of Defense Against Solar Radiation

Ludger Kolbe

Abstract Human skin has to cope constantly with the harmful effects of solar radiation. With skin cancer rates worldwide on the rise, effective photoprotection is an urgent need. Sun care products are formulated with high efficacy in the entire UVB and UVA range to deliver broadband protection. However, UV-filters in topical sun care products cannot completely block UV radiation. Although products with SPF 50 reduce the UV dose 50-fold, this means that still 2 % of the radiation penetrate into the skin. Nevertheless, skin cells contain protective enzymatic and non-enzymatic antioxidant systems that scavenge harmful reactive oxygen species, detoxify reactive metabolites, and repair UV-induced damage to DNA, proteins and lipids. Activating these endogenous cellular protection mechanisms by natural compounds contributes to a comprehensive skin protection from solar radiation. Several studies, mainly with carotenoids or flavonoids, have been published during the last two decades, proving the validity of the concept. Licochalcone A from Glycyrrhiza inflata has been shown to inhibit NFκB and activate Nrf2 pathways and, thus, provides strong anti-oxidative and anti-inflammatory efficacy. In conclusion, topical products containing licochalcone A minimize UVA and high energy visible light-induced oxidative stress and reduce erythema after exposure to excessive solar radiation.

Keywords Photoprotection · Licorice · Polyphenols · Carotenoids · Licochalcone A · Vitamin C · Vitamin E · NFκB · Nrf2 · Antioxidant · Anti-inflammatory

17.1 Introduction

As the outermost barrier of the body, the human skin has to cope constantly with the harmful effects of solar radiation. However, several cytoprotective defense and repair mechanisms help skin cells to reduce the damages. The most important

L. Kolbe (✉)
Front End Innovation, BF 519, Beiersdorf AG, Unnastrasse 48,
20245 Hamburg, Germany
e-mail: Ludger.Kolbe@Beiersdorf.com

G.T. Wondrak (ed.), *Skin Stress Response Pathways*,
DOI 10.1007/978-3-319-43157-4_17

defense against UV radiation is the production of melanin. The pigment absorbs UV rays and, thus, protects skin cells from further damage. The production of melanin increases substantially after exposure to sun light, leading to a protective UV absorbing tan. Enhanced melanin production usually starts three to four days after UV exposure (delayed tanning) and remains elevated for several weeks. In addition, the epidermal layer thickens after repetitive exposure to UV radiation (epidermal hyperplasia) to develop a denser stratum corneum, which more readily absorbs and disperses solar radiation and thus protects the viable epidermal skin cells underneath. Epidermal hyperplasia starts within days after sun exposure and lasts for up to one month. Consequently, these slow adaptive mechanisms cannot prevent skin damage induced by acute UV exposure. However, skin cells also contain protective enzymatic and non-enzymatic antioxidant systems that scavenge harmful reactive oxygen species (ROS), detoxify reactive metabolites, and repair UV-induced damage to DNA, proteins and lipids. These adaptive mechanisms are up regulated within hours (Talalay et al. 2007).

Nevertheless, the endogenous protection of human skin against solar UV radiation is not at all sufficient to fully protect the skin from acute and chronic skin damages. Especially in lighter skin types with little epidermal melanin, excessive sun exposure results in sunburn after just a short period of time. On a sunny summer day, sun exposure for 15 min might be enough for very sensitive individuals to get a sunburn. Usually the sunburn develops within 5 h, peaks within the first 24 h, but can last for several days. Chronic sun exposure, even at suberythemal doses, results in premature skin aging with wrinkle formation, dryness, loss of elasticity, hyperkeratosis and hyperpigmentation (Kligman and Kligman 1986). UVB radiation at wavelength ranging from 290 to 320 nm is mainly responsible for sunburn, whereas UVA radiation at 320–400 nm is mainly responsible for long-term photodamage of the skin. While premature skin aging is primarily a cosmetic problem, the development of skin cancer after long-term exposure to UV radiation is a major health issue. With skin cancer rates worldwide on the rise (Lomas et al. 2012), effective photoprotection is an urgent need. Recent publications show that consistent use of sunscreen product indeed reduces the risk for skin cancer (Iannacone et al. 2014) but sunscreen use remains low among the general population. A broadband UV protection system is of outmost importance to block both, damaging UVA and UVB rays. In ophthalmology, the damaging effect of blue-violet light has been known for quite some time (Downes 2016) and sunglasses blocking these wavelengths are available. The effects of blue-violet light on skin are currently under discussion, while the effect of solar infrared radiation A (IRA) on skin is another topic of debate. In dermatology, we just begin to understand the impact of non-UV radiation on skin physiology (Liebel et al. 2012; Kolbe 2012; Grether-Beck et al. 2014; Barolet et al. 2016). Blue light filters are not available for topical use and, if so, would not be accepted by the consumers since products containing these filters would turn the skin yellow.

