Pawan K. Maurya
Kamal Dua *Editors*

Role of Oxidative Stress in Pathophysiology of Diseases

Springer

Role of Oxidative Stress in Pathophysiology of Diseases

Pawan K. Maurya • Kamal Dua
Editors

Role of Oxidative Stress in Pathophysiology of Diseases

Springer

Editors
Pawan K. Maurya
Department of Biochemistry
Central University of Haryana
Mahendergarh, Haryana, India

Kamal Dua
Discipline of Pharmacy, Graduate School of Health
University of Technology Sydney
Sydney, NSW, Australia

ISBN 978-981-15-1567-5 ISBN 978-981-15-1568-2 (eBook)
https://doi.org/10.1007/978-981-15-1568-2

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

The publication of this book was finalised during the Coronavirus (COVID-19) pandemic. We would like to dedicate this book to all those who were affected by the pandemic, and in particular, to our health workforce around the world for their dedication and care during this difficult time

Contents

1 **Oxidative Stress and Oral Diseases** 1
Aravind Kumar Subramanian, Vivek Narayan, and R. Navaneethan

2 **The Role of Oxidative Stress in Chronic Liver Diseases** 13
Devaraj Ezhilarasan, Shanmugam Rajeshkumar, and Thangavelu Lakshmi

3 **The Role of Diverse Nanoparticles in Oxidative Stress: In Vitro and In Vivo Studies** 27
Shanmugam Rajeshkumar, Durairaj Sekar, Devaraj Ezhilarasan, and Thangavelu Lakshmi

4 **Oxidative Stress in Neurology and in Neurodegenerative Processes** 49
Gaurav Gupta, Sacchidanand Pathak, Sarita Rawat, Anurag Mishra, Yogendra Singh, Meenu Mehta, Saurabh Satija, Navneet Khurana, Terezinha de Jesus Andreoli Pinto, Shakti Shukla, Kavita Pabreja, and Dinesh Kumar Chellappan

5 **Role of Oxidative Stress in Complexity of Respiratory Diseases** 67
Maliheh Ghadiri, Amanda Eugenia Yung, and Mehra Haghi

6 **The Role of Synbiotics in Alleviating Oxidative Stress in Colorectal Cancer** 93
Monica Gulati, Sachin Kumar Singh, and Apoorva Singh

7 **Oxidative Stress and Immunological Complexities in Multidrug-Resistant Tuberculosis** 107
Ridhima Wadhwa, Nikita Sehgal, Naresh G, Taru Aggarwal, Saurabh Satija, Meenu Mehta, Gaurav Gupta, Dinesh K. Chellappan, Murtaza M. Tambuwala, Brain Oliver, Trudi Collet, Pawan K. Maurya, Philip M. Hansbro, and Kamal Dua

8 Infection-Induced Oxidative Stress in Chronic Respiratory Diseases 125
Shakti D. Shukla, Kanth Swaroop Vanka, Anne Chevalier, Wai Ching Chong, Kavita Pabreja, Madhur D. Shastri, and Ronan F. O'Toole

9 Oxidative Stress in Depression and Other Comorbid Disorders 149
Shvetank Bhatt, Dilip Kumar Pandey, Chandragouda R. Patil, and A. N. Nagappa

10 Oxidative Stress Monitoring in In Vitro and In Vivo Models 163
Thania Rios Rossi Lima, Bianca Camargo Penteado Sales, and Lilian Cristina Pereira

11 Effects of Yoga on Oxidative Stress During Aging 179
Riya Gupta and Pawan K. Maurya

12 Molecular Level Insight into the Involvement of Heat Shock Proteins in Oxidative-Stress-Mediated Human Diseases 195
Sangeeta Mitra, Angshuman Bagchi, and Rakhi Dasgupta

13 Oxidative Stress in Liver Diseases 209
Lubhan Singh, Sagarika Majhi, Kavita Pabreja, Poonam Negi, Rohit Goyal, Gaurav Gupta, Dinesh Kumar Chellappan, and Kamal Dua

14 Oxidative Stress and Inflammation Can Fuel Cancer 229
Foram U. Vaidya, Abu Sufiyan Chhipa, Nibha Sagar, and Chandramani Pathak

15 Role of Oxidative Stress in Chronic Kidney Disease 259
Kiran Dahiya, Rakesh Dhankhar, Priya Dahiya, Raunak Ahlawat, and Navdeep Hooda

16 Role of Oxidative Stress in the Pathophysiology of Type 2 Diabetes and Cardiovascular Diseases 277
Olorunfemi R. Molehin, Stephen A. Adefegha, and Anne A. Adeyanju

Correction to: Oxidative Stress and Immunological Complexities in Multidrug-Resistant Tuberculosis C1
Ridhima Wadhwa, Nikita Sehgal, Naresh G, Taru Aggarwal, Saurabh Satija, Meenu Mehta, Gaurav Gupta, Dinesh K. Chellappan, Murtaza M. Tambuwala, Brain Oliver, Trudi Collet, Pawan K. Maurya, Philip M. Hansbro, and Kamal Dua

About the Editors

Pawan K. Maurya is currently an Associate Professor at the Department of Biochemistry, Central University of Haryana. He is also Deputy Director of Deen Dayal Upadhyay Kaushal Kendra (DDUKK) at the same university. He holds a Ph. D. from the University of Allahabad and pursued research work as a Postdoctoral researcher at Taipei Medical University, Taiwan and the Federal University of Sao Paulo (UNIFESP), Brazil. His research focuses on oxidative stress, aging, telomere biology, biochemical diagnostics, nanomedicine, and clinical biochemistry. Dr. Maurya has received several national and international awards such as Science without Borders, Brazil (2014), and a Distinguished Service Award, Allahabad (2018). He has published 1 book, 10 book chapters, and over 60 research articles in respected international journals. He has also served on numerous technical program committees and organizing committees for various international conferences.

Kamal Dua is currently a lecturer at the Graduate School of Health (Pharmacy), University of Technology Sydney, Australia. He holds two doctoral degrees in Pharmaceutical Sciences and Immunology & Microbiology, Master of Pharmacy, MPharm (with a Major in Pharmaceutics), and Bachelor of Pharmacy (BPharm). He has also been awarded four professional diplomas in the areas of Clinical Research, Product Development, Drug Regulatory Affairs & Documentation and Intellectual Property Rights (IPR). He has received various awards and fellowships for his academic and professional achievements, including the Excellence Award in Pharmacy (2018); Young Scientist Award (2011); and the Sri. K. Sri Harsha Memorial Gold Medal & Rank Certificate (2004).

He also holds a conjoint lecturer position at the School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, Australia and Visiting Researcher at the Hunter Medical Research Institute (HMRI) and Centre for Inflammation, Centenary Institute, Australia. He is also a Visiting Academic at various other universities, including the School of Pharmaceutical Sciences, University of São Paulo, Brazil; School of Pharmaceutical Sciences, Shoolini University; School of Pharmaceutical Sciences, Lovely Professional University; and Saveetha Institute of Medical And Technical Sciences, Saveetha University, India.

His research experience spans 12 years which lies on the interdisciplinary boundary between the acquired extensive knowledge on the application of the principles of pharmacy (primarily formulation and pharmaceutics) to biomedical sciences (immunology and microbiology). He has published 90 peer-reviewed manuscripts in various respected pharmacy and pharmacology journals and has authored 2 books, 4 book chapters.

Oxidative Stress and Oral Diseases

1

Aravind Kumar Subramanian, Vivek Narayan, and R. Navaneethan

Abstract

Oral diseases such as dental caries, lichen planus, oral cancer, and most importantly chronic periodontitis are also believed to be linked to oxidative stress. In periodontitis, the incessant presence of inflammation releases free radicals and via various mechanisms such as DNA damage, lipid peroxidation, protein damage, oxidation of antiproteases, and release of pro-inflammatory cytokines causes free radical-induced damage. The role of free radicals in carcinogenesis has been studied for many decades. The free radicals cause DNA alterations such as mutations, DNA-based oxidation, mutation of tumor suppressor genes, and oxidative protein damage which facilitate the development of oral cancer. The inflammatory infiltrate present in oral lichen planus has CD4+ lymphocytes and is a source of reactive oxygen species which causes cellular damage. Moreover, the saliva itself has an antioxidant system which prevents free radical-mediated damage in certain oral diseases. Hence this chapter is intended to provide an insight about oxidative stress and its association with various oral diseases.

Keywords

Oxidative stress · Periodontitis · Free radicals · Oral diseases · Oral cancer

A. K. Subramanian (✉) · R. Navaneethan
Department of Orthodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

V. Narayan
Department of Oral Medicine and Radiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_1

1.1 Introduction

Oral diseases are numerous in number, and understanding their impact on general health is paramount. Diseases of the teeth and surrounding soft tissue are frequently the factors of bad health. General physicians often overlook this fact, thereby resulting in poor diagnosis and treatment. On the other hand, a dentist's judgment is insufficient on the grounds that by instruction and propensity he is basically worried about sparing teeth and his knowledge in pathology is limited to an exceptionally thin field. The teeth and the oral tissues are continuous with the rest of the body and is not a separate area. It is therefore essential that dentists and physicians collaborate with each other for the welfare of the patient in order to arrive at a proper diagnosis and initiate the appropriate treatment.

Oxidative stress happens as a condition of unsettling influence between free radical delivered and the ability of antioxidant system to balance [1]. Free radicals are chemicals with an unpaired electron, which are highly reactive and are potentially harmful [2]. The two basic types of free radicals produced are the reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS includes highly reactive oxygen-containing molecules such as hydroperoxyl radical, hydrogen peroxide, and singlet and triplet oxygen [2], while nitric oxide, nitric dioxide, and peroxynitrite comprise the RNS [1]. Biomarkers for oxidative stress can be studied and evaluated in various biological fluids such as blood, saliva, and urine, which is very helpful in understanding and diagnosis of various oral diseases and their association with oxidative stress.

1.2 The Role of Oxidative Stress in Oral Diseases

1.2.1 Chronic Periodontitis

Periodontitis is one of the most common oral diseases affecting the teeth and their supporting structures. Initially there is inflammation of the gingiva known as gingivitis (Fig. 1.1) which eventually progresses to loss of attachment and bone loss

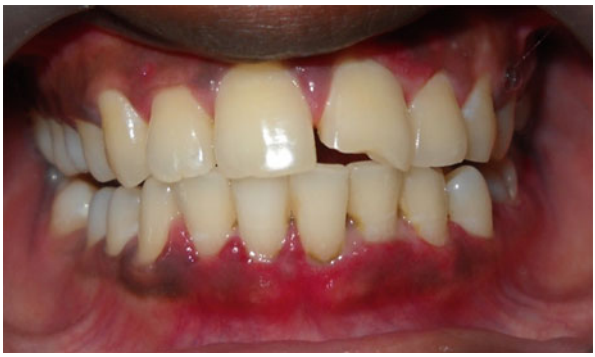


Fig. 1.1 Gingivitis of lower anterior gingiva

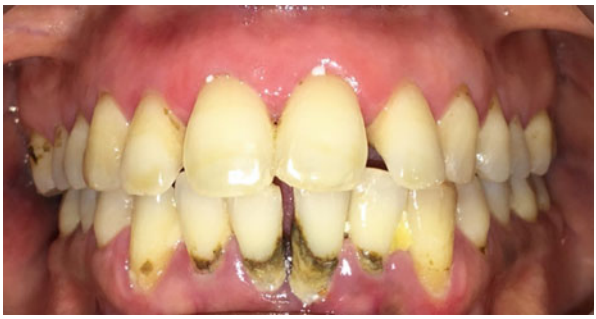


Fig. 1.2 Periodontitis of lower anterior gingiva

resulting in deepening of the gingival sulcus called as the periodontal pocket formation which is a common manifestation of periodontitis (Fig. 1.2). Exfoliation of the teeth occurs due to different types of periodontitis and can cause poor mastication resulting in malabsorption and malnourishment. Chronic periodontitis is also the cause for various systemic diseases of infective etiology by the mechanism of oral foci of infection. Periodontitis is a chronic inflammation affecting the supporting structures of the tooth, namely, gingiva, periodontal ligament, cementum, and the alveolar bone which are collectively called periodontium. About 800 species of bacteria are believed to be associated with periodontitis [3]. This bacterial spread also determines the efficiency of the host immune response. Bacteria, host immunity, and behavioral factors such as smoking are believed to play a vital role in causing periodontitis [3]. Patients suffering from severe periodontitis are 10.5–12% of the global population [4]. A systematic review, published before 2010, revealed that men were more prevalent compared to women (37.4% versus 28.1%); this might be due to the factor of smoking which is more common in men and can contribute to periodontitis [5]. This was supported by the National Health and Nutrition Examination Survey (NHANES) in 2011 who also found out the prevalence of the disease to be more in men than women [6]. The American Dental Association classifies periodontitis based on the periodontal attachment status as follows:

- Type I: Gingivitis
- Type II: Mild periodontitis
- Type III: Moderate periodontitis
- Type IV: Advanced periodontitis [7]

A systematic review in 2010 evaluated about the sexual dimorphism in destructive periodontal disease. In that review, it was found that periodontitis was more prevalent in males than females [5]. It also reports that periodontal surveys having more than 750 subjects describe a more occurrence of periodontitis in men. The world workshop for classifying periodontitis held in 2017 states that the factors smoking and diabetes have to be incorporated in the grading of periodontal disease, and a considerable amount of literature has found that the variables diabetes and smoking

have a significant weightage in causing periodontal disease [5]. The review concludes by stating that variations in sex might become useful in indicating the risk.

1.2.2 Pathogenesis

The disease commences by the accumulation of the bacteria at the dentogingival margin. Bacteria initiate the disease but the role of specific bacteria is obscure. The host immune response determines the composition of the biofilm, and inflammation precedes the overgrowth of the bacteria. The host immune mechanism responds by producing inflammatory cell infiltrate near the periodontal pocket. The usual clinical features include accumulation plaque which eventually leads to calculus formation in the supragingival and subgingival areas. This is accompanied by inflammation of the gingiva [8].

The initial stage is reaction of leukocytes and the endothelial cells to the biofilm formation. There are no obvious clinical signs observed at this stage yet histologic signs are evident. The junctional epithelium is triggered by the bacteria to produce cytokines and neuropeptides resulting in vasodilatation. Chemokines aid the neutrophils to be transferred to the inflammation site. Macrophages, lymphocytes, plasma cells, and mast cells appear after the neutrophils. Rete pegs formation from the epithelium occurs and activation of complement proteins takes place. This clinically appears as gingival inflammation and bleeding is present. The gingival crevicular fluid flow is increased [9].

The immune mechanism responsible so far was the innate immunity, and transition to acquired immunity takes place further. Accumulation of macrophages, plasma cells, T lymphocytes, and I_gG 1 and I_gG 3 subclasses of B lymphocytes is present. Impairment of flow of blood with increased collagenolytic activity occurs along with increased production of collagen by fibroblasts. Clinically this manifests as moderate to severe gingivitis characterized by gingival bleeding and color and contour changes. The final stage would be an advanced periodontitis characterized by irreversible attachment loss, bone loss, and the inflammation extending deeper into the alveolar bone [9].

1.2.3 The Role of Oxidative Stress

The course and severity of the disease is determined by the host immune response against the microorganisms [10]. The fundamental causative factor is the engagement of host and the bacterial enzymes in the destroying the periodontium [11]. Polymorphonuclear neutrophil (PMN) is the primary cell which is produced initially by the host immunity in response to the bacterial pathogens. PMNs arrive at the site of inflammation due to the pro-inflammatory cytokines secreted as a result of the immune mechanism. There is release of ROS and proteolytic enzymes catalyzed by the NADPH oxidase [12]. The presence of unpaired electron in the free radicals derived from oxygen makes it highly reactive in nature [13]. There is a rapid release

of oxygen from the PMN by the mechanism called the "respiratory burst," and this is catalyzed by NADPH oxidase enzyme during the process of phagocytosis [14]. The discharged free radicals are not target-specific, and there is complementary harm to the host tissues, and this can happen either by direct oxidation of the fundamental tissue parts or by actuation of transcription factors. ROS are also produced by osteoclasts in the bone and might have influence bone resorption [15]. Hence the PMN infiltration is considered to be the main event by the host immune system against the invasive microorganisms. PMNs thereby lead to ROS formation resulting in the destruction of periodontal tissues.

The role of oxygen tension appears to have a vital function in the production of ROS by the PMNs. This is because the PMNs require 1% of oxygen concentration and a pH of 7–7.5 approximately to produce the oxygen free radicals [16]. The function of transition metal ions is significant in producing highly reactive OH species. The presence of iron and copper in the gingival sulcus influences the growth and virulence of the periodontal pathogens. The OH free radicals are produced by Fenton and Haber-Weiss reaction. The extracellular matrix provides strength and support to the cells in the connective tissue. They have fibrous collagenous and non-collagenous network which surrounds the cell. The effect of ROS extends to the connective tissue constituents such as proteoglycans and glycosaminoglycans. Aggrecan is a type of proteoglycan having chondroitin sulfate/keratan sulfate glycosaminoglycan chains. Non-radical species such as hypochlorous acid and hydrogen peroxide and radical species such as OH have been implicated in aggrecan degradation by various in vitro studies. Reactive oxygen species play a vital function in degradation of periodontal tissues. The ROS degrades the connective tissue components and modifies the structures within the connective tissue, and this eventually results in the loss of function of the connective tissue of the periodontium.

1.3 Potentially Malignant Disorders (PMDs) and Oral Cancer

The term "potentially malignant disorders" was defined by the World Health Organization (WHO) as the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at a future date [17]. This terminology was a recent modification of older terminologies such as precancerous lesions and conditions. A precancerous lesion is a morphologically altered tissue which has a high capacity for transforming into malignancy, and precancerous condition is a state of the entire body which makes the host susceptible to acquiring malignancy. The potentially malignant disorders predispose the host to malignancies that affect the oral cavity. The development of the PMDs is due to a spectrum of etiological agents such as adverse habits which include tobacco in the form of smoking and chewing along with areca nut usage. Areca nut contains alkaloids such as arecoline and arecaidine which are believed to be the causative factor of a particular PMD. Some examples of PMDs include oral leukoplakia, oral lichen planus, and oral submucous fibrosis to name a few.

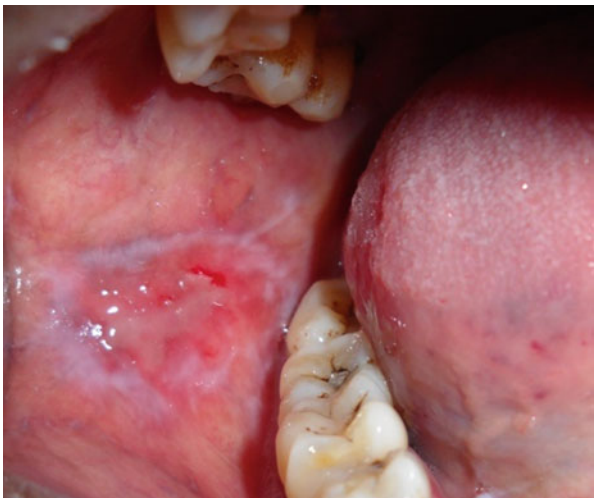


Fig. 1.3 Oral erosive lichen planus of the right buccal mucosa

1.3.1 Oral Lichen Planus

Oral lichen planus is defined as a chronic inflammatory mucocutaneous immune-mediated condition. Erosive-type oral lichen planus appears to have the malignant transformation potential, and the rate of it transforming into malignancy is sparse [18]. Clinically the lesion appears as having a mixed red and white appearance. The white component of the lesion appears as crisscross interlacing lines which are referred to as "Wickham's striae" and surrounds the erythematous eroded mucosa (Fig. 1.3). The lesion is often noticed in the buccal mucosa of the person. The person often has stomatodynia which gets intensified upon consuming spicy foods. The condition also affects the skin and has lesions appearing as purple, pruritic, polygonal papules. Oral lichen planus affects 1.27–2% of the population [17]. Patients with oral lichen planus have high levels of malondialdehyde and 4-hydroxy-2-neonenal which are products of lipid peroxidation [19]. The oxidative stress markers initiate a series of biological responses including initiation of apoptosis. This cellular apoptosis is brought about by B-cell lymphoma 2-associated X proteins, pro-inflammatory T-lymphocytes, nuclear localization, and activity of factor kappa B [20]. Reduction of antioxidants in saliva of patients with oral lichen planus is observed when compared with healthy controls in recent studies [21].

1.3.2 Oral Leukoplakia

Oral leukoplakia is the most common potentially malignant disorder of the oral mucosa. It is defined as a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer [22]. The common etiological agent is tobacco in the form of smoking. Smokeless forms available as chewing products also are responsible for causing the lesion. The

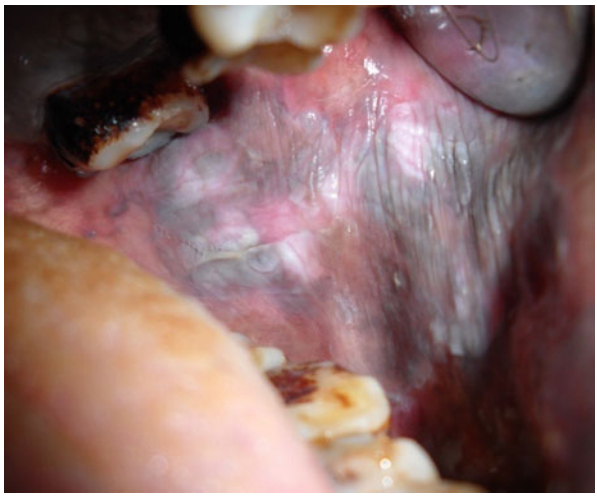


Fig. 1.4 Leukoplakia of the left buccal mucosa

prevalence of the lesion is 1.49–2.6% with a high occurrence among geriatrics especially in India due to excessive use of chewing varieties of tobacco along with areca nut [23]. Clinically the lesion commonly affects the buccal mucosa as the concentration of hot smoke in individuals with smoking habit is more concentrated in the commissures and the buccal mucosa (Fig. 1.4). The second likely place is the lateral borders of the tongue. The lesion is often bilateral in presentation and appears as thick non-scrapable white patch/plaque which is homogenous and having a characteristic "cracked mud" appearance. The lesion appears raised and the affected part of the mucosa is slightly roughened. Leukoplakia is of different types which are homogenous, speckled/nodular type, verrucous, erythroleukoplakia, and proliferative verrucous leukoplakia (PVL). Among these, the PVL and erythroleukoplakia have a greater propensity for malignant transformation. Salivary biomarkers of oxidative stress such as thiobarbituric acid and advanced glycation end products are significantly higher in individuals with leukoplakia than in those without the lesion, and the antioxidant levels were also found to be significantly lesser in individuals with leukoplakia [24]. Lipid peroxidation as a result of ROS has mutagenic effects. 8-Isoprostane (8-ISO) is an ROS and if present in high concentrations can make the individuals more susceptible to leukoplakia [25]. Lipid peroxidation can occur in cells with a cell membrane sensitive to ROS leading to production of mutagenic carbonyl compounds which can result in malignant transformation.