The avoidance of sun exposure during peak hours and wearing of protective clothing were the only protection measures for centuries. During the 1930s, topical products with UV filters that absorb or reflect UV radiation were developed. Today,

these products are formulated with high efficacy in the entire UVB and UVA range to deliver broadband sun protection. The sun protection factor (SPF) refers to the factor by which the UV filters of the product reduce the UV radiation penetrating into the skin (ISO 24444 2010). Hence, a product with a SPF 50 reduces the dose 50-fold, but this also means that 2 % of the radiation still penetrate into the skin. Consumers tend to apply less than the amount of product necessary, therefore, the actual SPF is often less than the labelled SPF on the product and even more residual radiation might penetrate into the skin. Since UV-filters in topical sun care products cannot completely block UV radiation, there are many approaches published in the literature aiming at boosting endogenous cellular protection mechanisms for a comprehensive sun protection.

17.2 Photoprotection by Retinoids and Nonsteroidal Anti-inflammatory Drugs

17.2.1 Retinoids

Several studies documented the positive effect of oral retinoids on skin cancer development. A study with patients at moderate risk to develop skin cancer, due to actinic keratosis, showed a reduced risk to develop new squamous cell carcinoma after oral retinol (Moon et al. 1997). Other studies with renal transplant patients found that oral acitretin significantly reduced new non-melanoma skin cancers (Bavinck et al. 1995; George et al. 2002; McKenna and Murphy 1999). In summary, these studies showed that oral retinoids prevent squamous cell carcinomas and probably prevent basal cell carcinomas as well as actinic keratosis. However, due to the severe side effects of oral retinoids the use of these compounds is limited to high-risk patients only.

17.2.2 Nonsteroidal Anti-inflammatory Drugs (NSAIDS)

NSAIDS inhibit inflammation mainly by inhibiting the production of prostaglandins from cyclooxygenase activity. Prostaglandin E_2 (PGE_2) has been identified as an important mediator of UV-damage to human skin (Halliday 2005). Two studies from Australia found a lower incidence of actinic keratosis and squamous cell carcinomas with regular use of NSAIDS (Butler et al. 2005; Grau et al. 2006). Another study confirmed the protective effect of NSAIDS on basal cell carcinoma and squamous cell carcinoma development (Clouser et al. 2009). In conclusion, oral NSAIDS seem to be photoprotective by inhibiting UV-induced inflammation and thus reduce UV-induced skin cancer (Elmets et al. 2010). However, NSAIDS are drugs with certain side effects and for that reason should not be used for photoprotection in general but for high-risk patients only.

17.3 Photoprotection with Natural Compounds

Plants use solar light to generate metabolic energy via photosynthesis and, therefore, developed highly effective molecular systems to harvest photons. At the same time, they had to develop very effective mechanisms to protect the light harvesting photosynthetic system and all other cellular components from excessive solar radiation. Plant-derived natural compounds are part of our daily diet and, therefore, these compounds might be active in humans after ingestion. Over the past decades, many photoprotective molecules, such as carotenoids, other terpenoids, flavonoids, and other polyphenolic compounds have been identified in various plant species. Several published studies provided evidence that individuals with a diet containing high amounts of vegetables and fruits are somewhat protected from the development of actinic keratosis (Hughes et al. 2009). Patients with a history of squamous cell carcinoma consuming large amounts of vegetables seem to be protected from the development of new skin cancers (Hughes et al. 2006). Therefore, plant-derived dietary components appear to have the potential to contribute to the protection of human skin from photodamage.

17.3.1 Caffeine and Green Tea Catechins

Caffeine is present in leaves, fruits and seeds of various plant species. It is well known for its stimulatory effect on the sympathetic nervous system and consumed in large amounts in teas and coffee (Fisone et al. 2004). Data of large prospective observational studies showed that those with the highest intake of caffeine developed less basal cell carcinoma than those with the lowest consumption (Song et al. 2012).

Green tea contains high levels of antioxidants, especially catechins, which are very effective radical scavengers (Nanjo et al. 1999; Weisburg et al. 2004; Higdon and Frei 2003; Khan and Mukhtar 2007). These polyphenolic compounds were studied intensively for their photoprotective properties. Oral green tea has been shown to enhance repair of UV-induced DNA damage in vitro (Katiyar 2011). In a 12-week, double-blind, placebo-controlled study, 60 female volunteers consumed either a beverage with green tea polyphenols (1402 mg total catechins/d) or a control beverage. UV-induced erythema decreased significantly in the intervention group after 6 and 12 weeks, respectively (Heinrich et al. 2011). Another study showed protection against UV radiation-induced cutaneous inflammation in an open oral intervention study with sixteen healthy human subjects, given green tea catechins (540 mg) and vitamin C (50 mg) daily for 12 weeks. (Rhodes et al. 2013). However, in a larger study with higher dose of green tea catechins (1080 mg/d) the group could not confirm the initial results (Farrar et al. 2015). The efficacy of topical application of green tea phenols was evaluated in a small study involving six subjects. DNA-damage (cyclobutane pyrimidine dimers) and erythema development showed a dose-dependent reduction after relatively high

amounts of green tea polyphenols (1–4 mg/2.5 cm^2). The UV-absorbing properties of green tea phenols likely contributed to the efficacy (Katiyar et al. 2000).

17.3.2 ß-Carotene and Other Carotenoids

Carotenoids are well known for their antioxidant activity, with the most prominent member of this chemical group being ß-carotene. A PubMed search with the search term "beta carotene antioxidant" at the beginning of 2016 revealed more than 9000 publications on this topic. Since ß-carotene is contained in many vegetable foodstuffs, it is consumed in relative large amounts with the normal diet. Therefore, ß-carotene can be detected in human skin and plasma (Stahl and Sies 2012). After sun exposure, ß-carotene levels in human skin decrease, leading to the conclusion that ß-carotene is protective and consumed by UV-induced ROS (Biesalski et al. 1996). Several human intervention studies showed the effectiveness of dietary carotenoids. Daily ingestion of 40 g tomato paste for 10 weeks elevated the plasma levels of lycopene, the tomato-specific carotenoid, and reduced erythema formation after irradiation with a solar light simulator. Significantly lower erythema intensity was found after 10 weeks but not after 4 weeks of treatment (Stahl and Sies 2002). A further study was conducted to examine whether tomato paste rich in lycopene protects against cutaneous photodamage (Rizwan et al. 2011). Lycopene was found to protect from acute and long-term photodamage. A recent study failed to reproduce significant results (Sokoloski et al. 2015) with only 20 volunteers; merely a tendency towards reduced erythema was seen. Already in 1972, the photoprotective efficacy of ß-carotene (180 mg/d) supplementation was demonstrated after 10 weeks (Mathews-Roth et al. 1972). Several studies showed protection against UV-induced erythema when ß-carotene was supplemented for at least 7 weeks and 12 mg or more per day (Lee et al. 2000; Stahl et al. 2000; Heinrich et al. 2003).

17.3.3 Polypodium Leucotomos Extract

Extracts from the tropical fern Polypodium leucotomos contain phenolic compounds like caffeic acid and ferulic acid. The extract has been shown to exert antioxidant and anti-inflammatory efficacy. In mice, P. leucotomos extract reduced the expression of COX-2, increased p53 expression and enhanced DNA repair. In a human study, the extracts showed antioxidant and anti-inflammatory activity and two oral doses of 7.5 mg/kg of the extract significantly reduced the sunburn reaction after irradiation with UVA + UVB as well as DNA damage (Middelkamp-Hup et al. 2004). The effects of Polypodium leucotomos extracts on human skin are currently studied intensively (Bhatia 2015; Gonzalez et al. 2011). While the oral efficacy was investigated in several studies (El-Haj and Goldstein

2015; Bhatia 2015), efficacy after topical treatment of human skin has not yet been shown convincingly.