1.3.3 Oral Cancer

Oral cancer ranks the sixth leading type of cancer in Asia [26]. Almost 274,300 new cases occur every year [27]. Oral cancer is a wide term under which many forms are categorized. It can take origin from any type of tissue. The malignancies arising from

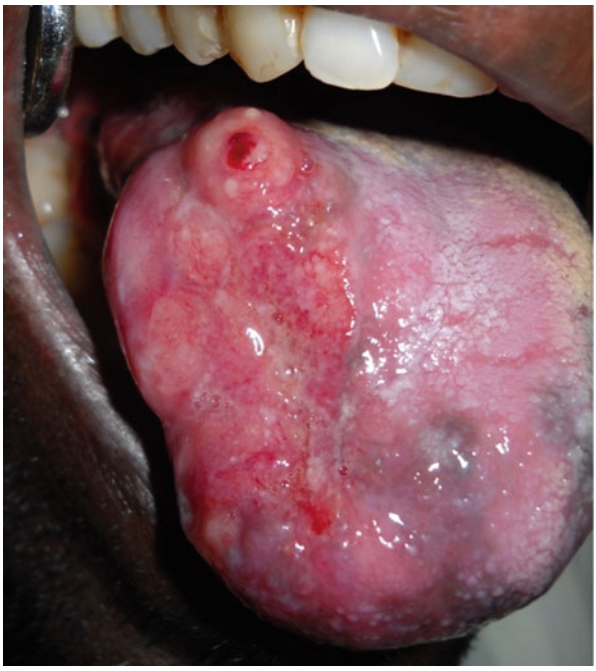


Fig. 1.5 Carcinoma of the right dorsolateral aspect of anterior two-thirds of tongue

an epithelial origin are called as carcinoma and those arising from the connective tissue are sarcoma. The treatment and prognosis for oral cancer often depend on the nature and more importantly the vehemency of the disease. It has a great influence on the day to day functions of the patient. Most of the patients visiting the hospital are often at the advanced stage of the disease. This unfortunate situation might be due to the nature of the disease and how the disease unfolds itself to the patient. Patients often do not notice the presence of the cancerous lesion in the early stages of the disease as it is asymptomatic and is hardly noticeable. Adverse habits such as smoking, chewing tobacco products along with areca nut and betel leaf, alcohol consumption along with low socioeconomic status can lead to oral malignancies. Most of the oral cancers are detected in individuals older than 40 years of age, and the average age of diagnosis of oral cancer is 60 years [27]. The most common type of oral cancer is oral squamous cell carcinoma. The lateral border of the tongue is usually involved (Fig. 1.5).

The imbalance between the oxidants and the antioxidants seems to be playing the etiological role in carcinogenesis [2]. Reactive oxygen species can cause tissue damage by the following mechanisms,

- Lipid peroxidation
- Protein damage
- DNA damage
- Stimulation of pro-inflammatory cytokine
- Oxidation of antiproteases [28]

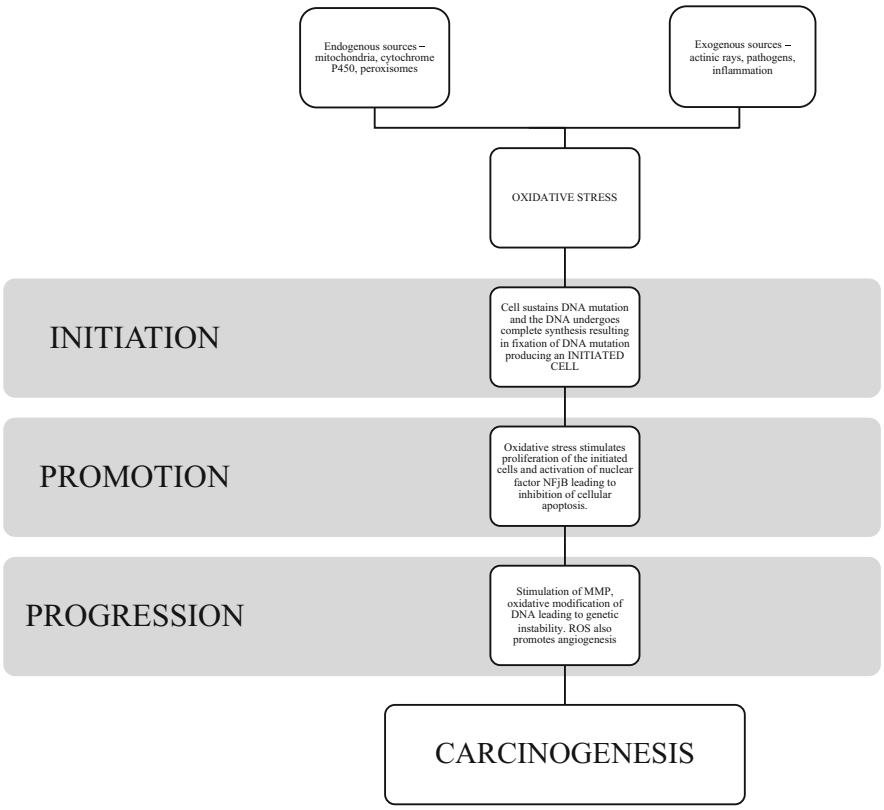


Fig. 1.6 A flowchart showing the process of carcinogenesis by the mechanism of oxidative stress. (*Courtesy Katakwar P. et al., oxidative stress marker in oral cancer 2016* [2])

ROS can be produced by endogenous and exogenous sources. Endogenous sources include mitochondria, peroxisomes, and cytochrome P450, and the exogenous sources include inflammation, actinic radiation, and pathogens. These sources trigger the oxidative stress and leads to damage to the genetic material, proteins, and lipids, gene mutations, chromosomal alterations, and expression of the mutated gene resulting in carcinogenesis (Fig. 1.6).

Carcinogenesis can be explained in three stages:

1. Initiation
2. Promotion
3. Progression

Initiation—the mutated DNA is present in a normal cell and the cell sustains it. Then the DNA undergoes the process of synthesis and this mutated DNA is now fixed. The mutated DNA in the cell now initiates the process of carcinogenesis.

Oxidative DNA changes happen in the cancer tissues due to the reactive oxygen species [29].

Promotion—this stage takes place by the proliferation and expansion of the initiated cells. This cancer development occurs by the combination of proliferation of the initiated cells and inhibition of apoptosis. ROS favors the expansion of the initiated cells by modulating the genes related to cellular proliferation and death [30]. It leads to activation of NFjB activation with induction of genes coding for proteins that inhibit apoptosis. Oxidative stress can stimulate cell division, thereby promoting tumor growth.

Progression—stimulation of matrix metalloproteinases, inhibition of proteases, and mutation are caused by the reactive oxygen species. DNA bases undergo oxidative modifications, and this makes the cell genetically instable and increases the chances of metastasis. ROS also influences angiogenesis in metastasis [31].

Tobacco in the form of smoking and chewing causes oxidative stress to increase, thereby disrupting the balance between the antioxidants and oxidative stress. There is also increased lipid peroxidation, oxidative damage to the DNA, and upsetting of the antioxidant defense which would favor malignant transformation. Chronic alcohol ingestion causes single nucleotide polymorphism of CYP2E1. This then leads to lipid peroxidation and mutation of the DNA [2].

1.4 Conclusion

The genes are often associated with oxidative stress, thereby predisposing individuals to a lot oral diseases. Some lifestyle diseases are multifactorial; hence other factors apart from oxidative stress also play a role in causing various diseases. Advancement in technology has aided us in identifying biomarkers for various oral diseases; hence, prompt diagnosis and initiating appropriate treatment are possible. Recent advancement in the field of molecular biology has enabled us to store a lot of genetic information about diseases and to aid in significant progress in the field. A lot of adverse habits are closely associated with oxidative stress and also cause elevation of the biomarkers for oxidative stress. Many environmental factors can release oxygen species which causes lipid peroxidation and DNA damage paving way for the development of carcinogenesis.

References

1. Kumar J, Teoh SL, Das S, Mahakknaukrauh P (2017) Oxidative stress in oral diseases: understanding its relation with other systemic diseases. Front Physiol 8:693. https://doi.org/10.3389/fphys.2017.00693
2. Katakwar P, Metgud R, Naik S, Mittal R (2016) Oxidative stress marker in oral cancer: a review. J Cancer Res Ther 12:438–446
3. Nazir MA (2017) Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim) 11(2):72–80

4. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W (2014) Global burden of severe periodontitis in 1990–2010: a systematic review and meta-regression. J Dent Res 93(11):1045–1053
5. Shiau HJ(2018) Periodontal disease in women and men. Curr Oral Health Rep 5:250. https://doi.org/10.1007/s40496-018-0195-x
6. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ (2012) Prevalence of periodontitis in adults in the United States: 2009 and 2010. J Dent Res 91(10):914–920. Represents one of the first national epidemiological surveys utilizing full mouth periodontal examination protocol
7. Nava-Villalba M, González-Pérez G, Liñan-Fernández M, Marco T-C (2013) Oxidative stress in periodontal disease and oral cancer, oxidative stress and chronic degenerative diseases - a role for antioxidants. IntechOpen, Rijeka, Croatia. https://doi.org/10.5772/52492
8. Novak JM, Novak KF (2006) Chronic periodontitis. In: Carranza FA (ed) Clinical periodontology. Saunders Elsevier, Philadelphia, pp 494–499
9. Fiorellini JP, Ishikawa SO, Kim DM (2006) Gingival inflammation. In: Carranza FA (ed) Clinical periodontology. Elsevier Saunders, Philadelphia, pp 355–361
10. Kinane DF, Lappin DF (2002) Immune processes in periodontal disease: a review. Ann Periodontol 7:62–71
11. Ding Y, Uitto V-J, Firth J et al (1995) Modulation of host matrix metalloproteinases by bacterial viruelence factors relevant in human periodontal diseases. Oral Dis 1:279–286
12. Waddington RJ, Moseley R, Embery G (2000) Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis 6:138–151
13. Halliwell B, Gutteridge JMC, Cross CE (1992) Free radicals, antioxidants and human disease: where are we now? J Clin Lab Med 119:598–620
14. Babior BM, Kipnes RS, Curnutte JT (1973) The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 52:741–744
15. Key LL, Wolf WC, Gundberg CM et al (1994) Superoxide and bone resorption. Bone 15:431–436
16. Gabig TG, Bearman SI, Babior BM (1979) Effects of oxygen tension and pH on the respiratory burst of human neutrophils. Blood 53:1133–1139
17. Mortazavi H, Baharvand M, Mehdipour M (2014) Oral potentially malignant disorders: an overview of more than 20 entities. J Dent Res Dent Clin Dent Prospects 8(1):6–14. https://doi.org/10.5681/joddd.2014.002
18. Czerninski R, Zeituni S, Maly A, Basile J (2015) Clinical characteristics of lichen and dysplasia *vs.* lichen planus cases and dysplasia cases. Oral Dis 21(4):478–560
19. Batu S, Ofluoglu D, Ergun S, Warnakulasuriya S, Uslu E, Güven Y, Tanyeri H (2016) Evaluation of prolidase activity and oxidative stress in patients with oral lichen planus and oral lichenoid contact reactions. J Oral Pathol Med 45(4):281–288
20. Li XR, Guo J, Li XM (2015) The expression and changes of apoptosis protein BCL-2 and BAX in oral lichen planus. Shanghai Kou Qiang Yi Xue 24(4):465–469
21. Darczuk D, Krzysciak W, Vyhouskaya P, Kesek B, Galecka-Wanatowicz D, Lipska W, Kacczmarzyk T, Gluch-Lutwin M, Mordyl B, Chomyszyn-Gajewska M (2016) Salivary oxidative status in patients with oral lichen planus. J Physiol Pharmacol 67(6):885–894
22. Warnakulasuriya S, Johnson NW, van der Waal I (2007) Nomenclature and classification of potential malignant disorders of the oral mucosa. J Oral Pathol Med 36(10):575–580
23. Petti S (2003) Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncol 39(8):770–780
24. Vlková B, Stanko P, Minàrik G, Tòthovà L, Szemes T, Bañasovà L, Novotnáková D, Hodosy J, Celec P (2012) Salivary markers of oxidative stress in patients with oral premalignant lesions. Arch Oral Biol 57(12):1651–1656
25. Senghore T, Li YF, Sung FC, Tsai MH, Hua CH, Liu CS, Huang RJ, Yeh CC (2018) Biomarkers of oxidative stress associated with the risk of potentially malignant oral disorders. Anticancer Res 38(9):5211–5216. https://doi.org/10.21873/anticanres.12844

26. Nayyar AS, Khan M, Ahmed I, Vijayalakshmi KR, Anitha M, Chendil V (2012) Changing biochemical markers and ongoing process of transformation pilot study. Int J Phys Math Sci 2:164–173
27. Epstein J, Elad S (2015) Oral and oropharyngeal cancer. In: Glick M (ed) Burket's oral medicine. PMPH-USA, Shelton, CT, pp 173–199
28. Shetti N, Patil R (2011) Antioxidants: its beneficial role against health damaging free radical. World J Sci Technol 1:46–51
29. Poulsen HE, Prieme H, Loft S (1998) Role of oxidative DNA damage in cancer initiation and promotion. Eur J Cancer Prev 7:9–16
30. Trueba GP, Sánchez GM, Giuliani A (2004) Oxygen free radical and antioxidant defence mechanism in cancer. Front Biosci 9:2029–2044
31. Maulik N (2002) Redox signaling of angiogenesis. Antioxid Redox Signal 4:805–815

The Role of Oxidative Stress in Chronic Liver Diseases

2

Devaraj Ezhilarasan, Shanmugam Rajeshkumar, and Thangavelu Lakshmi

Abstract

The liver plays a central role in the biotransformation of a variety of drugs and xenobiotics. During the biotransformation of drugs and chemicals, the liver generates various reactive intermediates and in turn attacks the hepatocyte membrane to generate free radicals such as superoxide, hydroxyl, peroxyl, hydrogen peroxide, peroxynitrite, peroxynitrous acid, etc. Hepatic stellate cells (HSCs), also known as perisinusoidal cells or Ito cells play a central role in the onset of various forms of chronic liver diseases. The free radicals liberated during biotransformation in the liver activate the quiescent HSCs into myofibroblast-like phenotype responsible for the excessive synthesis of extracellular matrix proteins that cause hepatic fibrosis, cirrhosis, portal hypertension, and hepatocellular carcinoma. On the other hand, hepatocytes also have several first-line intracellular antioxidant defenses such as superoxide dismutase, catalase, and glutathione and detoxifying enzymes to neutralize/protect the free radicals generated during oxidative stress. During chronic liver injury, the redox homeostasis is altered, and an enormous amount of free radicals are released with the concomitant decrease in the intracellular antioxidants causing oxidative stress. This chapter summarizes the molecular mechanisms of oxidative stress-induced chronic liver diseases.

D. Ezhilarasan (✉)
Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

Biomedical Research Unit and Laboratory Animal Research Centre, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

S. Rajeshkumar · T. Lakshmi
Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_2

Keywords

Chronic liver injury · Hepatic fibrosis · Hepatic stellate cells · Free radicals · Oxidative stress

2.1 Introduction

Chronic liver diseases (CLDs)-associated complications are responsible for significant morbidity and mortality worldwide. Oxidative stress due to chronic liver injury with excessive inflammation and mitochondrial dysfunction-mediated energy metabolism often contributed to a variety of liver diseases [1]. The liver is one of the highly susceptible organs due to its unique anatomic location and function [2]. When the liver is injured by a variety of etiological factors including acute or chronic ethanol intoxication, hepatotoxic drugs, aflatoxin contaminated food, hepatitis B and C virus (HBV and HCV) infections, metabolic diseases, etc. [3, 4], it undergoes deviation from normal architecture into various hepatotoxic manifestations like inflammation, steatosis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma (HCC) [5]. Therefore, CLDs cannot be approached as a single manifestation. Various forms of CLDs are depicted in Fig. 2.1. Ample evidence has confirmed the predominant role of oxidant and antioxidant imbalance in the pathogenesis of a variety of drugs and chemical-induced CLDs. Hence, this chapter discusses the role of oxidative stress in the onset of various forms of CLDs.

2.2 Free Radicals and the Liver

Biotransformation of hepatotoxic drugs and chemicals in the liver produces a variety of highly reactive metabolites including reactive oxygen/nitrogen species (ROS/RNS) [6, 7]. Physiological ROS production is critical for redox homeostasis. However, in pathological conditions, oxidative stress is said to occur when the intracellular antioxidant defense system such as antioxidants is overwhelmed by an increased intracellular ROS and other free radicals. Free radical is classically referred to as an atom or molecule that contains one or more unpaired electrons in an outermost shell of their orbitals and is capable of independent existence. The presence of unpaired electrons in an outermost shell makes free radicals highly unstable and electrophilic [2, 8]. When a free radical reacts with a non-radical to stabilize the outermost shell, the latter generally becomes a radical and thus a series of a chain of reaction is initiated. Some important free radicals known in biological systems are singlet oxygen, superoxide ($O_2^{\bullet -}$), hydroxyl, peroxyl, hydrogen peroxide, peroxynitrite, peroxynitrous acid, and nitric oxide (NO_2), and they act as a source for ROS [9–11]. Hepatocyte intracellular organelles like endoplasmic reticulum, mitochondria, and peroxisomes are the main sites for ROS production. A wide array of enzymatic systems including cytochrome P450 enzymes, cyclo oxygenases, lipoxygenases, xanthine oxidase, nicotinamide adenine dinucleotide phosphate

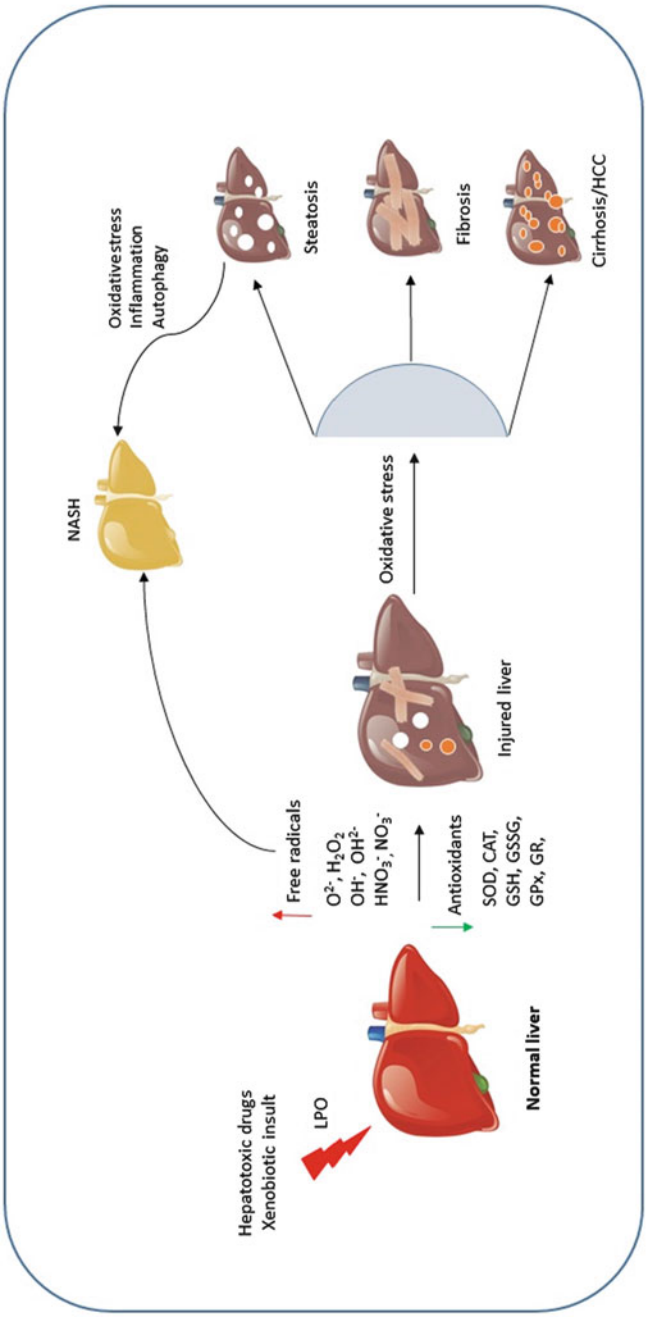


Fig. 2.1 Oxidative stress induced different forms of chronic liver diseases. *LPO* lipid peroxidation, superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxyl radicals (OH^{2-}), peroxynitrous acid (HNO_3^-), peroxynitrite (NO_3^-), *SOD* superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *GPx* glutathione peroxidase, *GR* glutathione reductase, *NASH* non-alcoholic steatohepatitis

oxidases, and NO_2 synthase from these organelles contribute to ROS production [12]. The free radicals primarily target polyunsaturated fatty acids of hepatocyte cell membrane as they are rich in double bonds and hence, hepatocyte membrane is more vulnerable to free radical attack during intracellular oxidative stress. Other cellular structures that are mainly affected by free radicals are macromolecules including structural and functional proteins, lipids, and DNA. On the other hand, overwhelmed ROS generation depletes the intracellular antioxidants that consequently fail to neutralize the excessive ROS leading to cellular injury. Undoubtedly, the excessive intracellular ROS and other free radicals with concomitant downregulation of antioxidants induce oxidative stress and have been associated with several forms of CLDs [13]. Therefore, natural and synthetic antioxidants are often employed to treat oxidative stress-induced CLDs.