17.3.4 Vitamin C and E

The fat-soluble Vitamin E and the water-soluble Vitamin C are a perfect combination for preventing UV-induced oxidative stress. Vitamin E molecules, i.e. tocopherols and tocotrienols, are excellent antioxidants preventing lipid oxidation (Thiele and Ekanayake-Mudiyanselage 2007). Vitamins C and E interact synergistically to protect each other and increase overall effectiveness (Halpner et al. 1998). Combinations of vitamin C and vitamin E are effective in photoprotection. Daily oral supplements of 3 g vitamin C and 2 g vitamin E increased the protection against photodamage in skin by approximately 1.5 times; either vitamin alone was ineffective (Fuchs and Kern 1998). Topical formulation of vitamins C and E deliver improved protection of skin against photodamage, achieving significantly greater protection than after oral application. A stable aqueous solution of 15 % vitamin C (L-ascorbic acid) and 1 % vitamin E (α-tocopherol) applied topically can provide fourfold photoprotection for skin (Lin et al. 2003). Topically applied vitamin C induced significant photoprotective effects at concentrations of at least 10 % in animals and humans, whereas a photoprotective effect has not been demonstrated by oral administration even at high doses in humans. Topical vitamin E reduced erythema, sunburn cells, chronic UV-B–induced skin damage and photocarcinogenesis in the majority of the published studies, whereas only high doses of oral vitamin E may affect the response to UV-B in humans. Combination of vitamins C and E, partly with other photoprotective compounds, did increase the photoprotective effects dramatically compared to monotherapies (Eberlein-König and Ring 2005).

17.3.5 Licochalcone A

Licorice is the extract from the dried roots and rhizomes of several plants of the genus Glycyrrhiza and is used as sweetener and as a traditional herbal medicine (Shibata 2000). It is added to candies, chewing gum, beverages (e.g., herbal teas), and herbal remedies for cough and stomach problems. Licorice candies are consumed worldwide and estimates suggest an annual consumption of about 1.5 kg/person (Spinks and Fenwick 1990). The Chinese pharmacopoeia accepts three species of licorice plants, Glycyrrhiza glabra, Glycyrrhiza uralensis, and Glycyrrhiza inflata, as sources for traditional medicines (State Pharmacopoeia Commission of the PRC 2010). The first written record of licorice use dates back to 2100 BC (Gibson 1978). Licorice is a rich source of antioxidant and anti-inflammatory substances. More than 20 triterpenoids and nearly 300 flavonoids

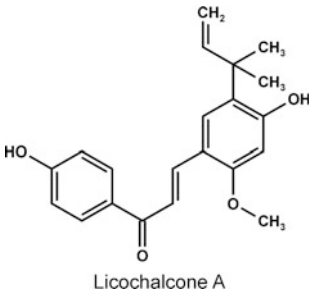

Fig. 17.1 Chemical structure of licochalcone A

have been identified so far (Wang et al. 2015). Glycyrrhiza inflata is one of the main botanical sources of licorice, and is chemically characterized by the presence of unusual chalcones, "retrochalcones", which are distinguished from ordinary chalcones by the absence of an oxygen functionality at the 2-position (Kondo et al. 2007). Licochalcones A was first isolated from Glycyrrhiza roots in 1975 and the structure of this compound (Fig. 17.1) was elucidated (Saitoh and Shibata 1975). From the above-mentioned three licorice plants, only G. inflata contains licochalcone A (Kondo et al. 2007).

17.3.5.1 In Vitro Efficacy of Licochalcone A

The retrochalcones from G. inflata are excellent antioxidants and superoxide scavenger (Haraguchi et al. 1998; Fu et al. 2013). In addition, licochalcone A has demonstrated anti-bacterial (Friis-Moller et al. 2002; Fukai et al. 2002), anti-fungal (Messier and Grenier 2011), anti-protozoan (Chen et al. 1993), and anti-tumor properties (Wang and Nixon 2001; Tsai et al. 2015). The anti-inflammatory activity of licochalcone A was first discovered when the compound was shown to inhibit mouse ear edema induced by arachidonic acid and phorbol 12-myristate 13-acetate (TPA) (Shibata et al. 1991). Concentrations of 500 μg/ear significantly reduced edema when the compound was applied no longer than 30 min after induction of the edema. The authors also found an inhibition of the tumor-promoting effect of TPA by licochalcone A. Various publications showed the comprehensive antioxidative and anti-inflammatory efficacy of licochalcone A. The compound is not only a classical radical scavenger, as shown by suppressing lipid peroxidation in several biological systems (Haraguchi et al. 1998), but also reduced the fMLP- and zymosan-induced oxidative burst and the migration of human mononuclear neutrophil granulocytes (Kolbe et al. 2006; Funatoshi-Tago et al. 2010). Licochalcone A was found to reduce the release of pro-inflammatory cytokines from various cell types. In human T cells, licochalcone A inhibited the proliferative activation by phytohemagglutinin and the production of TNF-α and IFN-γ (Barfod et al. 2002). In human dendritic cells, stimulated with LPS to produce the pro-inflammatory cytokines IL-6 and TNF-α, licochalcone A reduced both cytokines in a dose-dependent way down to control levels, or even below (Kolbe et al. 2006). In human fibroblasts, licochalcone

A inhibited PGE_2 and PGF2α production in response to interleukin 1β (IL-1β) stimulation (Furuhashi et al. 2005). However, licochalcone A had no effect on Cyclooxygenase (COX)-2 mRNA and protein expression in these cells and no effect on COX-1-dependent PGE_2 production. The authors concluded that licochalcone A induces an anti-inflammatory effect through the inhibition of COX-2-dependent PGE_2 and that this is quite different from the mechanism of corticosteroids. A recent study examined how licochalcone A inhibits UV-induced inflammatory responses in HaCaT cells (Song et al. 2015). Licochalcone A did not suppress COX-2 enzyme activity in vitro, but inhibited AP-1 activity and consequently reduced UV-induced COX-2 expression and PGE_2 release. In LPS stimulated mouse macrophages, licochalcone A dose-dependently inhibited the production of NO and PGE_2. Here, a reduction in iNOS and COX-2 expression was responsible for the inhibition (Kwon et al. 2008; Cui et al. 2008). In human EpiDerm™ skin models the inhibitory efficacy of licochalcone A on PGE_2 production was determined after UV-irradiation (Kolbe et al. 2006). PGE_2 release from human keratinocytes after UV-irradiation was reduced to control levels in the presence of 10 µg/mL licochalcone A-containing licorice extract (Fig. 17.2). Inhibiting prostaglandin production often results in increased leukotriene production, therefore, the release of Leukotriene B_4 (LTB_4) from human mononuclear neutrophil granulocytes was determined and licochalcone A was found to inhibit dose dependently the production of LTB_4.

In summary, Licochalcone A has a broad anti-oxidative and anti-inflammatory efficacy. It acts as a radical scavenger, inhibits the oxidative burst of neutrophils,

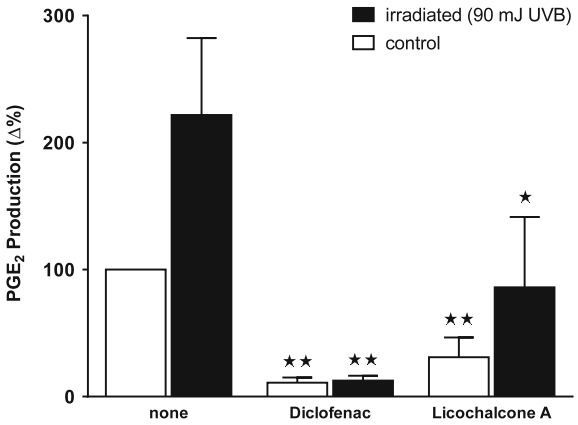

Fig. 17.2 Effects of licochalcone A on PGE_2 production of human keratinocytes. PGE_2 production of human keratinocytes in the EpiDerm™ skin model without UV irradiation (*open bars*) or after UV irradiation (90 mJ UVB/cm^2) (*closed bars*) was measured. Keratinocyte cultures were set up in the presence of the G. inflata extract corresponding to 10 µg/ml licochalcone A, or 50 ng/ml diclofenac, or without the active compounds (none). Data represent means ± SD of three independent experiments. Statistics: two-sided t test (active compound versus none); $*P < 0.05$; $**P < 0.01$

reduces release of pro-inflammatory cytokines and decreases the production of pro-inflammatory prostanoids by various cell types.