2.3 The Role of Intracellular Antioxidants/Antioxidant Response Element (ARE) in CLD

The liver contains several intracellular defenses in the name of antioxidants to protect them from free radicals attack. The first-line intracellular enzymatic antioxidants are the primary defense against free radicals, and these include superoxide dismutase (SOD) to dismutate $O_2^{\bullet -}$ radicals, catalase (CAT) to neutralize hydrogen peroxide radicals, glutathione reductase (GR), and glutathione peroxidases (GPx), etc. The non-enzymatic antioxidants are reduced glutathione (GSH), vitamins C and E, and β-carotene [2, 14]. Under oxidative stress conditions, intracellular antioxidant levels are often reported to decrease due to their over-utilization towards the suppression of various free radicals generated during the biotransformation of various drugs and chemicals in the liver [6, 15]. However, studies are also reported the increased levels of antioxidants during oxidative stress conditions. This was correlated with the induction of oxidative stress contributing the cell to induce the synthesis of antioxidants [16]. Both these hypotheses are accepted and reported widely. Therefore, at this juncture, we do not know whether increased or decreased intracellular antioxidants are responsible for oxidative stress conditions.

At the molecular level, transcription factors such as erythroid 2-related factor 2 (Nrf2) play a serious role in the oxidative stress condition and was shown to upregulate several cytoprotective genes [17, 18]. Under normal physiological conditions, Nrf2 gets activated via binding into kelch-like ECH-associated protein-1 (Keap1) in the cytoplasm, and the unbound or inactivated Nrf2 is easily degraded. Intracellular oxidative stress caused dissociation of Nrf2 from Keap1 by Keap1 modification or Nrf2 phosphorylation and is thus activated [19]. This activated Nrf2 translocates into the nucleus, offers cytoprotective effect, and interacts with ARE, promoting the expression of enzymic antioxidants and phase II detoxifying enzymes such as heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) [14, 17, 20]. Thus, Nrf2 is considered as a potential therapeutic target to attenuate oxidative stress-induced liver injury [21]. Several studies showed that activation of Nrf2 signaling pathway can ameliorate liver injury [22–25].

2.4 The Role of Oxidative Stress in CLDs

2.4.1 Oxidative Stress in Hepatic Steatosis

Hepatic steatosis is a multifactorial disease that occurs due to non-alcoholic fatty liver disease (NAFLD), ethanol consumption, drugs used in chemotherapy, and metabolic diseases, xenobiotics, infectious causes, and so on [26]. NAFLD is commonly considered one of the most common forms of CLDs, and it refers to various manifestations of liver damage including non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [27]. Day and James [28] classically categorized NASH as two-hit model. The "first hit" is hepatic steatosis that is a mild condition and is considered less harmful. The "second hit" includes excess fat accumulation and mitochondrial dysfunction that leads to inflammation and its associated damage due to oxidative stress [29]. In view of the above report, it is clear that oxidative stress is responsible for "second hit" and aggravates the pathogenesis of NASH. NAFLD is often implicated with the presence of ROS-meadiated mitochondrial dysfunction [30]. The mitochondrial respiratory chain is one of the major sources of intracellular ROS, which in turn damage mitochondrial DNA, lipids, and proteins. Lipid accumulation in hepatocytes increases the mitochondrial β-oxidation and interferes with the electron transport chain that results in electron leakage. The cytochrome C oxidase (VI complex)-mediated oxygen reaction and a proton reaction are also impaired, and this, in turn, causes direct interaction of an electron with oxygen forms ROS [30].

In experimental models of NASH, decreased GPx activity due to GSH depletion and impaired GPx transport to mitochondrial matrix from cytosol were reported [31]. Cardiolipin, an inner mitochondrial membrane phospholipid is reportedly susceptible to ROS attack. Interestingly, cardiolipin abnormalities have been implicated with a mitochondrial abnormality in several liver disease conditions, including NAFLD [27]. In an experimentally induced NAFLD model, administration of a high fed diet caused downregulation of NRF-dependent transcription signaling pathway, and cytoprotective enzymes like HO-1 and NQO1 with a concomitant increase in lipid peroxidation indicate the possibility of oxidative stress via NRF signaling pathway in NAFLDs [32, 33]. Since oxidative stress is associated with the progression of NAFLDs, antioxidants have been tried as a therapeutic candidate against different NAFLD experiment models, and such studies concretely proved that antioxidant treatments could decrease CLD progression through attenuation of oxidative stress [33–36]. Clinically, oxidative stress has also been reported in NAFLD patients, and therefore, antioxidants have also been tested in patients with NAFLDs [37, 38].

Oxidative stress-mediated hepatic steatosis is also well established in alcohol injury models. Acute alcohol intake increases oxidative stress and induces hepatic lipid accumulation through cytochrome P450 2E1 (CYP2E1)-mediated reactions. Direct involvement of CYP2E1 from mitochondria in ROS generation is reported both in experimental and clinical models of NASH [30]. The c-Jun N-terminal kinase (JNK) activation and ROS generation are significant events in acute alcohol

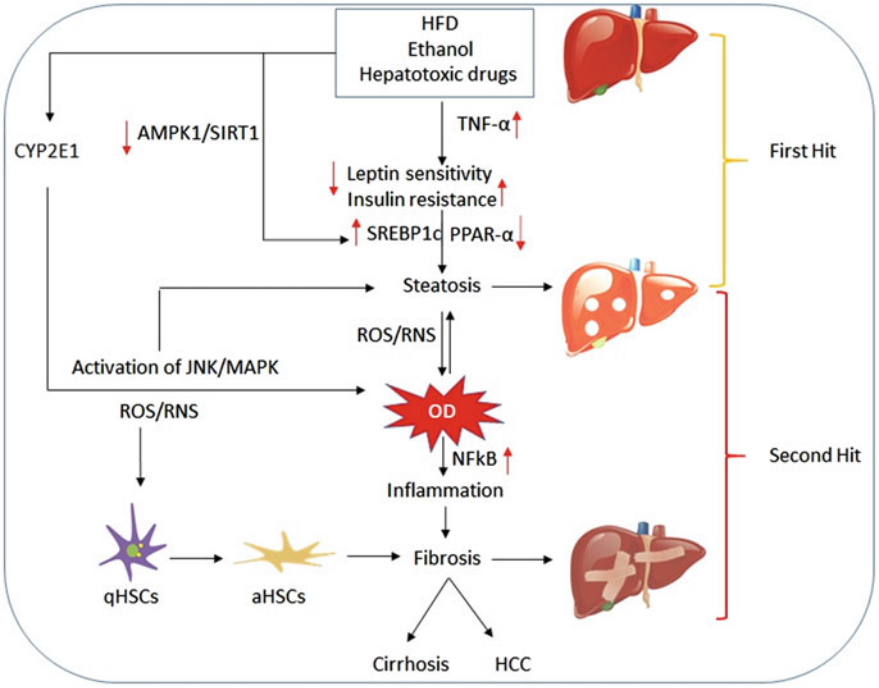


Fig. 2.2 Role of oxidative stress in the progression of steatotic liver injuries. *HFD* high fed diet, *CYP2El* cytochrome P450 2E1, *TNF-α* tumor necrosis factor alpha, *AMPK1* 5′ AMP-activated protein kinase 1, *SREBP1c* sterol regulatory element-binding protein 1c, *PPAR-α* peroxisome proliferator-activated receptor-alpha, *ROS* reactive oxygen species, *RNS* reactive nitrogen species, *JNK* c-Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *NF-KB* nuclear factor kappa B, *qHSC* quiescent hepatic stellate cells, *aHSCs* activated hepatic stellate cells, *HCC* hepatocellular carcinoma

intoxication-induced hepatic steatosis. Sterol regulatory element binding proteins (SREBP) is an important transcription factor which regulates the ROS-mediated inflammatory pathway and lipid synthesis via activation of hepatic nuclear factor kappa B [9, 10]. Acute alcohol intake also decreased autophagy and increased SREBP expression [39]. The mechanism of oxidative stress-induced fatty liver disease is depicted in Fig. 2.2.

2.4.2 Oxidative Stress in Hepatic Fibrosis

Liver fibrosis is a significant health problem that affects 100 million people worldwide with significant morbidity and mortality [40]. Liver fibrosis is a wound-healing response that is responsible for the deposition of an enormous amount of extracellular matrix (ECM) in the liver as a result of chronic liver injury [41]. Hepatic stellate cells (HSCs) are non-parenchymal cells that reside in the perisinusoidal space or

space of Disse is implicated in the hepatic fibrogenesis. In normal liver, quiescent HSCs (qHSCs) are responsible for the synthesis of normal ECM and retinoid storage. As a result of liver injury, qHSCs are activated by autocrine and/or paracrine signaling molecules from injured hepatocytes and acquired myofibroblast (MFB) like phenotype [2, 3, 42]. This phenotypic transdifferentiation is responsible for the synthesis of a variety of profibrogenic cytokines, i.e., transforming growth factor β receptor type II (TGFRIIβ), platelet-derived growth factor receptor β (PDGFRβ), and fibrosis markers such as fibril-forming collagens such as type I and III, vimentin, desmin, α-smooth muscle actin, and so on [43]. Fibrogenic signaling mediates the accumulation of excessive ECM in the perisinusoidal space which hinders liver metabolic functions and increases portal hypertension. It is a proven fact that oxidative stress is one of the main driven factors for the activation and phenotypic transdifferentiation of qHSCs into MFBs.

In vitro, experimental studies have shown that lipid peroxidation products can activate HSCs proliferation [44–46]. For instance, $O_2^{\bullet -}$ radical is involved in the progression of hepatic fibrosis, and entry of $O_2^{\bullet -}$ radical via chloride channels into HSCs is said to play a critical role in HSC activation [44]. In vivo, oxidative stress-induced fibrosis has been well reported in ethanol, carbon tetrachloride, thioacetamide, and dimethyl nitrosamine-induced experimental models [47]. For instance, during the biotransformation of ethanol, it first oxidizes into acetaldehyde by alcohol dehydrogenases, which subsequently oxidized to acetate by aldehyde dehydrogenases. Ethanol biotransformation in the liver produces an enormous amount of acetaldehyde and ROS, and these highly reactive intermediates, in turn, upregulate the TGF-β signaling and activate the HSCs to synthesize high amount of ECM [47, 48]. Accumulation of 4-hydroxynonenal (4-HNE), a lipid peroxidation product, has been reported experimentally as well as in patients with alcoholic liver diseases [49, 50]. Interestingly, unlike hepatocytes, HSCs are from mesenchymal origin do not contain strong intracellular antioxidant defense like hepatocytes, and hence they are more susceptible to get free radical attacks easily from injured hepatocytes as well as from Kupffer cells in the injured liver. Thus, studies have clearly shown that ROS accumulation could activate the HSCs and induce the fibrosis progression. Ironically, few studies have also reported that lipid peroxidation products are not responsible for HSCs activation [51, 52]. However, this concept is meagerly reported. Oxidative stress is now considered as a major contributor of HSCs activation and hepatic fibrosis progression. The mechanism of oxidative stress-induced HSCs activation and their consequence is presented in Fig. 2.3.

2.4.3 Oxidative Stress in Hepatocellular Carcinoma

HCC occurrence is increasing globally and ranks second in cancer-related mortality worldwide [53]. Multiple etiological factors including HBV and HCV infections, chronic ethanol consumption, NASH, obesity, diabetes, and aflatoxin-contaminated food are involved in the progression of HCC [54]. HCV infection induces excessive ROS and impairs the function of endogenous antioxidants, and therefore, HCV

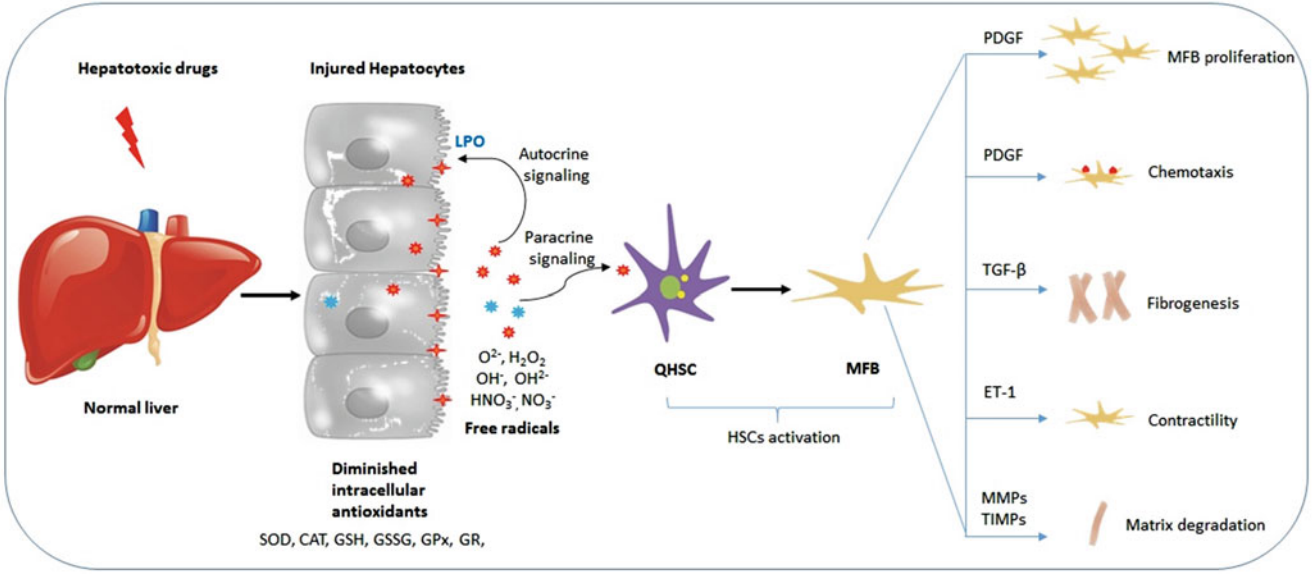


Fig. 2.3 Role of free radicals in oxidative stress induced hepatic fibrosis. *LPO* lipid peroxidation, superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxyl radicals (OH^{2-}), peroxynitrous acid (HNO_3^-), peroxynitrite (NO_3^-), *qHSCs* quiescent hepatic stellate cells, *MFB* myofibroblasts, *PDGF* platelet derived growth factors, *TGF-β* transforming growth factor β, *ET-1* endothelin-1, *MMPs* matrix metalloproteinases, *TIMPs* tissue inhibitor of metalloproteinases, *SOD* superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *GPx* glutathione peroxidase, *GR* glutathione reductase

infection is considered as the predominant cause of ROS mediated HCC progression [55]. ROS are capable of activating various signaling cascades responsible for angiogenesis, cancer cells invasion, metastasis, proliferation, and survival. In a recent preclinical study, thioredoxin reductase-1 (TrxR1), GR, and Nrf2 transcription factor null mouse have demonstrated to have a high susceptibility to HCC induced by diethylnitrosamine when compared to wild type indicating the possibility of antioxidant diminution in the progression of HCC [56]. Protein sequestosome 1/p62 (p62) acts as an intracellular defense against oxidative stress through Keap1/Nrf2 activation. Increased accumulation of p62 as inclusion bodies in HCC has been reported [57]. Oxidative stress-responsive miRNAs are recently identified in HCC cell lines. For instance, miR-34a-5p, miR-150-3p, miR-638, and miR-1915-3p are modulated in oxidative stress condition; therefore, the analysis of such miRNAs may provide a novel approach for the prognosis and diagnosis of HCC [58].

In clinical studies, oxidative stress was well correlated with the levels of NASH-HCC markers. The diminished antioxidant functions were observed in patients with NASH-HCC [59]. Interestingly, before tumor resection, HCC patients had increased oxidative stress and diminished GSH and antioxidant capacity [60]. Surgical resection is the main treatment of HCC; however, patients may develop oxidative stress-mediated liver inflammation after surgery, and coenzyme Q10 (ubiquinone), an antioxidant, level has significantly decreased in patients with HCC, thus confirming the oxidative stress. Therefore, coenzyme Q10 was used as an antioxidant therapy in HCC patients who underwent surgery [61]. Expression of Nrf2 and 8-hydroxydeoxyguanosine (8-OHdG) remarkably increased in the cancerous tissue of patients with HCC [62]. These studies are clearly indicating the concrete role of oxidative stress in the progression of HCC.

2.5 Conclusion

Undoubtedly, chronic toxic insult to the liver produces a variety of free radicals. When these free radical levels are increased in the intracellular milieu, it causes oxidative stress with a concomitant decrease in the intracellular antioxidant defense. The free radicals act as a signaling molecule and interfere with several cell signaling responsible for liver pathogenesis. More importantly, in the view of the "second hit" concept of steatosis, oxidative stress aggravates the simple fatty infiltration into complex diseases like hepatic inflammation, NASH, fibrosis, and HCC. In fibrosis conditions, free radicals from injured hepatocytes and Kupffer cells are responsible for the phenotypic transdifferentiation of the qHSCs into MFBs, thereby worsening the disease. Thus, oxidative stress injury plays a critical role in the onset of various forms of CLDs. Therefore, several experimental and clinical studies are now focusing on the verge of developing several antioxidants derived from synthetic and natural sources for CLDs.

References

1. Li X, Sun R, Liu R (2019) Natural products in licorice for the therapy of liver diseases: progress and future opportunities. Pharmacol Res 144:210–226
2. Ezhilarasan D (2018a) Oxidative stress is bane in chronic liver diseases: clinical and experimental perspective. Arab J Gastroenterol 19:56–64
3. Ezhilarasan D (2018b) Role of microRNAs in hepatic fibrosis progression. J Appl Pharm Sci 8:174–178
4. Ezhilarasan D (2019) Lead compounds with the potentials for the treatment of chronic liver diseases. In: Egbuna C, Kumar S, Ifemeje J, Ezzat S, Kaliyaperumal S (eds) Phytochemicals as lead compounds for new drug discovery: recent advances. Elsevier, Amsterdam, pp 195–210. ISBN: 978-0-12-817890-4
5. Cichoż-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20:8082–8091
6. Ezhilarasan D, Karthikeyan S, Vivekanandan P (2012) Ameliorative effect of silibinin against N-nitrosodimethylamine-induced hepatic fibrosis in rats. Environ Toxicol Pharmacol 34:1004–1013
7. Holmström KM, Finkel T (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 15:411–421
8. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247
9. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y (2015a) The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 16:26087–26124
10. Li X, Huang W, Gu J, Du X, Lei L, Yuan X, Sun G, Wang Z, Li X, Liu G (2015b) SREBP-1c overactivates ROS-mediated hepatic NF-κB inflammatory pathway in dairy cows with fatty liver. Cell Signal 27:2099–2109
11. Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 30:11–26
12. Senoner T, Schindler S, Stättner S, Öfner D, Troppmair J, Primavesi F (2019) Associations of oxidative stress and postoperative outcome in liver surgery with an outlook to future potential therapeutic options. Oxidative Med Cell Longev 2019:3950818
13. Jadeja RN, Devkar RV, Nammi S (2017) Oxidative stress in liver diseases: pathogenesis, prevention, and therapeutics. Oxidative Med Cell Longev 2017:8341286
14. Ma Q (2012) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426
15. Ezhilarasan D, Karthikeyan S (2016) Silibinin alleviates N-nitrosodimethylamine-induced glutathione dysregulation and hepatotoxicity in rats. Chin J Nat Med 14:40–47
16. Anitha M, Anitha R, Vijayaraghavan R, Senthil Kumar S, Ezhilarasan D (2019) Oxidative stress and neuromodulatory effects of deltamethrin and its combination with insect repellents in rats. Environ Toxicol 34:753–759
17. Ezhilarasan D, Karthikeyan S, Sokal E, Najimi M (2014) Silibinin treatment to hepatic stellate cells: an exploration of apoptosis and cellular senescence. J Pharm Res 8:1125–1130
18. Sun J, Fu J, Li L, Chen C, Wang H, Hou Y, Xu Y, Pi J (2018) Nrf2 in alcoholic liver disease. Toxicol Appl Pharmacol 357:62–69
19. Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, Sethi G, Perumal E (2018) Antioxidant response elements: discovery, classes, regulation and potential applications. Redox Biol 17:297–314
20. Al-Sawaf O, Clarner T, Fragoulis A, Kan YW, Pufe T, Streetz K, Wruck CJ (2015) Nrf2 in health and disease: current and future clinical implications. Clin Sci (Lond) 129:989–999
21. Jadeja RN, Upadhyay KK, Devkar RV, Khurana S (2016) Naturally occurring Nrf2 activators: potential in treatment of liver injury. Oxidative Med Cell Longev 2016:3453926

22. He W, Xu Y, Ren X, Xiang D, Lei K, Zhang C, Liu D (2019) Vitamin E ameliorates lipid metabolism in mice with nonalcoholic fatty liver disease via Nrf2/CES1 signaling pathway. Dig Dis Sci 64:3182. https://doi.org/10.1007/s10620-019-05657-9
23. Li J, Hu R, Xu S, Li Y, Qin Y, Wu Q, Xiao Z (2017) Xiaochaihutang attenuates liver fibrosis by activation of Nrf2 pathway in rats. Biomed Pharmacother 96:847–853
24. Shin JM, Lee KM, Lee HJ, Yun JH, Nho CW (2019) Physalin A regulates the Nrf2 pathway through ERK and p38 for induction of detoxifying enzymes. BMC Complement Altern Med 19:101
25. Xu D, Chen L, Chen X, Wen Y, Yu C, Yao J, Wu H, Wang X, Xia Q, Kong X (2017) The triterpenoid CDDO-imidazolide ameliorates mouse liver ischemia-reperfusion injury through activating the Nrf2/HO-1 pathway enhanced autophagy. Cell Death Dis 8:e2983
26. Idilman IS, Ozdeniz I, Karcaaltincaba M (2016) Hepatic steatosis: etiology, patterns, and quantification. Semin Ultrasound CT MR 37:501–510
27. Paradies G, Paradies V, Ruggiero FM, Petrosillo G (2014) Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease. World J Gastroenterol 20:14205–14218
28. Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114:842–845
29. Engin A (2017) Non-alcoholic fatty liver disease. Adv Exp Med Biol 960:443–467
30. Masarone M, Rosato V, Dallio M, Gravina AG, Aglitti A, Loguercio C, Federico A, Persico M (2018) Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. Oxidative Med Cell Longev 2018:9547613
31. Caballero F, Fernández A, Matías N, Martínez L, Fucho R, Elena M, Caballeria J, Morales A, Fernández-Checa JC, García-Ruiz C (2010) Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J Biol Chem 285:18528–18536
32. Chen YT, Lin YC, Lin JS, Yang NS, Chen MJ (2018) Sugary kefir strain Lactobacillus mali APS1 ameliorated hepatic steatosis by regulation of SIRT-1/Nrf-2 and gut microbiota in rats. Mol Nutr Food Res 62:e1700903
33. Deng Y, Tang K, Chen R, Nie H, Liang S, Zhang J, Zhang Y, Yang Q (2019) Berberine attenuates hepatic oxidative stress in rats with non-alcoholic fatty liver disease via the Nrf2/ARE signalling pathway. Exp Ther Med 17:2091–2098
34. Elvira-Torales LI, Martín-Pozuelo G, González-Barrio R, Navarro-González I, Pallarés FJ, Santaella M, García-Alonso J, Sevilla Á, Periago-Castón MJ (2019) Ameliorative effect of spinach on non-alcoholic fatty liver disease induced in rats by a high-fat diet. Int J Mol Sci 20: E1662
35. Veskovic M, Mladenovic D, Milenkovic M, Tosic J, Borozan S, Gopcevic K, Labudovic-Borovic M, Dragutinovic V, Vucevic D, Jorgacevic B, Isakovic A, Trajkovic V, Radosavljevic T (2019) Betaine modulates oxidative stress, inflammation, apoptosis, autophagy, and Akt/mTOR signaling in methionine-choline deficiency-induced fatty liver disease. Eur J Pharmacol 848:39–48
36. Yang DK, Jo DG (2018) Mulberry fruit extract ameliorates nonalcoholic fatty liver disease (NAFLD) through inhibition of mitochondrial oxidative stress in rats. Evid Based Complement Alternat Med 2018:8165716
37. Chashmniam S, Mirhafez SR, Dehabeh M, Hariri M, Azimi Nezhad M, Nobakht MGBF (2019) A pilot study of the effect of phospholipid curcumin on serum metabolomic profile in patients with non-alcoholic fatty liver disease: a randomized, double-blind, placebo-controlled trial. Eur J Clin Nutr 73:1224. https://doi.org/10.1038/s41430-018-0386-5
38. Yang J, Fernández-Galilea M, Martínez-Fernández L, González-Muniesa P, Pérez-Chávez A, Martínez JA, Moreno-Aliaga MJ (2019) Oxidative stress and non-alcoholic fatty liver disease: effects of Omega-3 fatty acid supplementation. Nutrients 11:E872
39. Yang L, Wu D, Wang X, Cederbaum A (2012) Cytochrome P4502E1, oxidative stress, JNK, and autophagy in acute alcohol-induced fatty liver. Free Radic Biol Med 53:1170–1180

40. Sánchez-Valle V, Chávez-Tapia NC, Uribe M, Méndez-Sánchez N (2012) Role of oxidative stress and molecular changes in liver fibrosis: a review. Curr Med Chem 19:4850–4860
41. Weiskirchen R, Weiskirchen S, Tacke F (2018) Recent advances in understanding liver fibrosis: bridging basic science and individualized treatment concepts. F1000Res 7:F1000. Faculty Rev-921
42. Ezhilarasan D, Evraerts J, Brice S, Buc-Calderon P, Karthikeyan S, Sokal E, Najimi M (2016) Silibinin inhibits proliferation and migration of human hepatic stellate LX-2 cells. J Clin Exp Hepatol 6:167–174
43. Ezhilarasan D, Sokal E, Najimi M (2018) Hepatic fibrosis: it is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int 17(3):192–197
44. den Hartog GJ, Qi S, van Tilburg JH, Koek GH, Bast A (2014) Superoxide anion radicals activate hepatic stellate cells after entry through chloride channels: a new target in liver fibrosis. Eur J Pharmacol 724:140–144
45. Nieto N, Friedman SL, Cederbaum AI (2002) Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1-derived reactive oxygen species. Hepatology 35:62–73
46. Novitskiy G, Traore K, Wang L, Trush MA, Mezey E (2006) Effects of ethanol and acetaldehyde on reactive oxygen species production in rat hepatic stellate cells. Alcohol Clin Exp Res 30:1429–1435
47. Ezhilarasan D, Lakshmi T, Karthikeyan S (2015) *In vivo* experimental models for hepatotoxin induced fibrosis – a toxicological view. Toxicol Int 22:1–9
48. Ceni E, Mello T, Galli A (2014) Pathogenesis of alcoholic liver disease: role of oxidative metabolism. World J Gastroenterol 20:17756–17772
49. Seth D, Haber PS, Syn WK, Diehl AM, Day CP (2011) Pathogenesis of alcohol-induced liver disease: classical concepts and recent advances. J Gastroenterol Hepatol 26:1089–1105
50. Smathers RL, Galligan JJ, Stewart BJ, Petersen DR (2011) Overview of lipid peroxidation products and hepatic protein modification in alcoholic liver disease. Chem Biol Interact 192:107–112
51. Fang HL, Lin WC (2008) Lipid peroxidation products do not activate hepatic stellate cells. Toxicology 253:36–45
52. Olynyk JK, Khan NA, Ramm GA, Brown KE, O'Neill R, Britton RS, Bacon BR (2002) Aldehydic products of lipid peroxidation do not directly activate rat hepatic stellate cells. J Gastroenterol Hepatol 17:785–790
53. Ponselvi Induja M, Ezhilarasan D, Ashok Vardhan N (2018) *Evolvulus alsinoides* methanolic extract triggers apoptosis in HepG2 cells. Avicenna J Phytomed 8:504–512
54. Sanyal AJ, Yoon SK, Lencioni R (2010) The etiology of hepatocellular carcinoma and consequences for treatment. Oncologist 15:14–22
55. Fu N, Yao H, Nan Y, Qiao L (2017) Role of oxidative stress in hepatitis C virus induced hepatocellular carcinoma. Curr Cancer Drug Targets 17:498–504
56. McLoughlin MR, Orlicky DJ, Prigge JR, Krishna P, Talago EA, Cavigli IR, Eriksson S, Miller CG, Kundert JA, Sayin VI, Sabol RA, Heinemann J, Brandenberger LO, Iverson SV, Bothner B, Papagiannakopoulos T, Shearn CT, Arnér ESJ, Schmidt EE (2019) TrxR1, Gsr, and oxidative stress determine hepatocellular carcinoma malignancy. Proc Natl Acad Sci U S A 116:11408. https://doi.org/10.1073/pnas.1903244116
57. Denk H, Stumptner C, Abuja PM, Zatloukal K (2019) Sequestosome 1/p62-related pathways as therapeutic targets in hepatocellular carcinoma. Expert Opin Ther Targets 23:393–406
58. Wan Y, Cui R, Gu J, Zhang X, Xiang X, Liu C, Qu K, Lin T (2017) Identification of four oxidative stress-responsive microRNAs, miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p, in hepatocellular carcinoma. Oxidative Med Cell Longev 2017:5189138
59. Shimomura Y, Takaki A, Wada N, Yasunaka T, Ikeda F, Maruyama T, Tamaki N, Uchida D, Onishi H, Kuwaki K, Nakamura S, Nouso K, Miyake Y, Koike K, Tomofuji T, Morita M, Yamamoto K, Okada H (2017) The serum oxidative/anti-oxidative stress balance becomes dysregulated in patients with non-alcoholic steatohepatitis associated with hepatocellular carcinoma. Intern Med 56:243–251

60. Cheng SB, Liu HT, Chen SY, Lin PT, Lai CY, Huang YC (2017) Changes of oxidative stress, glutathione, and its dependent antioxidant enzyme activities in patients with hepatocellular carcinoma before and after tumor resection. PLoS One 12:e0170016
61. Liu HT, Cheng SB, Huang YC, Huang YT, Lin PT (2017) Coenzyme Q10 and oxidative stress: inflammation status in hepatocellular carcinoma patients after surgery. Nutrients 9:E29
62. Ma-On C, Sanpavat A, Whongsiri P, Suwannasin S, Hirankarn N, Tangkijvanich P, Boonla C (2017) Oxidative stress indicated by elevated expression of Nrf2 and 8-OHdG promotes hepatocellular carcinoma progression. Med Oncol 34:57

The Role of Diverse Nanoparticles in Oxidative Stress: In Vitro and In Vivo Studies

3

Shanmugam Rajeshkumar, Durairaj Sekar, Devaraj Ezhilarasan, and Thangavelu Lakshmi

Abstract

Nanoparticles are a very advanced area of nanotechnology and play an important role in medical sciences and technology. Nanoparticles such as polymer and metal nanoparticles are widely used in applications for antioxidant activity. Nonenzymatic antioxidant assays using free radical–scavenging activity of nanoparticles have been investigated using different methods such as 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays, nitric oxide radical inhibition assays, superoxide anion–scavenging activity, reducing power, determination of total phenolic compounds and hydroxyl radical–scavenging assays. The investigated metal nanoparticle included gold, zinc oxide, copper, silver, zirconium oxide and selenium, and the polymer nanoparticles include chitosan and silica.

Keywords

Metal nanoparticles · Antioxidant activity · Polymers · Synthesis · Characterization

S. Rajeshkumar (✉) · D. Ezhilarasan · T. Lakshmi
Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, Tamil Nadu, India

D. Sekar
Dental Research Cell (DRC-BRULAC), Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, Tamil Nadu, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_3

3.1 Introduction

3.1.1 Nanoparticles Used in Antioxidant Activity

Nanotechnology is one of the most recent areas to be explored for its applications related to engineering, medicine and various other sciences. Nanoparticles are particles in the size range of 1–100 nm and have the most applications in nanotechnology. Nanoparticles in different forms play vital roles in biomedical applications. The different types or forms of nanoparticles include metal nanoparticles (gold, silver, zinc, copper, selenium, etc.), metal oxide nanoparticles (silver oxide, zinc oxide, copper oxide, cadmium oxide and zirconium oxide), polymer nanoparticles (chitosan, silica, polyethylene glycol, cellulose, polyvinyl alcohol and polyvinyl pyrrolidine), carbon nanotubes, magnetic nanoparticles, nanohydrogels, aerogels, graphene nanostructures, nanocomposites, nanoshells, nanohybrids and biomolecules (curcumin, beta cyclodextrins, etc.).

Previously, nanoparticles were synthesized using physical and chemical techniques such as chemical vapour deposition, microwave irradiation, sol–gel techniques, plasma synthesis techniques, mechanical milling, ultrasound techniques, the hydrothermal method, the solvothermal method, the electrodeposition process, electroexplosion and laser techniques. Because of the high cost and environmental factors, researchers have recently been exploring use of green materials for the synthesis of nanoparticles, using microorganisms such as *Bacillus subtilis*, *Klebsiella planticola*, *Klebsiella pneumoniae* and *Aspergillus niger*; plant extracts from *Coleus aromaticus*, *Pongamia pinnata*, etc.; and algal extracts of *Turbinaria conoides*, *Padina tetrastromatica*, etc. [1–6]. Synthesis of nanoparticles using biological methods is very simple and cost effective. The prepared nanoparticles have been characterized using various techniques such as scanning electron microscopy, atomic force microscopy, ultraviolet–visible light (UV-vis) spectroscopy, dynamic light scattering, transmission electron microscopy, Fourier transform infrared spectroscopy, gas chromatography with mass spectroscopy, zeta potential analysis, thermogravimetric analysis, elemental dispersive analysis and x-ray diffraction assays [7–9]. Figure 3.1 shows green synthesis of nanoparticles and their characterization.

These nanoparticles are used in diverse applications such as anticancer activity.

Different types of nanoparticles are used for antioxidant activity in vitro and in vivo. Among these nanoparticles, metal and metal oxide nanoparticles are majorly involved in the activity in different experimental procedures. Figure 3.2 shows the different types of nanoparticles involved in antioxidant activity.

3.1.1.1 Silver Nanoparticles

Silver nanoparticles are the major metal nanoparticles in use and are intensively used in antimicrobial applications for their antibacterial and antifungal activities. In

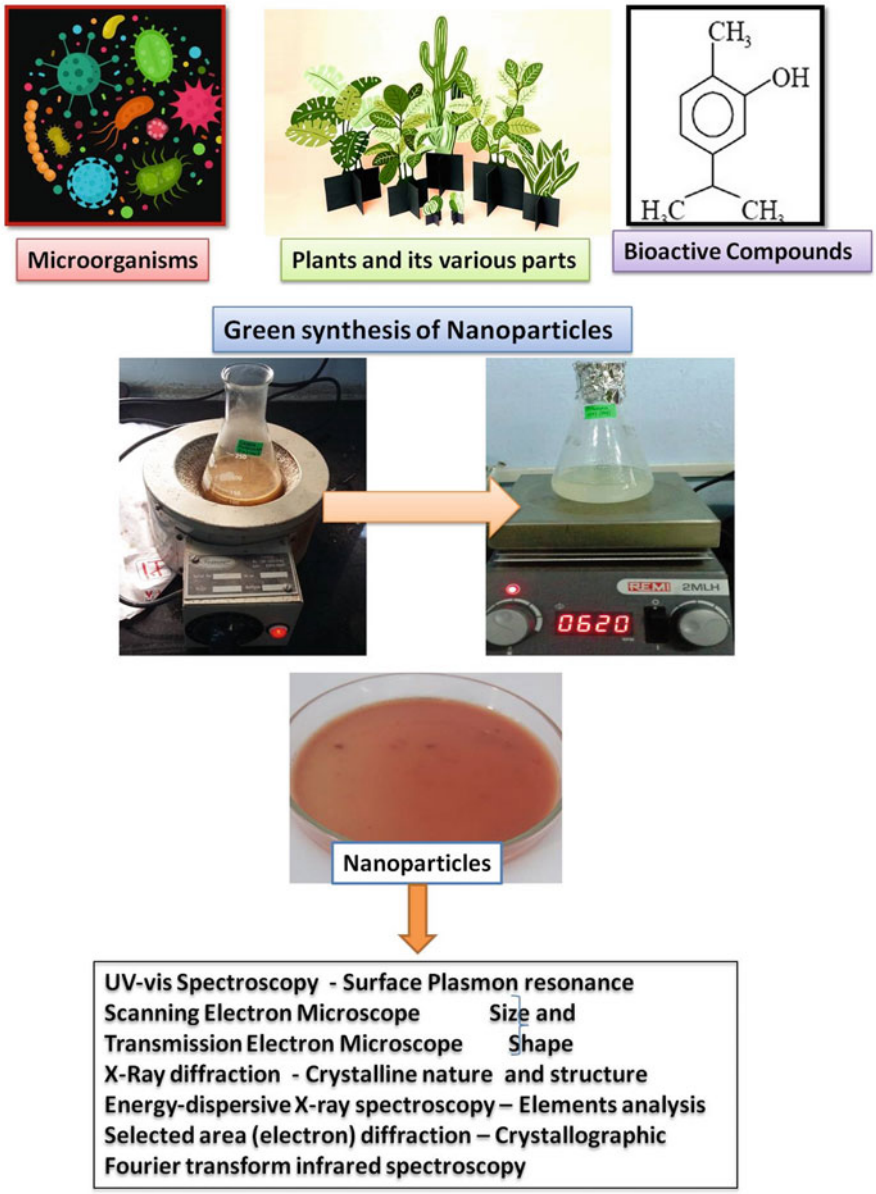


Fig. 3.1 Biosynthesis and characterization of nanoparticles

addition, silver nanoparticle have achieved very good results in anticancer and antioxidant activities [10–13]. Table 3.1 provides information on green synthesis of silver nanoparticles characterized using various techniques and antioxidant activities.

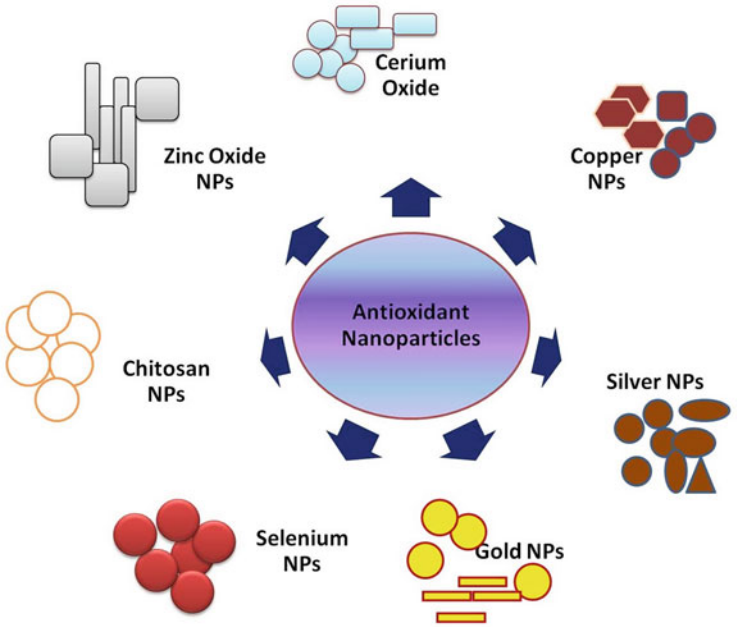


Fig. 3.2 Different nanoparticles (NPs) used in antioxidant activity

3.1.1.2 Gold Nanoparticles

Gold nanoparticles are widely used for delivery of drugs, proteins and genes in biomedical applications because of their surface plasmon resonance. These advanced metal nanoparticles also have applications in photothermal therapy, cancer imaging, identification of pathogens using immune chromatographic techniques, tissue imaging, anti-inflammatory activities and anticancer activities [39–41]. Table 3.2 provides information on gold nanoparticles and their antioxidant activities in various biochemical assays.

3.1.1.3 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have unique properties with many applications in many fields such as photocatalytic activity; antibacterial and antifungal activity against clinical, animal and plant pathogens; dye degradation and heavy metal degradation activity; and UV-filtering properties [10, 50–52]. Zinc oxide nanoparticles are one of the important types of semiconductor nanoparticles used in multitasking applications, including antioxidant activity, as shown in Table 3.3.

3.1.1.4 Antioxidant Activity of Other Nanoparticles

Apart from silver, gold and zinc nanoparticles, other nanoparticles such as chitosan, titanium dioxide, cerium oxide, selenium, magnetic nanoparticles, silicon dioxide and nickel oxide nanoparticles also show very good antioxidant activity in different

Table 3.1 Synthesis, characterization and antioxidant activity of silver nanoparticles (AgNPs)

Reducing agent	Characterization of NPs	Antioxidant activity: method, concentration, activity level	Reference
Aqueous leaf extract of *Ficus hispida* Linn.f.	TEM: 20 nm Shape: Spherical UV-vis spectroscopy: 423 nm	DPPH assay: 21.07 ± 0.02%	[14]
Aqueous leaf extract of *Cestrum nocturnum*	TEM: 20 nm Shape: Spherical UV-vis spectroscopy: 442 nm SEM: 15–28 nm	DPPH assay: 29.55%, hydrogen peroxide 45.41%, hydroxyl radical 20%, superoxide radical–scavenging activity 8%	[15]
Lippia alba extract	FTIR: 1595 and 1410/cm UV-vis spectroscopy: 408 nm	DPPH assay: 56.13 ± 4.79% FRAP assay: 2436.45 ± 137.16 μM	[16]
Mucuna birdwoodiana, *Phoebe lanceolata*, *Cratoxylum formosum*, *Scurrula parasitica*, *Ceratostigma minus*, *Myrsine africana* and *Lindera strychnifolia* plant extracts	UV-vis spectroscopy: 450 nm	DPPH assay: 29.2%, 35.2%, 44.4%, 42.4%, 35.8%, 28.3% and 30.2%	[17]
Acetic acid and polyvinyl alcohol, chitosan	TEM: 190–200 nm	DPPH assay: EC_{50} (in scavenging DPPH) 0.4 mg/mL FRAP assay: Hydroxyl radical–scavenging activity 77.43% and 85.9% at 1.5 mg/mL and 2 mg/mL, respectively Cytotoxic activity 5–200 μg/mL against Chinese hamster ovary (CHO-K1) cells	[18]
Caesalpinia pulcherrima stem extract	UV-vis spectroscopy: 410 nm TEM: 3–15 nm Crystalline structure	DPPH assay: IC_{50} 664 mg/mL Superoxide anion radical–scavenging activity 72 μg/mL ABTS radical–scavenging activity 216 μg/mL	[19]
Calophyllum tomentosum leaf extract	XRD: Crystalline structure UV-vis spectroscopy: 438 nm	DPPH assay: 90% Nitric oxide radical–scavenging assay: 78.46% Hydrogen peroxide–scavenging assay: 83.94% Reducing power activity 74%	[20]
Streptomyces violaceus MM72	TEM: 50 nm XRD: Crystalline structure	DPPH assay: 89.5% Total antioxidant activity 0.730 at 50 μg/mL FRAP assay: 1.83 AU at 1000 μg/mL concentration H_2O_2-scavenging activity 72.5% Nitric oxide–scavenging activity 60.1%	[21]

(continued)

Table 3.1 (continued)