17.3.5.2 Modulation of Anti-inflammatory and Anti-oxidative Pathways by Licochalcone A

The analysis of the mechanisms of the anti-inflammatory efficacy of licochalcone A focused on the inhibition of NFκB activation (Furusawa et al. 2009; Funakoshi-Tago et al. 2009). Licochalcone A was found to inhibit LPS-induced signaling through the inhibition of NFκB phosphorylation at serine 276 but licochalcone A had no effect on LPS-induced phosphorylation and degradation of IκBα indicating an inhibition downstream of IKK activation. It was shown that licochalcone A inhibited the interaction of NFκB p65 and the co-activator p300 leading to a reduction of NFκB transactivation. Interestingly, the inhibition of TNF-α-induced NFκB activation was found to be exerted by direct inhibition of IκB kinase complex activation (Funakoshi-Tago et al. 2009). Detailed analysis, using licochalcone A derivatives, revealed that the α,β-unsaturated ketone is very important for the effect of licochalcone A on the TNF-α and LPS signaling pathways. Reduced licochalcone A, lacking the double bond, had no effect on IKK activity. IKK was not inhibited by the addition of reduced licochalcone A (Funakoshi-Tago et al. 2010). The same group found that echinatin, a chalcone also from in G. inflata, was not able to inhibit TNFα-induced IKK activation, NFκB activation and chemokine expression (Funakoshi-Tago et al. 2009). In comparison to licochalcone A, echinatin lacks the 1,1-dimethyl-2-propenyl group. The authors concluded that not only the double bond but also the 1,1-dimethyl-2-propenyl group is required for IKK inhibition by licochalcone A (Funakoshi-Tago et al. 2010).

A key player in orchestrating the cytoprotective response in skin cells is the Nuclear Factor-E2-related factor 2 (Nrf2) (Schäfer and Werner 2015). Several studies demonstrated that Nrf2 activation efficiently protects cells from oxidative damage. Some studies suggested that PI3 K/Akt and MAPK pathways play a key role in regulating heme oxygenase 1 (HO-1) expression and Nrf2 dependent transcription (Zipper and Mulcahy 2000; Gong et al. 2004). In primary human fibroblasts, treatment with licochalcone A induced the nuclear translocation of Nrf2. This resulted in elevated HO-1 and glutamate-cysteine ligase (GCLM) expression leading to a higher ratio of reduced glutathione to oxidized glutathione and concomitant decrease of intracellular ROS concentration (Kühnl et al. 2015). Studies with RAW 264.7 cells revealed that inhibitors of PI3 K/Akt and ERK 1/2 abolished the nuclear translocation of Nrf2 by licochalcone A after chemically (tert-butyl hydroperoxide) induced oxidative stress. This suggest that licochalcone A induced HO-1 expression via the activation of PI3 K/Akt, ERK, and Keap1/Nrf2/ARE signaling (Lv et al. 2015), whereas JNK and p38 MAPK had no effect in these cells. Interestingly, in HaCaT cells, Licochalcone A effectively suppressed UV-induced phosphorylation of Akt and mTOR (Song et al. 2015). In this study, licochalcone A also did not influence the JNK or p38 MAPK signaling pathways. After carefully

analyzing potential molecular targets, the authors found that licochalcone A effectively inhibits UV-induced PI3 K, MEK1, and B-Raf kinase activity, but not C-Raf. The effects of licochalcone A on the PI3/AKT, mTOR pathway were confirmed by another group (Tsai et al. 2015) in SiHa cells. They found induction of autophagy and apoptosis by licochalcone A, treatment with autophagy-specific inhibitors enhanced licochalcone A induced apoptosis. In transgenic mice, the effect of licochalcone A on JNK was analyzed in great detail (Yao et al. 2014). Licochalcone A inhibited JNK1-mediated, but not JNK2-mediated, c-Jun phosphorylation in vivo and in vitro. Licochalcone A competed with JIP1 for binding with JNK1. In some tumor cells, Licochalcone A seem to activate other MAPK pathways. In head and neck squamous cell carcinoma cells (FaDu cells) licochalcone A induced TNF-related apoptosis-inducing ligand (TRAIL) expression via ERK 1/2 and p38 MAPK pathways (Park et al. 2015). The stimulation of TRAIL expression induces apoptosis in cancer cells without toxicity to normal cells. In HeLa cells, licochalcone A enhanced TRAIL-induced apoptosis through increased expression of TRAIL-R2 (Szliszka et al. 2012). Two recent papers demonstrated that licochalcone A might not only activate detoxification enzymes through the Keap1/Nrf2 pathway but also by inhibiting the arylhydrocarbon receptor (AhR) pathway. Licochalcone A was shown to be a strong AhR receptor antagonist (Hajirahimkhan et al. 2015; Dunlap et al. 2015). Taken together, these mechanisms might explain the chemo-preventive efficacy of licochalcone A (Shibata 1994; Bode and Dong 2015).

In summary, Licochalcone acts via activation of the Nrf2 pathway and inhibition of NFκB and AhR pathways. The role of MAP-Kinases in Licochalcone signaling is not yet fully understood.