Reducing agent	Characterization of NPs	Antioxidant activity: method, concentration, activity level	Reference
Phyllanthus acidus extract (leaf and twig)	SEM and DLS: 48.36 and 164.30 nm Shape: Spherical	DPPH assay: EC_{50} of leaf extract AgNPs 58.83 ± 1.65 μg/mL Nitric oxide radical–scavenging assay: EC_{50} of twig extract AgNPs 60.75 ± 1.59 μg/mL Hydroxy radical–scavenging assay: EC_{50} 43.02 ± 1.62 μg/mL	[22]
Citrus limon, *Citrus sinensis* and *Citrus limetta* fruit waste (peel) extracts	TEM: 9–46 nm	DPPH assay: Scavenging activity 87.43%, 67.50% and 95.13% Antimicrobial activity: Agar well diffusion technique Cytotoxic activity against human lung cancer cell line A549	[23]
Garlic, turmeric and green tea extracts	UV-vis spectroscopy: 450 nm XRD: Crystalline structure SEM and TEM: 8 nm Shape: Spherical	DPPH and ABTS hydroxyl radical–scavenging assays: Turmeric-mediated AgNPs showed maximum activity	[24]
White tea extract	TEM: 19.77 ± 3.82 nm for white tea/AgNPs XRD: Crystalline structure	DPPH assay: 88.09 ± 0.08% Cytotoxicity studies on MOLT-4 cells	[25]
Commercial green tea extract (*Camellia sinensis*)	UV-vis spectroscopy: 410 nm DLS: 34.68 ± 4.95 nm	–	[26]
Aconitum toxicum Reichenb. Rhizome alcoholic extract	UV-vis spectroscopy: 70 and 55 nm	DPPH assay: 81.11% for extract 1, 84.32% for extract 2	[27]
Quercetin, rutin and gallic acid, protocatechuic acid, caffeic acid and hesperidin supplied from sigma-Aldrich and Fluka	UV-vis spectroscopy: 407 nm SEM: Spherical nanoparticles, size 76.01 nm	DPPH and ABTS assays: Quercetin showed greater activity than rutin	[28]
Tropaeolum majus L. leaf extract	UV-vis spectroscopy: 463 nm	BHT assay: 98.9% DPPH assay: Ethanol extract 52.5%, aqueous extract 66.1% ABTS assay: Ascorbic acid 81.46%, aqueous extract 56.6%, ethanol extract 43.4% Total antioxidant activity: 550 and 530 μg/mL	[29]
Chitosan–polyethylene glycol hydrogel	UV-vis spectroscopy: 404–408 nm SEM: 99.1 ± 2.3 nm	DPPH assay: Ascorbic acid 22–92%, AgNPs 15–57%, chitosan hydrogel 11–43%, AgNP-loaded chitosan hydrogel 26–85%	[30]

Allium ampeloprasum L. leaf extract	UV-vis spectroscopy: 420 and 440 nm TEM: 2 and 43 nm Shapes: Spherical, quasispherical, hexagonal, ellipsoidal and irregular	DPPH assay: 62.2–82.4% ABTS assay: 64.5–96.8%	[31]
Phenolic compounds purchased from sigma-Aldrich	UV-vis spectroscopy: 420 nm TEM: ≤15 nm	ABTS assay: AgNPs: $R = 0.956$, $p < 0.0001$	[32]
Novel L-arginine–dextran-70 functionalized with RF and HSA	TEM: 41.60 nm UV-vis spectroscopy: ~476 nm	Antioxidant activity of $SNPs^{Arg}$ systems ($SNPs^{Arg}$, $SNPs^{Arg}$/HSA, $SNPs^{Arg}$/RF, $SNPs^{Arg}$/RF/HSA) was monitored by chemiluminescence Antioxidant activity of $SNPs^{Arg}$ was about 11.3%, and that of the $SNPs^{Arg}$/RF/HSA system was increased to 21.24%	[33]
g-C_3N_4 nanosheet–decorated Ag_2S composites	UV-vis spectroscopy (g-C_3N_4 sample): 440 nm UV-vis spectroscopy (Ag_2S): 675 nm	DPPH method: g-C_3N_4/Ag_2S composite exhibited greater DPPH radical–scavenging activity (IC_{50} 1.58 μM) than bare Ag_2S (IC_{50} 2.81 μM)	[34]
Fusarium oxysporum	SEM and AFM: 30–45 nm	–	[35]
Memecylon umbellatum Burm.f. (4-*N*-methyl benzoic acid)	UV-vis spectroscopy: 430 nm High-resolution TEM: 7–23 nm	Superoxide radical–scavenging activity: EC_{50} 66.68 μg/mL (74.76%) DPPH radical–scavenging activity: EC_{50} 53.46 mg/mL (81.57%) Anticancer activity against breast cancer cell line: IC_{50} 42.19 μg/mL	[36]
Trichoderma atroviride	UV-vis spectroscopy: 390–400 nm FTIR: 1115.4 and 3450/cm	DPPH-scavenging activity in a concentration-dependent manner (IC_{50} 45.6 μg/mL)	[37]
Gallic acid and chitosan	TEM: 26.23 ± 9.92 nm	–	[38]

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), *AFM* atomic force microscopy, *BHT* butylated hydroxy toluene, *DLS* differential light scattering, *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate, EC_{50} half-maximal effective concentration, *FRAP* ferric-reducing ability of plasma, *FTIR* Fourier transform infrared, *g-C_3N_4* graphitic carbon nitride, *HSA* human serum albumin, IC_{50} half-maximal inhibitory concentration, *RF* riboflavin, *SEM* scanning electron microscopy, *$SNPs^{Arg}$* L-arginine–dextran-70–based-silver nanoparticles, *TEM* transmission electron microscopy, *UV-vis* ultraviolet–visible light, *XRD* x-ray diffraction

Table 3.2 Antioxidant activity of gold nanoparticles (AuNPs)

Reducing agent	Characterization of NPs	Antioxidant activity: method, concentration, activity level	Reference
Extra-virgin olive oil	TEM: 15 nm UV-vis spectroscopy: 540 nm	ABTS and DPPH assays, Folin–Ciocalteau method Gallic acid: Reference standard Coefficients: ABTS $R^2 = 0.999$, DPPH $R^2 = 0.996$, Folin–Ciocalteau $R^2 = 0.992$	[42]
Citric acid	TEM: 12 ± 1.5 nm Spherical and uniformly dispersed UV-vis spectroscopy: 525 nm Cyclic voltammetry	DPPH assay: IC_{30} 1.89 E−6 M, IC_{50} 1.15 E−5 M	[43]
Panax ginseng leaf extract	UV-vis spectroscopy: 517 nm (DPPH assay)	DPPH assay: IC_{50} 16.06 μg/mL MTT assay: HDF and murine melanoma B16BL6 cell lines (tyrosinase activity assay)	[44]
DMAHF	UV-vis spectroscopy: 534 nm FTIR TEM: 6–8 nm	DPPH assay	[45]
Chitosan	UV-vis spectroscopy: 551 nm Shapes: Spherical and irregular TEM, XRD, FTIR	Hydroxyl radical–scavenging assay, FRAP assay (0.266 ± 0.007), DPPH assay ($n = 3$, $p \leq 0.05$), ABTS assay	[46]
Starch	UV-vis spectroscopy: 525 nm Size 22 nm TEM: Tert-BHP limit of detection 39 μM	AuNP nanosensor–based peroxyl radical–scavenging assay Classic ORAC assay	[47]
Aqueous leaf extract of *Delonix regia*	UV-vis spectroscopy: 541 and 432 nm TEM: 6–40 nm Shapes: Spherical and irregular	DPPH: SC_{50} 7.35 ABTS assay: SC_{50} 6.21	[48]
Sodium citrate	TEM: 13 nm	Histological assessment Effective: 50 nm AuNPs	[49]

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), *DMAHF* 4′-*N*,*N*-dimethylamino-3-hydroxyflavone, *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate, *FRAP* ferric-reducing ability of plasma, *FTIR* Fourier transform infrared, *HDF* human dermal fibroblast, IC_{30} 30% of maximal inhibitory concentration, IC_{50} half-maximal inhibitory concentration, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *ORAC* oxygen radical absorbance capacity, SC_{50} sample concentration reducing DPPH or ABTS concentration to half its initial value, *TEM* transmission electron microscopy, *tert-BHP* tert-butyl hydroperoxide, *UV-vis* ultraviolet–visible light, *XRD* x-ray diffraction

Table 3.3 Antioxidant activity of zinc oxide nanoparticles (ZnONPs)

Reducing agent	Characterization of NPs	Antioxidant activity: method, concentration, activity level	Reference
Citrus pectin powder, chitosan, sodium alginate	UV-vis spectroscopy TEM: 46 nm DLS, XRD, FTIR	DPPH assay IC_{50} values 47.5 and 65 μg/mL Antibacterial activity against gram-positive and gram-negative organisms and yeast Anticancer activity against Ehrlich ascites carcinoma	[53]
Inducible nitric oxide synthase (nos2) gene	TEM: 45 nm	qPCR analysis Western blot analysis	[54]
Psidium guajava leaf extract	UV-vis spectroscopy: 345 nm SEM: Spherical shapes	DPPH assay: 77.80–81.35%	[55]
Thymus vulgaris leaf extract	Size 50–60 nm Shape: Irregular TEM, XRD, EDX, DLS, FTIR	DPPH assay: $\leq$75% antibacterial activity against selected foodborne pathogens	[56]
Mangifera indica leaf extract	UV-vis spectroscopy, TEM, SEM, XRD, EDX Size 45–60 nm Shape: Spherical and hexagonal quartzite	DPPH assay Cytotoxicity assays: A549 lung cancer cell line	[12]
Curcumin	FTIR, field emission SEM, XRD, UV-vis spectroscopy	DPPH assay: 24.25% Stability of curcumin improved ($p < 0.05$)	[57]
Synthesized by aqueous and polyol method	XRD: 10 and 40 nm UV-vis spectroscopy TEM: 10 and 15 nm	DPPH assay: IC_{50} values 39.38 and 43.33 Metal chelation: IC_{50} values 54.17 and 51.6 ABTS assay: IC_{50} values 38.31 and 39.15 Antibacterial activity	[58]
Pithecellobium dulce peel extract	Hexagonal crystalline structure Shape: Spherical Size 11.5 $\pm$ 2 nm	Photocatalytic activity Antifungal activity	[59]
Tecomaca stanifolia leaf extract	UV-vis spectroscopy: 380 nm Shape: Spherical Size 70–75 nm	DPPH assay Anticancer activity IC_{50} 65 μg/mL (A549 cell line)	[60]
Malus pumila and *Juglen regia* plant extracts	UV-vis spectroscopy, TEM, XRD, FTIR, DLS, SEM, EDX Size 12 and 16 nm	DPPH assay Antibacterial activity	[61]
Artemisia haussknechtii leaf extract	UV-vis spectroscopy, TEM, GC-MS, FTIR, AFM, SEM, EDX, powder XRD Size 50–60 nm Shape: Hexagonal wurtzite	Total antioxidant capacity DPPH assay Disc diffusion assay	[62]

(continued)

Table 3.3 (continued)

Reducing agent	Characterization of NPs	Antioxidant activity: method, concentration, activity level	Reference
Oleic acid, gluconic acid, tween 80	Shapes: (1) flower-like nanorods and nanoflakes; (2) nanogranules, size 20–30 nm; (3) assembled hierarchical structure	H_2O_2 free radical–scavenging activity, ABTS assay, DPPH assay	[63]
Ricinus communis plant seed extract	Shape: Crystalline hexagonal arrangement Size 20 nm Powder XRD, FTIR, XRD, TEM	Antioxidant activity DPPH assay, FRAP assay Anticancer activity: IC_{50} of ZnONPs in MDA-MB-231 breast cancer cells: 7.103 μg/mL Antifungal activity	[64]
Coccinia abyssinica tuber extract	Size 10.4 nm Shape: Hexagonal (analysed using TEM)	DPPH assay IC_{50} 127.74 μg/mL Well diffusion assay	[65]
Codonopsis lanceolata root extract	Size and shape: 500 nm with flower-like structure confirmed by XRD and TEM UV-vis spectroscopy: 365 nm	Photocatalytic degradation activity	[66]
Vitamin E and C mixture	DLS, TEM, inductive coupled plasma mass spectrometry Size 35 nm	Lipid peroxidation activity in Nile tilapia tissues	[67]
Aqueous extract of chironji leaves	XRD, TEM and UV-vis techniques: 363 nm Shape: Hexagonal wurtzite	DPPH assay IC_{50} 8025 μg/mL Antibacterial activity Photocatalytic degradation activity	[68]
Copditis rhizome extract	Size 8.50 nm Shapes: Spheres and rods SEM, EDX, FTIR, XRD, TEM, TGA, SAED, UV-vis spectroscopy	DPPH assay: 1 mg/mL (52.34%), >0.5 mg/mL (51.57%), >0.25 mg/mL (51.19%), >0.125 mg/mL (38.12%) Cytotoxicity against RAW 264.7 cells Antibacterial activity	[69]
Water extract of *Garcinia xanthochymus*	UV-vis spectroscopy: 370 nm SEM: Spongy cave-like structures XRD: Pure wurtzite structure	DPPH assay Photocatalytic degradation activity	[70]
Polygala tenuifolia root extract	UV-vis spectroscopy, FTIR, TGA TEM: 33.03–73.48 nm Shape: Spherical	DPPH assay: 45.47%	[71]

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), *AFM* atomic force microscopy, *DLS* differential light scattering, *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate, *EDX* energy-dispersive x-ray, *FRAP* ferric-reducing ability of plasma, *FTIR* Fourier transform infrared, *GC-MS* gas chromatography with mass spectrometry, IC_{50} half-maximal inhibitory concentration, *qPCR* quantitative polymerase chain reaction, *SAED* selected area electron diffraction, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *TGA* thermogravimetric analysis, *UV-vis* ultraviolet–visible light, *XRD* x-ray diffraction

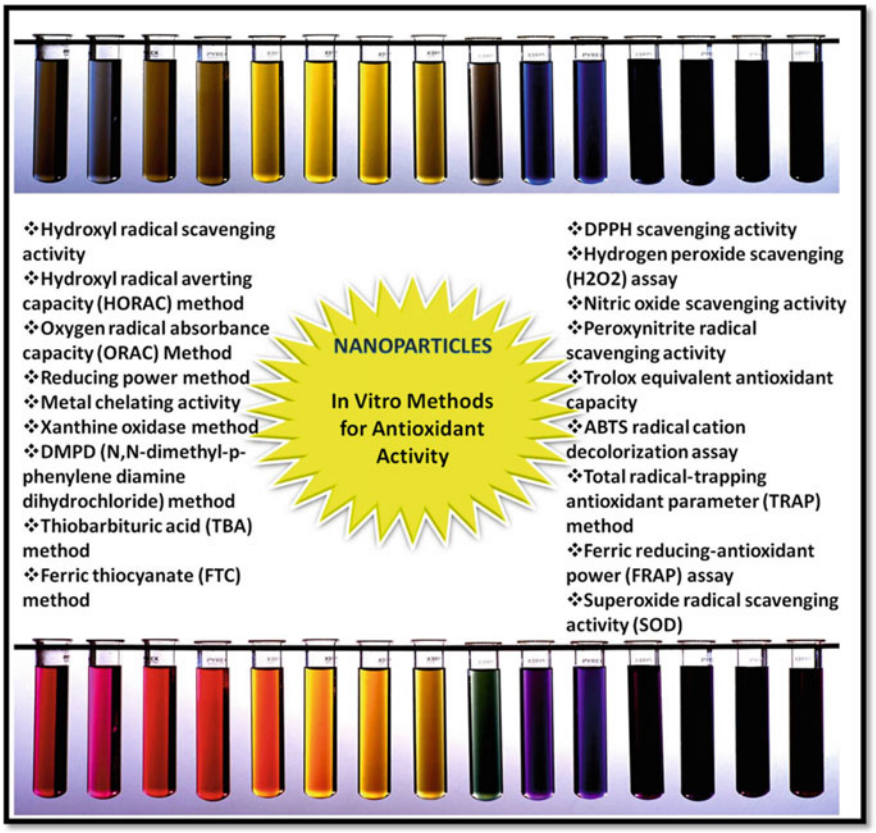


Fig. 3.3 In vitro antioxidant activity of nanoparticles using various assays. *ABTS* 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate

assays. Figure 3.3 shows different antioxidant assays used for free radical–scavenging nanoparticles.

3.1.1.5 Antioxidant Activity of Polymer, Magnetic and Oxide Nanoparticles

Chitosan is an important bioactive product, obtained from crab shells and prawn shells. It shows good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Candida albicans*, and it has shown good scavenging activity of 76% in a 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay [72]. Super-para iron oxide nanoparticles synthesized using *Stevia* leaf extract had a spherical shape and were 25 nm in size on high-resolution transmission electron microscopy (TEM) analysis. They showed good antioxidant activity in a DPPH assay and a half-maximal inhibitory concentration (IC_{50}) of 65 μg/mL [73]. Manganese oxide nanoparticles prepared using mature seeds of

Atropa belladonna L. showed a crystalline structure (on x-ray diffraction (XRD)) and a spherical shape with a size of 30 nm, confirmed by TEM. The free radical–scavenging activity of MnO_2 nanoparticles, investigated using a DPPH assay with plantlets at 200 mg/L with an IC_{50} of 134.6 μg/mL and Fe^{2+}-chelating activity, also showed the same tendency [74].

Selenium nanoparticles synthesized using pectin showed DPPH radical–scavenging activity of 92%, a Trolox-equivalent antioxidant capacity assay value of 222.18 μmol Trolox per gram of the sample and a ferric-reducing ability of plasma (FRAP) assay value of 127.51 μmol Fe^{2+} per gram of the sample [75]. A hyperbranched polysaccharide from *Lignosus rhinocerotis* also showed good activity in a DPPH assay (24.29%, 23.28%, 44.84%, 52.31% and 43.22%) and in an ABTS radical–scavenging assay (83.18% and 81.54%) [76].

Pisonia alba leaf extract–mediated cerium oxide nanoparticles with the characteristics of a cubic fluorite crystal structure (on XRD), UV-vis spectroscopy values of 258 and 317 nm, and a 12 nm size on TEM showed good antifungal activity and moderate antioxidant activity in a DPPH assay and FRAP assay [77].

3.1.1.6 Antioxidant Activity of Nanoparticles In Vivo

In a recent research article, Qin et al. showed that layered double hydroxide (LDH) nanoparticles possessed a DPPH-scavenging effect, a hydroxyl radical (OH)–scavenging effect and a pro-oxidative Cu^{2+}-chelating effect. This was mainly due to folic acid coupling with the LDH nanoparticles; moreover, folic acid–LDH was successful in increasing glycogen levels in muscle and hepatic glycogen. It was suggested that a folic acid–LDH antioxidant could have indications for use as a novel antioxidant or an antifatigue nutritional supplement [78].

An in vivo study by Zhang et al. revealed that nano-gold loaded with resveratrol (Res-GNPs) showed a better antitumour effect than resveratrol alone. This was due to the fact that the gold nanoparticles could transport more resveratrol to cells and to mitochondria; thus, the gold nanoparticles coupled with resveratrol reduced the cancer effect both in vitro and in vivo [79].

The above studies clearly indicate that nanoparticles, when coupled with antioxidants, provide more protection for healthy cells and provide anticancer effects.

In in vitro studies on sulphoraphane-modified selenium nanoparticles, Krug et al. showed anticancer action in several cancer cell cultures. They also showed that this high antitumour activity and selectivity with regard to diseased and healthy cells is an extremely promising treatment for cancer cells [80]. The different parameters analysed to determine the in vivo antioxidant activity of the nanoparticles are shown in Fig. 3.4.

Khan et al. studied the effects of cobalt-doped tin oxide (Co-doped SnO_2) nanoparticles and revealed that in breast carcinoma cells, green-synthesized Co-doped SnO_2 nanoparticles showed potential antioxidant activity in a DPPH assay and also showed significant anticancer and antitumour activity in both in vitro and in vivo conditions. The multipurpose properties of synthesized

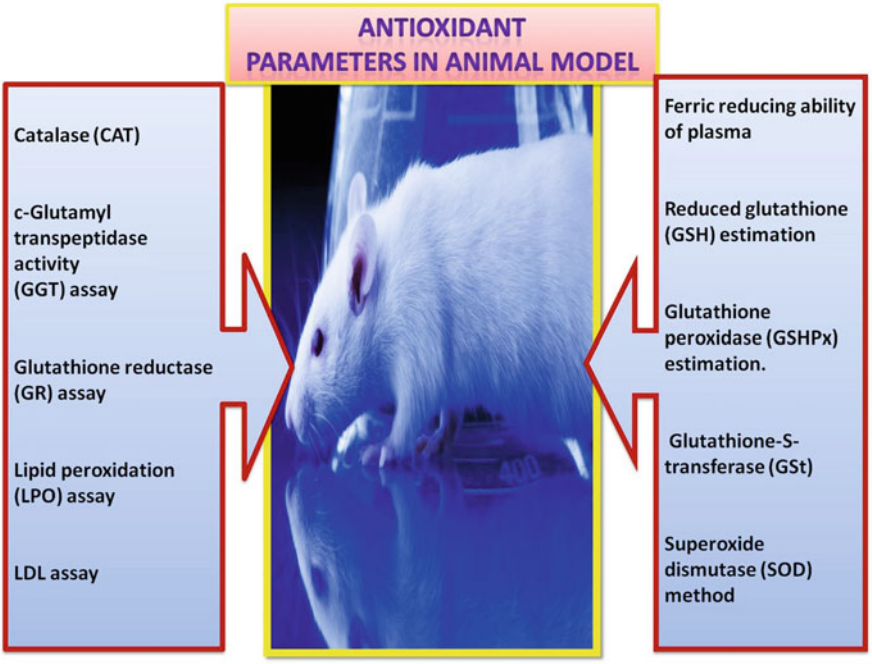


Fig. 3.4 In vivo antioxidant activity of nanoparticles. *LDL* low-density lipoprotein

nanoparticles demonstrated in this study showed that they could be useful for pharmaceutical and nanomedicine applications [81].

A research study by Tang et al. demonstrated the characterization of epigallocatechin-3-gallate (EGCG)–functionalized chitin (CH) derivative nanoparticles (CE-HKNPs) and compared their antitumour activity with that of free Honokiol (HK). The result showed that the CE-HKNPs were effective, inhibiting the cell proliferation of $HepG_2$ cells and decreasing the mitochondrial membrane potential. Moreover, in both in vitro and in vivo conditions they did not elicit any side effects in the cells. It was suggested that CE-HKNPs are an effective delivery system against liver cancer cells [82]

A recent article by Shanmugasundaram et al. described a Sprague Dawley (SD) rat model in which hepatoprotective experiments were conducted against diethyl nitrosamine (DEN)–stimulated liver cancer cells using biocompatible nanoparticles of silver (AgNPs), gold (AuNPs) and their alloy (Ag/AuNPs), synthesized from microbes. The animals treated with nanoparticles showed significant tumour reduction in in vivo studies, and this was also confirmed by other studies. The results showed anticancer activity only in DEN-stimulated liver cells, due to the synthesized AgNPs, AuNPs and Ag/AuNPs. In nanodrug development, microbial biocompatible nanoparticles have been shown to have potential as an effective drug [83].

Sulaiman et al. described an experiment, using an *Oleo europaea* leaf extract, in which copper oxide (CuO) nanoparticles (CuNPs) were synthesized. Because of the stability of the antioxidant effect, the free radical–scavenging activity of the CuNPs against 2,2-diphenyl-1-picryl-hydrazyl was assured. In mice, immune responses were observed in both the thymus and the spleen. After CuNP treatment the thymus, spleen and serum showed reductions in the adenosine deaminase (ADA) enzyme. In a dose-dependent manner, application of CuNPs against AMJ-13 and SKOV-3 cancer cells induced cell death by apoptosis. Normal dermal fibroblast cells showed less significant cytotoxic effects. Thus, CuNPs have the ability to act as an anticancer agent [84].

In contrast, Nemmer et al. found that exposure to cerium oxide nanoparticles (CeO_2NPs) induced lung toxicity. In their study, a noticeable increase in neutrophils in the bronchoalveolar lavage fluid, along with an increase in tumour necrosis factor (TNF) and a drop in the activity of the antioxidant catalase, were stimulated by CeO_2NPs. Increased plasma levels of C-reactive protein and TNF were also noted [85]. In this in vivo study it was found that thrombosis was due to acute pulmonary oxidative damage and systemic inflammation.

Qiao et al. studied andrographolide (ADG), a diterpenoid separated from *Andrographis paniculata* with a range of pharmacological activities including antitumour, anti-inflammatory, anticancer and hepatoprotective effects. They showed that a freeze-dried ADG nanosuspension (ADG-NS) could remain highly stable [86].

Pramanik et al. performed in vitro and in vivo studies on biotin-enriched gold nanoparticles targeted for delivering an anticancer active copper complex, copper (II) diacetyl-bis (N_4-methylthiosemicarbozane), tethered to 20 nm gold nanoparticles (AuNPs) and additionally decorated with biotin for target achievement. They revealed very good anticancer activity against HeLa cells derived from cervical cancer cells; less activity was observed against HaCaT cells. In an in vivo comparison with a nanoparticle conjugate without biotin, using a HeLa cell xenograft tumour model, the biotin-enriched nanoparticle conjugate showed a greater reduction in tumour volume than the control (without biotin), suggesting significant targeting [87].

3.2 Mechanisms of Action

Different metal nanoparticles, polymer nanoparticles, metal-coated polymer nanoparticles and bioactive compound–coated/decorated nanoparticles act as nanoantioxidants. The major mechanisms of action of these nanoparticles mimic the behaviour of catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and chain-breaking activity. Examples of these nanoparticles and their mechanisms of action in different assays are cerium oxide nanoparticles, which show catalase-like behaviour in hydrogen peroxide disappearance on spectrophotometric analysis [88], polyvinyl pyrrolidone–coated gold nanoparticles, which decrease H_2O_2 in spectrometric analysis and show catalase-like behaviour [89],

and gold nanorods, gold with platinum nanorods, core shells and gold with palladium nanorods, which shows catalase-like behaviour in H_2O_2 assays, spectrophotometric analysis and O_2 evaluation using dark electrodes [90].

Nanoparticles such as manganese oxide nanoflowers and grapheme oxide–supported selenium nanoparticles have shown glutathione peroxidase–like behaviour in a glutathione reductase–coupled assay using spectrophotometric analysis [91, 92].

The chain-breaking mechanism is the major action in various antioxidants (also called radical-trapping antioxidants) such as flavonoids, vitamin C, vitamin E and many synthetic alternatives.

A chain-breaking or slowdown mechanism of action was found in some nanoparticles, such as oleic acid–coated cerium oxide nanoparticles, when an AAPH-derived radical-scavenging (oxygen radical absorbance capacity (ORAC)) assay was performed [93]. Polyacrylic acid–protected platinum nanoparticles were analysed using a DPPH assay with spectrophotometric analysis. Inhibition of linoleic acid peroxidation was observed with electron paramagnetic resonance (EPR) detection of AAPH-derived radical–scavenging activity [94]. Zirconium oxide nanoparticles and polyethylene glycol–coated melanin nanoparticles have also shown chain-breaking activity, confirmed by a DPPH assay [95, 96].

Superoxide dismutase–like behaviour is the major mechanism in many antioxidant nanomaterials and xanthine/xanthine oxidase and cytochrome C analysed by spectrophotometric analysis, potassium oxide reaction, EPR study of reactions with potassium oxide with 5-diethoxyphosphoryl 5-methyl-1-pyrroline-*n*-oxide (DEPMPO) and oxide evaluation. The nanomaterials involved in these actions are fullerene, multiwalled carbon nanotubes, trismalanyl C-60, dimercaptosuccinic acid–coated Co_3O_4 nanoparticles, polyvinyl pyrrolidone–coated gold nanoparticles, glycine-coated copper nanoparticles, polyethylene glycol–coated manganese and carbon nanoclusters, palladium nanoparticles, platinum nanopowder and Mn_3O_4 nanoflowers [89, 91, 97–105].

References

1. Malarkodi C, Annadurai G (2013) A novel biological approach on extracellular synthesis and characterization of semiconductor zinc sulfide nanoparticles. Appl Nanosci 3:389–395. https://doi.org/10.1007/s13204-012-0138-0
2. Paulkumar K, Rajeshkumar S, Gnanajobitha G et al (2013) Biosynthesis of silver chloride nanoparticles using *Bacillus subtilis* MTCC 3053 and assessment of its antifungal activity. ISRN Nanomater 2013:1–8. https://doi.org/10.1155/2013/317963
3. Rajeshkumar S, Malarkodi C (2014) In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. Bioinorg Chem Appl 2014:1–10. https://doi.org/10.1155/2014/581890
4. Rajeshkumar S, Malarkodi C, Vanaja M, Paulkumar K (2014) Green-chemical fabrication of silver nanoparticles by marine macro algae and its fungicidal activity. Int Res J Pharm Biosci 1:1–7

5. Vanaja M, Annadurai G (2013) *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. Appl Nanosci 3:217–223. https://doi.org/10.1007/s13204-012-0121-9
6. Vanaja M, Gnanajobitha G, Paulkumar K et al (2013) Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: influence of physicochemical factors. J Nanostruct Chem 3:17. https://doi.org/10.1186/2193-8865-3-17
7. Happy A, Soumya M, Kumar SV, Rajeshkumar S, Sheba RD, Lakshmi T, Nallaswamy VD (2019) Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*. Biochem Biophys Rep 17:208–211. https://doi.org/10.1016/j.bbrep.2019.01.002
8. Menon S, Ks SD, Santhiya R et al (2018) Selenium nanoparticles: a potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces 170:280–292. https://doi.org/10.1016/j.colsurfb.2018.06.006
9. Santhoshkumar J, Kumar SV, Rajeshkumar S (2017a) Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. Resour Technol 3:459–465. https://doi.org/10.1016/j.reffit.2017.05.001
10. Agarwal H, Menon S, Venkat Kumar S, Rajeshkumar S (2018) Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. Chem Biol Interact 286:60–70. https://doi.org/10.1016/j.cbi.2018.03.008
11. Rajeshkumar S, Bharath LV (2017) Mechanism of plant-mediated synthesis of silver nanoparticles—a review on biomolecules involved, characterisation and antibacterial activity. Chem Biol Interact 273:219–227. https://doi.org/10.1016/j.cbi.2017.06.019
12. Rajeshkumar S, Kumar SV, Ramaiah A et al (2018) Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzym Microb Technol 117:91–95. https://doi.org/10.1016/j.enzmictec.2018.06.009
13. Rajeshkumar S, Rinitha G (2018) Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel *Persea americana* seeds. OpenNano 3:18–27. https://doi.org/10.1016/j.onano.2018.03.001
14. Ramesh AV, Rama D, Battu G, Basavaiah K (2018) A facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of *Ficus hispida* Linn.f. for catalytic, antioxidant and antibacterial applications. South Afr J Chem Eng 26:25–34. https://doi.org/10.1016/j.sajce.2018.07.001
15. Kumar A, Srivastava R, Singh P, Bahadur V (2018) Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. J Ayurveda Integr Med. https://doi.org/10.1016/j.jaim.2017.11.003
16. Nunes MR, de Souza Maguerroski Castilho M, de Lima Veeck AP et al (2018) Antioxidant and antimicrobial methylcellulose films containing *Lippia alba* extract and silver nanoparticles. Carbohydr Polym 192:37–43. https://doi.org/10.1016/j.carbpol.2018.03.014
17. Ahn E, Jin H, Park Y (2019) Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts. Mater Sci Eng C 101:204–216. https://doi.org/10.1016/j.msec.2019.03.095
18. Hajji S, Ben S, Hamza-Mnif I et al (2019) Biomedical potential of chitosan–silver nanoparticles with special reference to antioxidant, antibacterial, hemolytic and in vivo cutaneous wound healing effects. Biochim Biophys Acta Gen Subj 1863:241–254. https://doi.org/10.1016/j.bbagen.2018.10.010
19. Moteriya P, Chanda S (2018) Biosynthesis of silver nanoparticles formation from *Caesalpinia pulcherrima* stem metabolites and their broad spectrum biological activities. J Genet Eng Biotechnol 16:105–113. https://doi.org/10.1016/j.jgeb.2017.12.003
20. Govindappa M, Hemashekhar B, Arthikala M et al (2018) Anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using *Calophyllum tomentosum* leaves extract. Results Phys 9:400–408. https://doi.org/10.1016/j.rinp.2018.02.049

21. Sivasankar P, Seedevi P, Poongodi S (2018) Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by *Streptomyces violaceus* MM72. Carbohydr Polym 181:752–759. https://doi.org/10.1016/j.carbpol.2017.11.082
22. Sripriya N, Vasantharaj S, Mani U et al (2019) Encapsulated enhanced silver nanoparticles biosynthesis by modified new route for nano-biocatalytic activity. Biocatal Agric Biotechnol 18:101045. https://doi.org/10.1016/j.bcab.2019.101045
23. Ahmed S, Kaur G, Sharma P et al (2018) Fruit waste (peel) as bio-reductant to synthesize silver nanoparticles with antimicrobial, antioxidant and cytotoxic activities. J Appl Biomed 16:221–231. https://doi.org/10.1016/j.jab.2018.02.002
24. Selvan DA, Mahendiran D, Kumar RS, Rahiman AK (2018) Garlic, green tea and turmeric extracts–mediated green synthesis of silver nanoparticles: phytochemical, antioxidant and in vitro cytotoxicity studies. J Photochem Photobiol B Biol 180:243–252. https://doi.org/10.1016/j.jphotobiol.2018.02.014
25. Haghparasti Z, Shahri MM (2018) Green synthesis of water-soluble nontoxic inorganic polymer nanocomposites containing silver nanoparticles using white tea extract and assessment of their in vitro antioxidant and cytotoxicity activities. Mater Sci Eng C 87:139–148. https://doi.org/10.1016/j.msec.2018.02.026
26. Rolim WR, Pelegrino MT, de Araújo LB et al (2019) Green tea extract mediated biogenic synthesis of silver nanoparticles: characterization, cytotoxicity evaluation and antibacterial activity. Appl Surf Sci 463:66–74. https://doi.org/10.1016/j.apsusc.2018.08.203
27. Anca N, Stefania D, Fierascu I et al (2018) Phytosynthesis of gold and silver nanoparticles enhance in vitro antioxidant and mitostimulatory activity of *Aconitum toxicum* Reichenb. rhizomes alcoholic extracts. Mater Sci Eng C 93:746–758. https://doi.org/10.1016/j.msec.2018.08.042
28. Bhutto AA, Kalay Ş, Sherazi STH, Culha M (2018) Quantitative structure–activity relationship between antioxidant capacity of phenolic compounds and the plasmonic properties of silver nanoparticles. Talanta 189:174–181. https://doi.org/10.1016/j.talanta.2018.06.080
29. Valsalam S, Agastian P, Valan M et al (2019) Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J Photochem Photobiol B Biol 191:65–74. https://doi.org/10.1016/j.jphotobiol.2018.12.010
30. Masood N, Ahmed R, Tariq M, Ahmed Z (2019) Silver nanoparticle impregnated chitosan–PEG hydrogel enhances wound healing in diabetes induced rabbits. Int J Pharm 559:23–36. https://doi.org/10.1016/j.ijpharm.2019.01.019
31. Khoshnamvand M, Huo C, Liu J (2019) Silver nanoparticles synthesized using *Allium ampeloprasum* L. leaf extract: characterization and performance in catalytic reduction of 4-nitrophenol and antioxidant activity. J Mol Struct 1175:90–96. https://doi.org/10.1016/j.molstruc.2018.07.089
32. Della Pelle F, Scroccarello A, Sergi M et al (2018) Simple and rapid silver nanoparticles based antioxidant capacity assays: reactivity study for phenolic compounds. Food Chem 256:342–349. https://doi.org/10.1016/j.foodchem.2018.02.141
33. Voicescu M, Ionescu S, Manoiu VS et al (2019) Synthesis and biophysical characteristics of riboflavin/HSA protein system on silver nanoparticles. Mater Sci Eng C 96:30–40. https://doi.org/10.1016/j.msec.2018.10.087
34. Ayodhya D, Veerabhadram G (2019) Synthesis and characterization of g-C_3N_4 nanosheets decorated Ag_2S composites for investigation of catalytic reduction of 4-nitrophenol, antioxidant and antimicrobial activities. J Mol Struct 1186:423–433. https://doi.org/10.1016/j.molstruc.2019.03.048
35. Ukkund SJ, Ashraf M, Udupa AB et al (2019) Synthesis and characterization of silver nanoparticles from *Fusarium oxysporum* and investigation of their antibacterial activity. Mater Today Proc 9:506–514. https://doi.org/10.1016/j.matpr.2018.10.369

36. AlSalhi MS, Elangovan K, Jacob A et al (2019) Synthesis of silver nanoparticles using plant derived 4-N-methyl benzoic acid and evaluation of antimicrobial, antioxidant and antitumor activity. Saudi J Biol Sci 26:970–978. https://doi.org/10.1016/j.sjbs.2019.04.001
37. Saravanakumar K, Wang M (2018) *Trichoderma* based synthesis of anti-pathogenic silver nanoparticles and their characterization, antioxidant and cytotoxicity properties. Microb Pathog 114:269–273. https://doi.org/10.1016/j.micpath.2017.12.005
38. Guzmán K, Kumar B, Cumbal L et al (2019) Ultrasound-assisted synthesis and antibacterial activity of gallic acid–chitosan modified silver nanoparticles. Prog Org Coat 129:229–235. https://doi.org/10.1016/j.porgcoat.2019.01.009
39. Chithrani DB (2010) Nanoparticles for improved therapeutics and imaging in cancer therapy. Recent Pat Nanotechnol 4:171–180
40. Grzelczak M, Perez-Juste J, Mulvaney P, Liz-Marzan LM (2008) Shape control in gold nanoparticle synthesis. Chem Soc Rev 37:1783–1791. https://doi.org/10.1039/b711490g
41. Santhoshkumar J, Rajeshkumar S, Venkat Kumar S (2017b) Phyto-assisted synthesis, characterization and applications of gold nanoparticles—a review. Biochem Biophys Rep 11:46. https://doi.org/10.1016/j.bbrep.2017.06.004
42. Della F, Vilela D, Cristina M et al (2015) Antioxidant capacity index based on gold nanoparticles formation. Application to extra virgin olive oil samples. Food Chem 178:70–75. https://doi.org/10.1016/j.foodchem.2015.01.045
43. Razzaq H, Saira F, Yaqub A et al (2016) Interaction of gold nanoparticles with free radicals and their role in enhancing the scavenging activity of ascorbic acid. J Photochem Photobiol B Biol 161:266–272. https://doi.org/10.1016/j.jphotobiol.2016.04.003
44. Jiménez-Pérez ZE, Singh P, Kim Y et al (2018) Applications of *Panax ginseng* leaves–mediated gold nanoparticles in cosmetics relation to antioxidant, moisture retention, and whitening effect on B16BL6 cells. J Ginseng Res 42:327–333. https://doi.org/10.1016/j.jgr.2017.04.003
45. Karmakar A, Mallick T, Fouzder C, Mukhuty A (2019) Antioxidant flavone functionalized fluorescent and biocompatible metal nanoparticles: exploring their efficacy as cell imaging agents. Nano-Struct Nano-Objects 18:100278. https://doi.org/10.1016/j.nanoso.2019.100278
46. Pu S, Li J, Sun L et al (2019) An in vitro comparison of the antioxidant activities of chitosan and green synthesized gold nanoparticles. Carbohydr Polym 211:161–172. https://doi.org/10.1016/j.carbpol.2019.02.007
47. Esin S, Bekdeşer B, Apak R (2019) A novel colorimetric sensor for measuring hydroperoxide content and peroxyl radical scavenging activity using starch-stabilized gold nanoparticles. Talanta 196:32–38. https://doi.org/10.1016/j.talanta.2018.12.022
48. Dauthal P, Mukhopadhyay M (2018) Antioxidant activity of phytosynthesized biomatrix-loaded noble metallic nanoparticles. Chin J Chem Eng 26:1200–1208. https://doi.org/10.1016/j.cjche.2017.12.014
49. Kirdaite G, Leonaviciene L, Bradunaite R et al (2019) Antioxidant effects of gold nanoparticles on early stage of collagen-induced arthritis in rats. Res Vet Sci 124:32–37. https://doi.org/10.1016/j.rvsc.2019.02.002
50. Agarwal H, Kumar SV, Rajeshkumar S (2017) A review on green synthesis of zinc oxide nanoparticles—an eco-friendly approach. Resour Technol 3:406. https://doi.org/10.1016/j.reffit.2017.03.002
51. Dastjerdi R, Montazer M (2010) A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties. Colloids Surf B Biointerfaces 79:5–18. https://doi.org/10.1016/j.colsurfb.2010.03.029
52. Naveed Ul Haq A, Nadhman A, Ullah I et al (2017) Synthesis approaches of zinc oxide nanoparticles: the dilemma of ecotoxicity. J Nanomater 2017:1–14. https://doi.org/10.1155/2017/8510342
53. El-Batal AI, Mosalam FM, Ghorab MM et al (2018) Antimicrobial, antioxidant and anticancer activities of zinc nanoparticles prepared by natural polysaccharides and gamma radiation. Int J Biol Macromol 107:2298–2311. https://doi.org/10.1016/j.ijbiomac.2017.10.121

54. Koner D, Banerjee B, Hasan R, Saha N (2019) Antioxidant activity of endogenously produced nitric oxide against the zinc oxide nanoparticle–induced oxidative stress in primary hepatocytes of air-breathing catfish, *Clarias magur*. Nitric Oxide 84:7–15. https://doi.org/10.1016/j.niox.2018.12.010
55. Gupta R, Malik P, Das N, Singh M (2019) Antioxidant and physicochemical study of *Psidium guajava* prepared zinc oxide nanoparticles. J Mol Liq 275:749–767. https://doi.org/10.1016/j.molliq.2018.11.085
56. Zare M, Namratha K, Thakur MS, Byrappa K (2019) Biocompatibility assessment and photocatalytic activity of bio-hydrothermal synthesis of ZnO nanoparticles by *Thymus vulgaris* leaf extract. Mater Res Bull 109:49–59. https://doi.org/10.1016/j.materresbull.2018.09.025
57. Guo X, Li W, Wang H et al (2018) Preparation, characterization, release and antioxidant activity of curcumin-loaded amorphous calcium phosphate nanoparticles. J Non-Cryst Solids 500:317–325. https://doi.org/10.1016/j.jnoncrysol.2018.08.015
58. Soren S, Kumar S, Mishra S et al (2018) Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. Microb Pathog 119:145–151. https://doi.org/10.1016/j.micpath.2018.03.048
59. Madhumitha G, Fowsiya J, Gupta N et al (2019) Green synthesis, characterization and antifungal and photocatalytic activity of *Pithecellobium dulce* peel–mediated ZnO nanoparticles. J Phys Chem Solids 127:43–51. https://doi.org/10.1016/j.jpcs.2018.12.005
60. Sharmila G, Thirumarimurugan M, Muthukumaran C (2019b) Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: characterization and evaluation of its antioxidant, bactericidal and anticancer activities. Microchem J 145:578–587. https://doi.org/10.1016/j.microc.2018.11.022
61. Ullah A, Kareem A, Nami SAA et al (2019) *Malus pumila* and *Juglen regia* plant species mediated zinc oxide nanoparticles: synthesis, spectral characterization, antioxidant and antibacterial studies. Microb Pathog 129:233–241. https://doi.org/10.1016/j.micpath.2019.02.020
62. Alavi M, Karimi N, Salimikia I (2019) Phytosynthesis of zinc oxide nanoparticles and its antibacterial, antiquorum sensing, antimotility, and antioxidant capacities against multidrug resistant bacteria. J Ind Eng Chem 72:457–473. https://doi.org/10.1016/j.jiec.2019.01.002
63. Zare M, Namratha K, Byrappa K et al (2018) Surfactant assisted solvothermal synthesis of ZnO nanoparticles and study of their antimicrobial and antioxidant properties. J Mater Sci Technol 34:1035–1043. https://doi.org/10.1016/j.jmst.2017.09.014
64. Shobha N, Nanda N, Shivanna A et al (2019) Synthesis and characterization of zinc oxide nanoparticles utilizing seed source of *Ricinus communis* and study of its antioxidant, antifungal and anticancer activity. Mater Sci Eng C 97:842–850. https://doi.org/10.1016/j.msec.2018.12.023
65. Safawo T, Sandeep BV, Pola S, Tadesse A (2018) Synthesis and characterization of zinc oxide nanoparticles using tuber extract of anchote (*Coccinia abyssinica* (Lam.) Cong.) for antimicrobial and antioxidant activity assessment. OpenNano 3:56–63. https://doi.org/10.1016/j.onano.2018.08.001
66. Lu J, Ali H, Hurh J et al (2019) The assessment of photocatalytic activity of zinc oxide nanoparticles from the roots of *Codonopsis lanceolata* synthesized by one-pot green synthesis method. Optik 184:82–89. https://doi.org/10.1016/j.ijleo.2019.03.050
67. Alkaladi A (2019) Vitamins E and C ameliorate the oxidative stresses induced by zinc oxide nanoparticles on liver and gills of *Oreochromis niloticus*. Saudi J Biol Sci 26:357–362. https://doi.org/10.1016/j.sjbs.2018.07.001
68. Suresh D, Nethravathi PC, Udayabhanu et al (2015) Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using *Cassia fistula* plant extract and their photodegradative, antioxidant and antibacterial activities. Mater Sci Semicond Process 31:446–454. https://doi.org/10.1016/j.mssp.2014.12.023