17.3.5.3 In Vivo Efficacy of Licochalcone A

Several studies, revealing the efficacy of licochalcone in inflammatory skin condition have been published (Weber et al. 2006; Wananukul et al. 2012; Angelova-Fischer et al. 2013; Angelova-Fischer et al. 2014). The photoprotective efficacy in vivo was determined in three different experimental settings. In the first study, using solar simulated radiation, the effect of licochalcone A on the sunburn reaction on the back of 12 human volunteers was determined. A licochalcone A-containing formulation (0.05 % of a licochalcone A-rich licorice extract) applied immediately and 5 h after irradiation with solar simulated radiation reduced the developing erythema significantly relative to the vehicle-treated test sites. At 5 h and 24 h after exposure, skin redness was visibly reduced (Kolbe et al. 2006). To investigate whether the effect of licochalcone A on ROS production in vitro translates into the inhibition of oxidative processes in vivo, a human intervention study was performed using ultraweak photon emission (UPE) as readout parameter. The UPE method detects photons generated mainly by oxidative processes in the skin (Khabiri et al. 2008; Hagens et al. 2008). The UVA-evoked UPE detects the photons from human skin after irradiation with a short pulse of UVA. Irradiation of

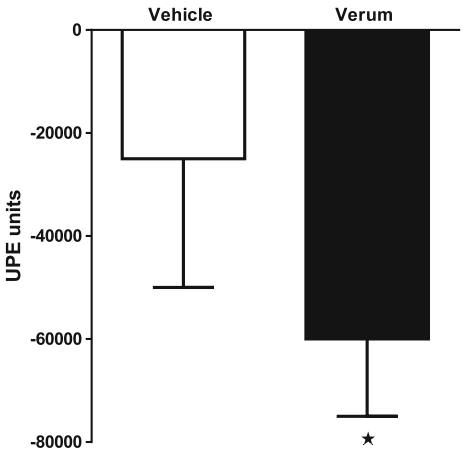

Fig. 17.3 Effect of topically applied licochalcone A on UVA-induced photon emission of the skin. In a study with 22 healthy volunteers, a test formulation containing licochalcone A-rich licorice extract and a corresponding vehicle without licorice extract were applied on the inner forearm for 2 weeks. The UVA-induced photon emission of untreated, verum and vehicle areas were quantified in vivo by utilizing a photomultiplier system. The total number of photons was counted and normalized to the corresponding measurements before the application of products. Data were analyzed using Wilcoxon's signed rank test for original data, $*P < 0.05$

endogenous chromophores, e.g. porphyrins and flavins, with UVA leads to the formation of reactive oxygen species (Dalle Carbonare and Pathak 1992). In the study, 22 volunteers applied a formulation containing licochalcone A and the corresponding vehicle twice daily to their inner forearms. Two weeks of treatment with the licochalcone A containing lotion significantly reduced the amount of skin-derived photons induced by a short stimulus of UVA radiation compared to untreated and vehicle-treated skin areas (Kühnl et al. 2015). The study confirmed that licochalcone A significantly reduced oxidative stress in vivo (Fig. 17.3). In the third study, the photoprotective efficacy in the visible range (400–700 nm, maximum at 440 nm) of a sunscreen formulation containing licochalcone A was investigated using resonance Raman spectroscopy (Vandersee et al. 2015) in a double blind, vehicle controlled pilot study performed on six healthy volunteers (Darvin et al. 2016). The sunscreens containing licochalcone A, or its vehicle, were topically applied on the volunteers' forearms and after 1 h the initial carotenoid values were measured. After irradiation with 100 J/cm^2 the measurements were repeated. In unprotected skin areas and areas treated with the vehicle, the carotenoid content dropped significantly by 15 %. The carotenoid content in areas treated with licochalcone A containing sunscreen remained unchanged, illustrating the anti-oxidative potency of licochalcone also against visible light induced oxidative stress (Fig. 17.4).