69. Nagajyothi PC, Sreekanth TVM, Lee J, Duk K (2014) Mycosynthesis: antibacterial, antioxidant and antiproliferative activities of silver nanoparticles synthesized from *Inonotus obliquus* (Chaga mushroom) extract. J Photochem Photobiol B Biol 130:299–304. https://doi.org/10.1016/j.jphotobiol.2013.11.022
70. Nethravathi PC, Shruthi GS, Suresh D et al (2015) *Garcinia xanthochymus* mediated green synthesis of ZnO nanoparticles: photoluminescence, photocatalytic and antioxidant activity studies. Ceram Int 41:8680–8687. https://doi.org/10.1016/j.ceramint.2015.03.084
71. Nagajyothi PC, Ju S, Jun I et al (2015) Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using *Polygala tenuifolia* root extract. J Photochem Photobiol B Biol 146:10–17. https://doi.org/10.1016/j.jphotobiol.2015.02.008
72. Mubarakali D, Lewisoscar F, Gopinath V et al (2018) An inhibitory action of chitosan nanoparticles against pathogenic bacteria and fungi and their potential applications as biocompatible antioxidants. Microb Pathog 114:323–327. https://doi.org/10.1016/j.micpath.2017.11.043
73. Khatami M, Alijani HQ, Fakheri B et al (2019) Super-paramagnetic iron oxide nanoparticles (SPIONs): greener synthesis using *Stevia* plant and evaluation of its antioxidant properties. J Clean Prod 208:1171–1177. https://doi.org/10.1016/j.jclepro.2018.10.182
74. Tian H, Ghorbanpour M, Kariman K (2018) Manganese oxide nanoparticle–induced changes in growth, redox reactions and elicitation of antioxidant metabolites in deadly nightshade (*Atropa belladonna* L.). Ind Crop Prod 126:403–414. https://doi.org/10.1016/j.indcrop.2018.10.042
75. Qiu W, Wang Y, Wang M, Yan J (2018) Construction, stability, and enhanced antioxidant activity of pectin-decorated selenium nanoparticles. Colloids Surf B Biointerfaces 170:692–700. https://doi.org/10.1016/j.colsurfb.2018.07.003
76. Cai W, Hu T, Bakry AM et al (2018) Effect of ultrasound on size, morphology, stability and antioxidant activity of selenium nanoparticles dispersed by a hyperbranched polysaccharide from *Lignosus rhinocerotis*. Ultrason Sonochem 42:823–831. https://doi.org/10.1016/j.ultsonch.2017.12.022
77. Sharmila G, Muthukumaran C, Saraswathi H et al (2019a) Green synthesis, characterization and biological activities of nanoceria. Ceram Int 45:12382. https://doi.org/10.1016/j.ceramint.2019.03.164
78. Qin L, Wang W, You S, Dong J, Zhou Y, Wang J (2014) In vitro antioxidant activity and in vivo antifatigue effect of layered double hydroxide nanoparticles as delivery vehicles for folic acid. Int J Nanomedicine 9:5701
79. Zhang D, Zhang J, Zeng J, Li Z, Zuo H, Huang C, Zhao X (2019) Nano-gold loaded with resveratrol enhance the anti-hepatoma effect of resveratrol in vitro and in vivo. J Biomed Nanotechnol 15(2):288–300
80. Krug P, Mielczarek L, Wiktorska K, Kaczyńska K, Wojciechowski P, Andrzejewski K, Ofiara K, Szterk A, Mazur M (2018) Sulforaphane-conjugated selenium nanoparticles: towards a synergistic anticancer effect. Nanotechnology 30(6):065101
81. Khan SA, Kanwal S, Rizwan K, Shahid S (2018) Enhanced antimicrobial, antioxidant, in vivo antitumor and in vitro anticancer effects against breast cancer cell line by green synthesized un-doped SnO_2 and Co-doped SnO_2 nanoparticles from *Clerodendrum inerme*. Microb Pathog 125:366–384
82. Tang P, Sun Q, Yang H, Tang B, Pu H, Li H (2018) Honokiol nanoparticles based on epigallocatechin gallate functionalized chitin to enhance therapeutic effects against liver cancer. Int J Pharm 545(1–2):74–83
83. Shanmugasundaram T, Radhakrishnan M, Gopikrishnan V, Kadirvelu K, Balagurunathan R (2017) Biocompatible silver, gold and silver/gold alloy nanoparticles for enhanced cancer therapy: in vitro and in vivo perspectives. Nanoscale 9(43):16773–16790
84. Sulaiman GM, Tawfeeq AT, Jaaffer MD (2018) Biogenic synthesis of copper oxide nanoparticles using *Olea europaea* leaf extract and evaluation of their toxicity activities: an in vivo and in vitro study. Biotechnol Prog 34(1):218–230

85. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH (2017) The acute pulmonary and thrombotic effects of cerium oxide nanoparticles after intratracheal instillation in mice. Int J Nanomedicine 12:2913
86. Qiao H, Chen L, Rui T, Wang J, Chen T, Fu T, Li J, Di L (2017) Fabrication and in vitro/in vivo evaluation of amorphous andrographolide nanosuspensions stabilized by d-α-tocopheryl polyethylene glycol 1000 succinate/sodium lauryl sulfate. Int J Nanomedicine 12:1033
87. Pramanik AK, Palanimuthu D, Somasundaram K, Samuelson AG (2016) Biotin decorated gold nanoparticles for targeted delivery of a smart-linked anticancer active copper complex: in vitro and in vivo studies. Bioconjug Chem 27(12):2874–2885
88. Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, King JE, Seal S, Self WT (2010) Nanoceria exhibit redox state–dependent catalase mimetic activity. Chem Commun 46(16):2736–2738
89. He W, Zhou YT, Wamer WG, Hu X, Wu X, Zheng Z, Boudreau MD, Yin JJ (2013) Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. Biomaterials 34(3):765–773
90. Li J, Liu W, Wu X, Gao X (2015) Mechanism of pH-switchable peroxidase and catalase-like activities of gold, silver, platinum and palladium. Biomaterials 48:37–44
91. Singh N, Savanur MA, Srivastava S, D'Silva P, Mugesh G (2017) A redox modulatory Mn_3O_4 nanozyme with multi-enzyme activity provides efficient cytoprotection to human cells in a Parkinson's disease model. Angew Chem Int Ed 56(45):14267–14271
92. Huang Y, Liu C, Pu F, Liu Z, Ren J, Qu X (2017) A GO–Se nanocomposite as an antioxidant nanozyme for cytoprotection. Chem Commun 53(21):3082–3085
93. Lee SS, Song W, Cho M, Puppala HL, Nguyen P, Zhu H, Segatori L, Colvin VL (2013) Antioxidant properties of cerium oxide nanocrystals as a function of nanocrystal diameter and surface coating. ACS Nano 7(11):9693–9703
94. Watanabe A, Kajita M, Kim J, Kanayama A, Takahashi K, Mashino T, Miyamoto Y (2009) In vitro free radical scavenging activity of platinum nanoparticles. Nanotechnology 20 (45):455105
95. Balaji S, Mandal BK, Ranjan S, Dasgupta N, Chidambaram R (2017) Nano-zirconia—evaluation of its antioxidant and anticancer activity. J Photochem Photobiol B Biol 170:125–133
96. Ju KY, Lee Y, Lee S, Park SB, Lee JK (2011) Bioinspired polymerization of dopamine to generate melanin-like nanoparticles having an excellent free-radical-scavenging property. Biomacromolecules 12(3):625–632
97. Liu GF, Filipović M, Ivanović-Burmazović I, Beuerle F, Witte P, Hirsch A (2008) High catalytic activity of dendritic C_{60} monoadducts in metal-free superoxide dismutation. Angew Chem Int Ed 47(21):3991–3994
98. Ali SS, Hardt JI, Quick KL, Kim-Han JS, Erlanger BF, Huang TT, Epstein CJ, Dugan LL (2004) A biologically effective fullerene (C_{60}) derivative with superoxide dismutase mimetic properties. Free Radic Biol Med 37(8):1191–1202
99. Dong J, Song L, Yin JJ, He W, Wu Y, Gu N, Zhang Y (2014) Co_3O_4 nanoparticles with multi-enzyme activities and their application in immunohistochemical assay. ACS Appl Mater Interfaces 6(3):1959–1970
100. Liu Y, Wu H, Li M, Yin JJ, Nie Z (2014) pH dependent catalytic activities of platinum nanoparticles with respect to the decomposition of hydrogen peroxide and scavenging of superoxide and singlet oxygen. Nanoscale 6(20):11904–11910
101. Ge C, Fang G, Shen X, Chong Y, Wamer WG, Gao X, Chai Z, Chen C, Yin JJ (2016) Facet energy versus enzyme-like activities: the unexpected protection of palladium nanocrystals against oxidative damage. ACS Nano 10(11):10436–10445
102. Samuel EL, Marcano DC, Berka V, Bitner BR, Wu G, Potter A, Fabian RH, Pautler RG, Kent TA, Tsai AL, Tour JM (2015) Highly efficient conversion of superoxide to oxygen using hydrophilic carbon clusters. Proc Natl Acad Sci U S A 112(8):2343–2348

103. Ragg R, Schilmann AM, Korschelt K, Wieseotte C, Kluenker M, Viel M, Völker L, Preiß S, Herzberger J, Frey H, Heinze K (2016) Intrinsic superoxide dismutase activity of MnO nanoparticles enhances the magnetic resonance imaging contrast. J Mater Chem B 4 (46):7423–7428
104. Korschelt K, Ragg R, Metzger CS, Kluenker M, Oster M, Barton B, Panthöfer M, Strand D, Kolb U, Mondeshki M, Strand S (2017) Glycine-functionalized copper (II) hydroxide nanoparticles with high intrinsic superoxide dismutase activity. Nanoscale 9(11):3952–3960
105. Liu Y, Ai K, Ji X, Askhatova D, Du R, Lu L, Shi J (2017) Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury in ischemic stroke. J Am Chem Soc 139(2):856–862

Oxidative Stress in Neurology and in Neurodegenerative Processes

4

Gaurav Gupta, Sacchidanand Pathak, Sarita Rawat, Anurag Mishra, Yogendra Singh, Meenu Mehta, Saurabh Satija, Navneet Khurana, Terezinha de Jesus Andreoli Pinto, Shakti Shukla, Kavita Pabreja, and Dinesh Kumar Chellappan

Abstract

Aging is one of the principal risk factors that play an important role in several human conditions and pathogenesis, primarily neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD). A progressive loss of neurons, reduced motor or behavioral functions, and abnormally aggregated proteins define these conditions. An unbalanced redox environment, including the generation of excessive reactive oxygen species (ROS) or system deficiency, causes oxidative stress (OS). The brain is one of the principal organs that are particularly susceptible to ROS because of its elevated oxygen demand and the presence of abundant peroxidation-sensitive

G. Gupta (✉) · S. Pathak · S. Rawat · A. Mishra
School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India

Y. Singh
Department of Pharmaceutical Sciences, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, India

M. Mehta · S. Satija · N. Khurana
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

T. de Jesus Andreoli Pinto
Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

S. Shukla
Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute (HMRI) & School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia

K. Pabreja
School of Medicine and Public Health, University of Newcastle, Callaghan, NSW, Australia

D. K. Chellappan
Department of Life Sciences, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_4

lipid cells. Previous studies have reported that widespread neurodegenerative disease pathophysiology involves OS. Cellular antioxidants are known to alter such redox status, target destruction, and regulate oxidative mechanisms engaged in cell proliferation, gene expression, signal transduction, and cell death pathway. Oxidants and antioxidants are important in maintaining free balance, metabolized, environmental-related radicals and the body's antioxidant mechanisms. In biological systems, several complex natural antioxidant mechanisms occur that work together to prevent prooxidant damage. The objective of this chapter is to demonstrate that free radicals are engaged in neurodegenerative disease pathophysiology and that antioxidants and scavenging products help in the prevention and cure of such disease conditions. This chapter also examines the role of antioxidants in neurodegenerative illnesses, in their chemoprevention and therapy.

Keywords

Oxidative stress · Neurodegenerative diseases · Antioxidants · Reactive oxygen species

4.1 Introduction

Neurodegenerative illnesses are a heterogeneous group of disorders that are portrayed by slow progressive loss of neurons. The etiology of neurodegenerative maladies has not yet been completely explained. However, expanded oxidative pressure has been proposed as one of the potential fundamental etiologies in different neurodegenerative ailments. Aggregate oxidative pressure may initiate cell harm, an impedance of the deoxyribonucleic acid (DNA) repair system, and mitochondrial dysfunction, all of which have been reported as key factors in speeding up the advancement of neurodegenerative processes [1–3]. Therefore, much of the research efforts in this field have been channeled toward identifying novel and effective therapies and strategies that can ensure protection against oxidative harm and conceivably treat neurodegenerative ailments. This chapter attempts to examine the crucial pathophysiological pathways involved in oxidative stress in the advancement of neurodegenerative conditions, particularly Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Alzheimer's disease (AD) [4–6]. In addition, the chapter will also provide an outline of the currently available information and evidence for the treatment of neurodegenerative maladies and their future perspectives.

4.2 Oxidative Stress

Reactive oxygen species (ROS) are usually generated in the cells of all biological life-forms, in response to normal physiological processes and homeostasis mechanisms that occur within the cells. In physiological conditions, low to direct

convergences of ROS are associated with several events, for example, immune response, inflammation, synaptic plasticity, learning, and memory. The overabundance of ROS creates a toxic environment that could directly affect the functioning of cellular structures like mitochondria, resulting in a myriad of diseases and conditions. However, the human body is equipped with an assortment of antioxidant agents that serve to balance the impact of oxidants. These include enzyme frameworks like the superoxide dismutase (SOD) and the glutathione (GSH) frameworks. When this equilibrium is affected, either due to the excessive generation of ROS or due to the depletion of antioxidant substances, it could lead to oxidative impairment, resulting eventually in the overall disability of cellular capacities. This phenomenon is seen in numerous pathophysiological conditions, including mitochondrial dysfunction and other cellular abnormalities. The central nervous system, especially the brain, is powerless against oxidative pressure and harm on account of its high oxygen utilization, low antioxidant agent barriers, and high abundance of polyunsaturated fats [7–11].

4.3 Mitochondrial Oxidative Stress and Its Role in Neurodegenerative Diseases

Mitochondria are key multifunctional organelles that play various significant roles within the cell (Fig. 4.1). They are crucial substances in thermogenesis, calcium homeostasis, maintenance of key cell metabolites, and redox flagging. Neurons are excitable cells that are progressively sensitive to the effects of oxidative harm and are in contrast to other dividing cells. They are increasingly inclined to becoming defective as they mature. Thus, it becomes significant that neuronal integrity and their survival is primarily maintained and preserved by antioxidant defense systems in the body [12–14]. All neurodegenerative conditions share general basic highlights, for example, the aggregation of specific proteins, initiation of oxidative harm, and eventual lead-up to mitochondrial dysfunction. A large number of the genes related to PD or ALS have close associations with mitochondria. In addition, the conglomeration of misfolded proteins (β-amyloid, tau, and α-synuclein) are also known to restrain the mitochondrial framework that prompts oxidative pressure. Thus, the identification of common mechanisms underlying such neurodegenerative ailments, including mitochondrial dysfunction, will build our comprehension of the basic prerequisites for neuronal survival that can expedite future neuroprotective treatment strategies [15–18].

4.4 Epidemiology

Alzheimer's disease is reported to be one of the most widely recognized diseases among all existing neurological disorders. Approximately, around 50–60% of subjects with dementia present clinical proof of presenile or decrepit types of AD. The ongoing Canadian investigation on well-being and maturing estimated

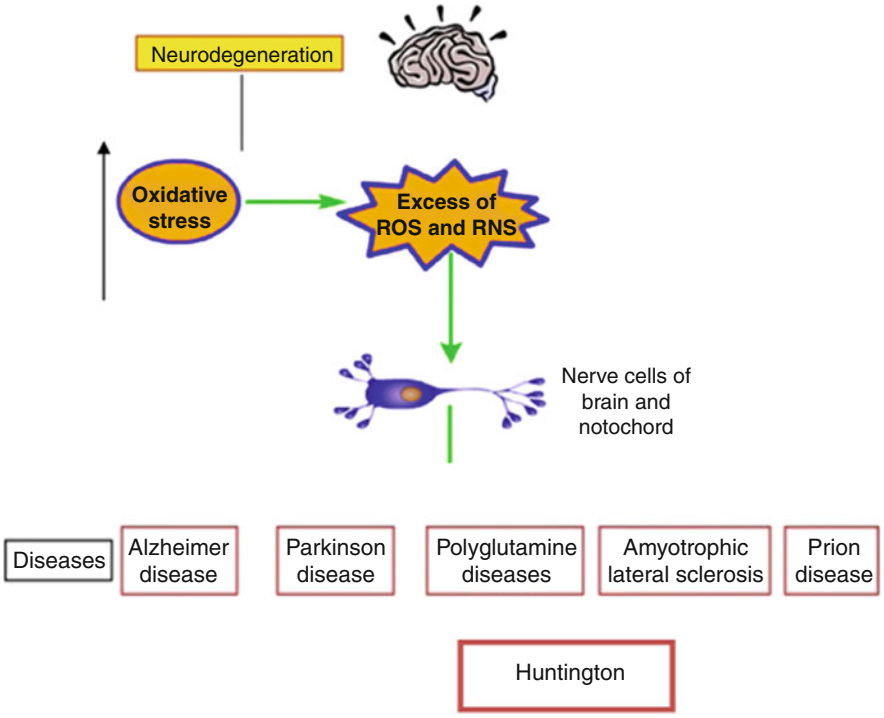


Fig. 4.1 Role of oxidative stress in neurodegenerative disease

that the prevalence of dementia in people who were 65 years and over was 8.0%. From this population, approximately 5.1% could experience the ill effects of presenile or decrepit dementia of Alzheimer type. After the age of 75, AD is found to predominantly impact women. PD and multiple sclerosis (MS) are commonly viewed as less common conditions with a predominance of under 1.0%. Creutzfeldt-Jakob illness (CJD) and ALS are relatively rare and are expressed less frequently [19–22].

A recent study in India investigated the prevalence of neurological disorders in six samples from different districts, including rustic populations in each of the six studies and urban population in two of them, through house-to-house observations. The unrefined frequency rate for every 100,000 population varied from 967 to 4070, with an average of 2394. Corresponding to this result, the projected effects of neurological problems in India were faced by about 30 million people from the present population. Nevertheless, this is not the whole spectrum of the question since in the studies, no variables are included, namely neuroinfections, injuries, neoplasms, and metabolic issues. The low rate of commonness observed in the study on Kashmir could be due to the exclusion of migraine, the most famous of all diseases in the region. The fluctuations in prevalence levels observed across the study areas could be due to the contrast in ID, the meaning of the disease, and the

avoidance of some neurologic problem, as well as some predominant local problem. Both age groups are affected by neurology problems. In certain populations, the age-specific prevalence rates are more and more prominent. Expanding rates are found in the city of Bangalore in elderly people claiming that the geriatric population has the weight of nervous issues, where age rates per 100,000 are explicit: for age <15: 2653, for ages 31–40: 3932, for ages >60: 5012. Neurological problems in the rustic population are increasingly frequent as contrasted with the 1.9:1 urban population. In Bangalore and Malda, the predominance of women was higher than in men. In India, the predominance is similar to Nigeria and Ethiopia; however, the prevalence rate is less than in Sicily and Tunisia [23–27].

The lifetime probability for PD is determined to be 2% for populations and 1.3% for individuals. The value for parkinsonism is somewhere in the range of 3.7–4.4%. Parkinsonism is a term used to describe conditions depicting akinesia that do not meet idiopathic PD clinical or pathological criteria [28, 29].

ALS, otherwise called motor neuron disease, is an uncommon neuromuscular ailment with a prevalence rate of around 1 out of every 100,000 individuals in the population. High inhibitions are characterized by the degeneration of motor neurons and are typically unaffected by acumen and features like PD. The National Institute for Neurological Disorders and Stroke estimates that from changes associated with the superoxide dismutase 1 protein, 5–10% of all ALS cases are due to inherited characteristics [30, 31].

The epidemiologic assertion of HD relies on the determination, by a nervous system specialist, of the family ancestry and its general setup. Expansive territorial investigations that used existing clinical and institutional records, on the other hand, revealed relatively lower rates. Huntington's illness is an uncommon neuropsychiatric disease with the pervasiveness of 5–10 for each 100,000 population among the Caucasian people. In Japan, a much lower pervasiveness of around one tenth of the commonness of the Caucasian populace is observed [32–34].

4.5 Pathophysiology

4.5.1 Alzheimer's Disease (AD)

The pathophysiology of Alzheimer's disease can be explained with the help of three hypotheses.

4.5.1.1 Amyloid Cascade Hypothesis

Neuritic plaques are extracellular injuries found in the cerebrum and cerebral vasculature. Amyloid precursor protein (APP) is the forerunner of amyloid plaque. Plaques from AD cerebrums, to a great extent, comprise a protein called βAP. βAP is formed from APP. The actual mechanisms and functions of APP are not clear yet. In a general sense, it is known to have a critical role in the generation of βAP. βAP, in turn, initiates the formation of plaques and plaque-initiated neurodegeneration. It happens in the following three stages [35–37]:

1. A sticky layer forms on the neurons.
2. Catalysts cut the APP into several parts of protein, including beta-amyloid.
3. Fragments of beta-amyloid unite in clusters to form plaques.

In AD, these clusters initiate the degeneration of neurons and influence the hippocampus and different regions of the cerebral cortex.

4.5.1.2 Neurofibrillary Tangles (NFTs)

NFTs are ordinarily found in the cells of the hippocampus and cerebral cortex in people with AD and are made up of unusually hyperphosphorylated tau protein. Tau protein gives support to microtubules, cell transportation, and the skeletal supportive network. At the point when tau fibers experience strange phosphorylation at a particular site, they cannot tie successfully to microtubules and microtubule breakdown. Without an intact arrangement of microtubules, the cells cannot work appropriately and eventually die [38, 39].