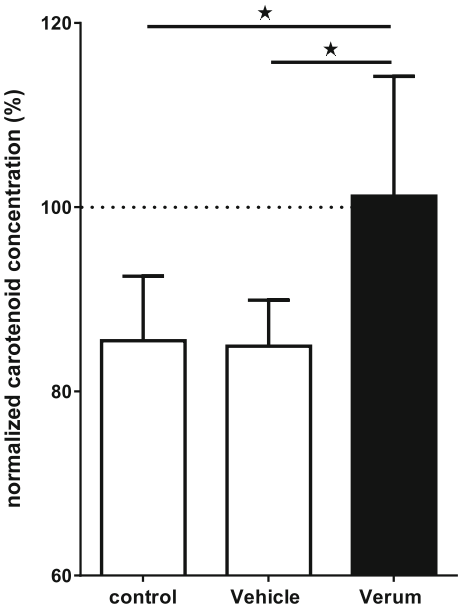

Fig. 17.4 Cutaneous carotenoids degradation after the irradiation with blue light. The protective efficacy of a sunscreen formulation containing Lica A was investigated in vivo in the visible range (400–700 nm, with a maximum at 440 nm) using resonance Raman spectroscopy in a double blind pilot study performed on six healthy volunteers. The sunscreens were topically applied to the forearms and 1 h later the absorption the initial carotenoid values were measured. After irradiation with 100 J/cm^2 the measurements were repeated. Pre-irradiation cutaneous carotenoid values were set to 100 %. Data were analyzed using Wilcoxon's signed rank test for original data, $*P < 0.05$ for verum versus control and verum versus vehicle

In summary, Licochalcone showed photoprotective efficacy in vivo against solar simulated radiation induced erythema and UVA- as well as VIS-induced oxidative stress.

17.4 Photoprotection with Natural Compounds—Systemic Versus Topical Application

Given the fact that protection by topical sunscreens even at SPF 50 still allows 2 % of UV-rays and all of the high energy visible light to penetrate into the skin, there is a need for complementary protection measures. Consequently, an additional line of defense against harmful sunrays within the skin by cellular protection mechanisms would enhance the overall resulting skin protection against solar radiation. Stimulating the endogenous cytoprotective mechanisms, e.g. by activating Nrf2, results in a higher resistance of skin cells to solar radiation induced oxidative stress.

However, biological photoprotection by small molecules, whether they are natural or synthetic, can only be complimentary to the classical sun protection by UV-filters.

Published studies on antioxidants mainly focus on the oral delivery of the actives because the investigators mostly were interested in systemic health benefits (Rani et al. 2016). With regard to endogenous photoprotection, oral treatment has certain disadvantages. Systemic treatment results in long pre-treatment periods before effective concentrations of the active are reached in the skin. In addition, high doses of active ingredients might be needed to achieve beneficial effects and this might lead to side effects in other organs. On the contrary, some of the most active antioxidants are very colorful, like lycopene or ß-carotene, and for this reason cannot be included in high concentrations in topical products. A diet rich in carotenoids and polyphenols may contribute to endogenous photoprotection in the long term. However, the long period of 7–10 weeks until protection becomes significant and the low level of protection make it impossible to rely on oral photoprotection alone. In conclusion, nutritional supplementation of photoprotective compounds can only be complementary to topical photoprotection.

Topical sun care products containing UV filters almost instantly protect against solar radiation. However, as mentioned above, even with a SPF 50 product 2 % of the sun light still penetrates into the skin. Therefore, adding active ingredients that stimulate the cellular protective mechanisms offer a second line of defense against UV radiation within the skin, coping with potential damage caused by residual penetrating solar radiation. Since topical sun protection products are applied directly on the target organ this allows effective concentrations of supplementary photoprotective ingredients much faster than via oral intake. Effective concentrations might be obtained within hours or days, not in weeks as shown for oral application. In addition, even with lower concentrations of the active ingredient in the product, compared to products for oral treatment, topical treatment can result in much higher effective doses in the skin. Most studies on endogenous photoprotection focused on increasing the minimal erythema dose as read-out for efficacy. However, effective endogenous photoprotection should not be seen as a method to provide additional sunburn protection. Prevention of sunburn, measured as the sun protection factor (SPF), should solely be achieved by UV-filters.

There are several ways to determine photoprotective efficacy of natural compounds in vivo, e.g. by measuring oxidized metabolites like isoprostane or 4-hydroxynonenal as markers of oxidative stress, assessing cyclobutane pyrimidine dimers, 6–4 photoproducts and 8-oxo-deoxyguanin as markers for DNA damage, or determining pro-inflammatory markers like TNFα, IL-6 and PGE_2, to name a few. These parameters are suitable as endpoints to determine photoprotective efficacy, but some more need to be defined.

17.5 Concluding Remarks

UV filters are the backbone of every effective sun care product; however, adding small natural compounds that activate endogenous cytoprotective mechanisms can significantly contribute to overall photoprotection. While formulating some of these natural compounds remains a challenge, some already made their way into topical suncare products. The era of biological photoprotection has just begun.

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