4.5.1.3 Cholinergic Hypothesis

The cholinergic theory of AD came to fruition because of the combined perceptions of shortfalls in choline acetyltransferase and acetylcholine (ACh). ACh is significant in memory and learning. It is well known that a reduction in cholinergic neurons and primarily cholinergic neurotransmission prompts a decrease in psychological and noncognitive capacities. In addition, the utilization of cholinesterase inhibitors (CIs) does not have a noteworthy impact on the greater part of Alzheimer's affected patients who are continuing with the treatment, showing the nearness of other significant procedures in the transformation of the illness [40–42].

4.5.2 Parkinson

4.5.2.1 Low Dopamine Levels

Researchers have studied the effects of low or falling dimensions of dopamine, in the synapse, with PD. This happens when cells that produce dopamine burn out. Dopamine assumes a role in sending messages to the parts of the cerebrum that control development and coordination. Low dopamine levels can make it harder for individuals to control their developments. As dopamine levels fall in an individual with PD, their manifestations steadily turn out to be progressively extreme [43, 44].

4.5.2.2 Low Norepinephrine Levels

Norepinephrine, another neurotransmitter, possesses a significant role in controlling numerous biological functions, for example, the dissemination of blood. In PD, the nerve endings that produce this neurotransmitter die. This may clarify why individuals with PD experience development issues, as well as weakness, clogging, and orthostatic hypotension, when circulatory strain switches on standing up, prompting light-headedness [45–47].

4.5.2.3 Lewy Bodies

An individual with PD may have clusters of protein in their cerebrum, known as Lewy bodies. Lewy body dementia is an alternate condition, which has joined with PD [48–50].

4.5.2.4 Genetic Factors

In most instances, PD seems to run in families; however, it is not constantly inherited. Analysts are now in an attempt to recognize the explicit hereditary components that may prompt PD. Moreover, it creates the impression that not one but rather various variables are involved. Consequently, it is thought that a blend of hereditary and ecological elements may prompt the condition. Other conceivable external factors could also be involved. These could be poisons, for example, pesticides, solvents, metals, and different contaminations [51–53].

4.5.3 Huntington's Disease

HD is brought about by a faulty gene (mhTT) on chromosome number 4. An ordinary duplication of the gene produces huntingtin, a protein. The defective gene is bigger than it ought to be. This prompts the unreasonable production of cytosine, adenine, and guanine (CAG), the structural parts of DNA. Typically, CAG repeat somewhere around ten or more times, but in HD, they repeat 36 or more times. Such a change results in a bigger type of huntingtin. This is dangerous as it makes harmful synapses. Some synapses are sensitive to the bigger type of huntingtin, particularly those identified with development, thinking, and memory. It undermines these factors and eventually causes dangerous effects. Researchers are yet to identify how precisely this occurs [54–56].

4.5.4 Risk Modifiers

4.5.4.1 Alcohol

Excessive utilization of alcohol has been linked to causing dementia due to its harmful nature. A study conducted in France reported that older people who consumed wine constantly have the possibility of acquiring AD, compared to other age groups. In addition, the researcher in the Rotterdam Study reported that consumption of moderate amounts of liquor would have the equivalent advantageous impact. The advantages of liquor are attributed to the presence of antioxidants [57–60].

4.5.4.2 Education and Early-Life Experience

The risks of acquiring AD is fundamentally higher among inadequately taught people when compared to people who are educated on this. It is found that the amount of formal instruction acquired plays a major role and has a defensive impact.

Whether generally instructive accomplishment is a surrogate for other hereditary or natural impact stays obscure. For instance, phonetic capacity during the second decade of life is an indicator of consequent intellectual impedance. It was found that the mental capacity scores in the youth were lower than normal among youngsters, who in the long run acquired AD after the age of 65. These findings would infer that the determinants of such illnesses are usually set up at an early age and, thus, may influence the capacity to accomplish instructive accomplishment. Thus, individuals who have acquired beneficial factors during the early part of their lives have a lower danger of an ensuing disease as grown-ups [61–64].

4.5.4.3 Mental and Leisure Activity

The amount of time spent preoccupied with physical and mental exercises during late life has been related to a lower risk of AD. The risk is further reduced in people with complex action designs, which include continuous scholarly activities, active lifestyle, and regular physical exercises. The Canadian Study of Health and Aging identified that higher beneficial impact was acquired through regular physical exercises [65–68].

4.5.4.4 Smoking

Several findings have reported and demonstrated that smokers have a two- to fourfold higher risk of acquiring AD. This is also observed in people without an APO-ε4 allele. In these investigations, smoking is thought to build the danger of dementia through perplexing cooperation with the cerebral vessels. A related study had reported that there was an 80% decrease in the levels of Aβ 1–42 peptide in transgenic mice treated with nicotine and sucrose. Cortical insoluble peptide Aβ 1–40 and Aβ 1–42 peptide were found to be diminished fundamentally in the nicotine-treated mice. On the other hand, abstinence from nicotine could offer a potential advantage [69–72].

4.5.4.5 Down Syndrome

The neuropathological changes in AD were acquired in people with Down's syndrome by 40. Not all patients are psychotic, however. Symptoms associated with AD are doubled or tripled with Down family history. Schupf et al. examined a relationship from an alternative perspective and found that mothers with children with Down's disease before 35 years were more at risk of AD than mothers who had children with mental disorders of a different sort [73–75].

4.5.4.6 Depression

A discouraged state of mind could precede the state of Alzheimer's illness. In a study conducted among geriatric subjects, it has been reported that a discouraged state of mind was related to a higher risk of dementia. Thus, it could be a predictive factor or could be an early sign of the ailment [76–78].

4.5.4.7 Traumatic Head Injury

Reports have shown that severe head injuries increase the risk of AD. Several studies conducted in human subjects and in rodents have provided considerable evidence on this [79–81].

4.5.4.8 Cardiovascular Disease and Related Vascular Risk Factors

Hyperlipidemia, obesity, diabetes, and related factors are generally attributed to coronary disease or stroke as potential predictors of AD. The link between Alzheimer's and these cardiovascular variables could most closely be established during the middle ages, years before dementia begins. It stays dubious whether these elements are connected exclusively to stroke and coronary illness [82–84].

4.5.4.9 Anti-inflammatory Agents

Alzheimer's ailment was observed to be less prevalent among people who consumed anti-inflammatory agents. It is believed that there is a significant positive interaction between anti-inflammatory agents and Aβ1–42 that provides this protective effect toward AD. Moreover, some mitigating medications seem to modify gamma-secretase, which is required for the production of Aβ peptides, without fundamentally affecting other APP pathways [85, 86].

4.5.5 Risk Factors for Parkinson

The advancing age and sex of a person are two unavoidable variables that influence the risk of PD.

4.5.5.1 Age

In the vast majority of people with PD, signs after 60 years have been found. In 5–10% of cases, they seem to have occurred prior to this age. If the signs begin before age 50, it is classified as "early beginning" PD.

4.5.5.2 Sex

Men appear to be 50% more likely than women to develop PD. Nonetheless, one study had discovered that the probability of developing AD in women increases when they age [87, 88].

4.5.6 Genetic Factors and Family History

Family history of AD has been a major factor in the development of this disorder. It is reported that there is a higher probability of developing AD when there is a close family member who already has the disorder. As indicated by the Parkinson's Foundation, around 20–22% of cases are likely because of inherited genetic variables. The others are "sporadic."

4.5.6.1 Autosomal Dominant

The condition results in a change in only one gene, which is affected, in 1–2% of people with PD. Variations in the quality of alpha-synuclein (SNCA) and leucine-rich recurrent kinase 2 (LRRK2) are also possible factors. As indicated by the Parkinson's Foundation, men belonging to the North African Arab communities have a higher probability of having this autosomal dominant property [89–91].

4.5.6.2 Autosomal Recessive

It is reported that PD could also develop in cases where there are autosomal recessive genes. This could probably happen when there are two duplicates in a specific factor. These progressions may include factors or genes such as PRKN.

4.5.7 Risk-Factor Modifier Genes

These genes influence the risk of PD but do not cause manifestations. This gene synthesizes the compound glucocerebrosidase, which is implicated in PD. Not every person with a gene transformation will develop PD. Persons with a PD family history are more prone or vulnerable to developing PD, as reported by several researchers [92–94].

In light of the outcomes in this precise audit, hereditary components seem to assume the most significant involvement in the development of HD. Among the potential hazard factors, CAG rehash length in the allele was observed to be a moderately significant hazard factor for the development of HD. Studies have reported that CAG length is fundamentally connected with the rate of developing HD. It has been also predicted that a higher number of CAG rehashes were related to shorter survival [95].

4.6 Therapeutic Options

4.6.1 Antioxidants

Antioxidants have been proven to be therapeutic in the treatment and prevention of many of the above ailments. There are numerous studies that support this claim. Cell reinforcements and atoms that respond especially in the presence of ROS, thereby inactivating them, have been of much interest as antioxidants, thereby restoring health and longevity. Various studies have provided substantial evidence of protection in diseases like cancer and other neurological disorders. Antioxidants principally form part of our normal dietary necessities and in various cases have been found to be disease preventive. Some of these are nutrient C, ubiquinone, β-carotene, curcumin, and several red wine micronutrients, which have a great substance of flavonoid and phenolic mixes.

In spite of the huge volume of positive research and findings that support antioxidants, there have been relatively limited clinical studies. Potent antioxidants

like α-tocopherol (nutrient E) have been widely studied in both PD and AD. The findings were encouraging. Several constructive outcomes were observed in the AD trial, with an expansion in the mean survival time for the treated subjects. Further studies are currently ongoing [96–98].

One of the outcomes of ROS is the inception of excitotoxicity, forming by activation of glutamate receptors and various medicines to promote these receptors. This has shown that neurodegenerative disorder treatment is viable. Memantine, which focuses on the *N*-methyl-D-aspartate (NMDA) receptor, has obtained FDA endorsement for use in AD. Memantine moderates the advancement of the ailment and has the advantage in patients with reasonably serious to severe form. Amantadine, a fractional NMDA foe, has been utilized in the treatment of PD for quite a while with humble clinical additions, including expanded survival times. Riluzole is the principal medication used to treat ALS with any demonstrated adequacy. The impact of riluzole is that it can broaden the mean survival time of patients by a few months. Riluzole is proven to hinder glutamate discharge (and in this manner to diminish excitotoxicity) by meddling with sodium channels [79, 84, 90].

4.6.2 Modulation of Metal–Protein Interactions

The basic reasons for oxidative pressure are the breakdown of metal homeostasis, metalloenzyme degradation, or breakdown of antioxidant protection. Pharmacological treatment focusing on these factors requires a distinguishing proof of atoms that will repress the pernicious impact of deviant metal interactions. One of the strategies is to focus on a metal–protein weakening compound (MPAC). This should not be mistaken with "chelation treatment," a term related to the evacuation of mass metals, for example, in Wilson's illness (Cu) and β-thalassemia (Fe). The breakdown in metal homeostasis in these illnesses prompts tissue immersion with metals. The goal of the MPAC is to upset an irregular metal–protein collaboration to accomplish an unpretentious repartitioning of metals and a resulting standardization of metal circulation [76, 82, 89, 93].

In a related study, patients were given intramuscular infusions of desferrioxamine twice a day for 5 days for a week for 2 years. The treatment routine brought a significant decrease in usual abilities when contrasted with control patients. In light of the associations between Aβ and metals, we have researched the potential utilization of MPACs in treating AD. A study utilized a hydrophobic moderate metal chelator (clioquinol (CQ, 5-chloro-7-iodo-8-hydroxyquinoline)) equipped for intersecting the blood–brain barrier (BBB) as a prototypic MPAC. CQ can accommodate metal particles with moderate partiality. CQ was given orally to Tg2576 transgenic mice113 in a blinded trial. The outcomes demonstrated a 49% lessening of parameters when compared with nontreated controls. Following 9 weeks of study, the general well-being and body weight parameters were increasingly steady in the treated animals. Treatment with CQ did not prompt an orderly lessening in metal dimensions, which is most likely because of the medication's moderate restricting affinities. Strikingly, in a similar report, triethylenetetramine

(TETA), a hydrophilic high-partial metal chelator that is unequipped for intersecting the BBB and that has been utilized to treat Wilson's illness, did not restrain Aβ affidavit, showing that fundamental consumption of metal particles (chelation treatment) is probably not going to be a powerful restorative system for the treatment of AD [78, 85, 91, 96, 99].

4.7 Summary

The brain has a poor ability to adapt to oxidative pressure and shows minimal regenerative capacities. Studies have been on the rise that substantiate the therapeutic use of several elements, along with main proteins, in neurodegenerative diseases like ALS, PD, and AD. Oxidative stress has been implicated with dangerous impact across different pathways, which necessitates further research in this direction.

References

1. Al-Amin MM, Chowdury MIA, Saifullah ARM, Alam MN, Jain P, Hossain M et al (2019) Levocarnitine improves AlCl3-induced spatial working memory impairment in Swiss albino mice. Front Neurosci 13:278
2. Alonso-Andres P, Martin M, Albasanz JL (2019) Modulation of adenosine receptors and antioxidative effect of beer extracts in in vitro models. Nutrients 11(6):1258
3. Antognazza MR, Abdel Aziz I, Lodola F (2019) Use of exogenous and endogenous photomediators as efficient ROS modulation tools: results and perspectives for therapeutic purposes. Oxid Med Cell Longev 2019:2867516
4. Barcelos IP, Troxell RM, Graves JS (2019) Mitochondrial dysfunction and multiple sclerosis. Biology 8(2):37
5. Baszczynski O, Watt JM, Rozewitz MD, Guse AH, Fliegert R, Potter BVL (2019) Synthesis of terminal ribose analogues of adenosine 5′-diphosphate ribose as probes for the transient receptor potential cation channel TRPM2. J Org Chem 84(10):6143–6157
6. Birla H, Keswani C, Rai SN, Singh SS, Zahra W, Dilnashin H et al (2019) Neuroprotective effects of Withania somnifera in BPA induced-cognitive dysfunction and oxidative stress in mice. Behav Brain Funct 15(1):9
7. Bordoni M, Pansarasa O, Dell'Orco M, Crippa V, Gagliardi S, Sproviero D et al (2019) Nuclear phospho-SOD1 protects DNA from oxidative stress damage in amyotrophic lateral sclerosis. J Clin Med 8(5). pii: E729
8. Borriello M, Iannuzzi C, Sirangelo I (2019) Pinocembrin protects from AGE-induced cytotoxicity and inhibits non-enzymatic glycation in human insulin. Cells 8(5). pii: E385
9. Chandola C, Casteleijn MG, Chandola UM, Gopalan LN, Urtti A, Neerathilingam M (2019) CD44 aptamer mediated cargo delivery to lysosomes of retinal pigment epithelial cells to prevent age-related macular degeneration. Biochem Biophys Rep 18:100642
10. Chen JG, Jiang QC, Wen B, Wang RY, Wu YG, Li X (2018) [Effects of deoxygedunin on Alzheimer-like pathologic dysfunction induced by D-galactose combined with AlCl3]. Zhongguo Ying Yong Sheng Li Xue Za Zhi 34(6):496–500
11. Chen XQ, Qiu K, Liu H, He Q, Bai JH, Lu W (2019) Application and prospects of butylphthalide for the treatment of neurological diseases. Chin Med J 132(12):1467–1477
12. Cheng CS, Liu TP, Chien FC, Mou CY, Wu SH, Chen YP (2019) Codelivery of plasmid and curcumin with mesoporous silica nanoparticles for promoting neurite outgrowth. ACS Appl Mater Interfaces 11(17):15322–15331

13. Chiricosta L, Gugliandolo A, Tardiolo G, Bramanti P, Mazzon E (2019) Transcriptomic analysis of MAPK signaling in NSC-34 motor neurons treated with vitamin E. Nutrients 11 (5):1081
14. Clementi ME, Lazzarino G, Sampaolese B, Brancato A, Tringali G (2019) DHA protects PC12 cells against oxidative stress and apoptotic signals through the activation of the NFE2L2/HO-1 axis. Int J Mol Med 43(6):2523–2531
15. Collin F (2019) Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. Int J Mol Sci 20(10):2407
16. Dator RP, Solivio MJ, Villalta PW, Balbo S (2019) Bioanalytical and mass spectrometric methods for aldehyde profiling in biological fluids. Toxics 7(2):32
17. Day SM, Yang W, Wang X, Stern JE, Zhou X, Macauley SL et al (2019) Glucagon-like peptide-1 cleavage product improves cognitive function in a mouse model of Down syndrome. eNeuro 6(2):ENEURO.0031-19.2019
18. de Oliveira NKS, Almeida MRS, Pontes FMM, Barcelos MP, de Paula da Silva CHT, Rosa JMC et al (2019) Antioxidant effect of flavonoids present in Euterpe oleracea Martius and neurodegenerative diseases: a literature review. Cent Nerv Syst Agents Med Chem 19(2):75–99
19. Deb S, Phukan BC, Mazumder MK, Dutta A, Paul R, Bhattacharya P et al (2019) Garcinol, a multifaceted sword for the treatment of Parkinson's disease. Neurochem Int 128:50–57
20. Ding X, Liu X, Wang F, Wang F, Geng X (2019) Role of senescence and neuroprotective effects of telomerase in neurodegenerative diseases. Rejuvenation Res. https://doi.org/10.1089/rej.2018.2115
21. Doonan LB, Hartigan A, Okamura B, Long PF (2019) Stress-free evolution: the Nrf-coordinated oxidative stress response in early diverging metazoans. Integr Comp Biol 59 (4):799–810
22. Doria M, Nury T, Delmas D, Moreau T, Lizard G, Vejux A (2019) Protective function of autophagy during VLCFA-induced cytotoxicity in a neurodegenerative cell model. Free Radic Biol Med 137:46–58
23. Dos Santos TM, Siebert C, de Oliveira MF, Manfredini V, Wyse ATS (2019) Chronic mild hyperhomocysteinemia impairs energy metabolism, promotes DNA damage and induces a Nrf2 response to oxidative stress in rats brain. Cell Mol Neurobiol 39(5):687–700
24. Engin AB, Engin A (2019) Nanoparticles and neurotoxicity: dual response of glutamatergic receptors. Prog Brain Res 245:281–303
25. Faienza MF, D'Amato G, Chiarito M, Colaianni G, Colucci S, Grano M et al (2019) Mechanisms involved in childhood obesity-related bone fragility. Front Endocrinol 10:269
26. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, D'Arcangelo D et al (2019) Beneficial role of phytochemicals on oxidative stress and age-related diseases. Biomed Res Int 2019:8748253
27. Fuentes-Villalobos F, Farkas C, Riquelme-Barrios S, Armijo ME, Soto-Rifo R, Pincheira R et al (2019) DISC1 promotes translation maintenance during sodium arsenite-induced oxidative stress. Biochim Biophys Acta Gene Regul Mech 1862(6):657–669
28. Fukui K, Shirai M, Ninuma T, Kato Y (2019) Anti-obesity effects of tocotrienols and bran in high-fat diet-treated mice. Nutrients 11(4). pii: E830
29. Furukawa Y, Sawamoto A, Yamaoka M, Nakaya M, Hieda Y, Choshi T et al (2019) Effects of carbazole derivatives on neurite outgrowth and hydrogen peroxide-induced cytotoxicity in Neuro2a cells. Molecules 24(7). pii: E1366
30. Gao S, Zhang X, Song Q, Liu J, Ji X, Wang P (2019) POLD1 deficiency is involved in cognitive function impairment in AD patients and SAMP8 mice. Biomed Pharmacother 114:108833
31. Gargiulo-Monachelli G, Meyer M, Lara A, Garay L, Lima A, Roig P et al (2019) Comparative effects of progesterone and the synthetic progestin norethindrone on neuroprotection in a model of spontaneous motoneuron degeneration. J Steroid Biochem Mol Biol 192:105385
32. Grimaldi G, Catara G, Palazzo L, Corteggio A, Valente C, Corda D (2019) PARPs and PAR as novel pharmacological targets for the treatment of stress granule-associated disorders. Biochem Pharmacol 167:64–75

33. Gundogdu G, Dodurga Y, Kucukatay V (2019) The sulfite molecule enhances homocysteine toxicity in SH-SY5Y cells. Mol Biol Rep 46(4):4017–4025
34. Hadrian K, Melkonyan H, Schlatt S, Wistuba J, Wasmuth S, Heiligenhaus A et al (2019) Age-related distribution and potential role of SNCB in topographically different retinal areas of the common marmoset Callithrix jacchus, including the macula. Exp Eye Res 185:107676
35. Halon-Golabek M, Borkowska A, Herman-Antosiewicz A, Antosiewicz J (2019) Iron metabolism of the skeletal muscle and neurodegeneration. Front Neurosci 13:165
36. Haque MM, Murale DP, Kim YK, Lee JS (2019) Crosstalk between oxidative stress and tauopathy. Int J Mol Sci 20(8). pii: E1959
37. He P, Yan S, Wen X, Zhang S, Liu Z, Liu X et al (2019) Eriodictyol alleviates lipopolysaccharide-triggered oxidative stress and synaptic dysfunctions in BV-2 microglial cells and mouse brain. J Cell Biochem 120(9):14756–14770
38. Hou S, Wang L, Zhang G (2019) Mitofusin-2 regulates inflammation-mediated mouse neuroblastoma N2a cells dysfunction and endoplasmic reticulum stress via the Yap-Hippo pathway. J Physiol Sci 69(5):697–709
39. Huang C, Ng OT, Chu JM, Irwin MG, Hu X, Zhu S et al (2019) Differential effects of propofol and dexmedetomidine on neuroinflammation induced by systemic endotoxin lipopolysaccharides in adult mice. Neurosci Lett 707:134309
40. Ikram M, Saeed K, Khan A, Muhammad T, Khan MS, Jo MG et al (2019) Natural dietary supplementation of curcumin protects mice brains against ethanol-induced oxidative stress-mediated neurodegeneration and memory impairment via Nrf2/TLR4/RAGE signaling. Nutrients 11(5). pii: E1082
41. Jakaria M, Azam S, Haque ME, Jo SH, Uddin MS, Kim IS et al (2019) Taurine and its analogs in neurological disorders: focus on therapeutic potential and molecular mechanisms. Redox Biol 24:101223
42. Jang Y, Kwon I, Cosio-Lima L, Wirth C, Vinci DM, Lee Y (2019) Endurance exercise prevents metabolic distress-induced senescence in the hippocampus. Med Sci Sports Exer 51 (10):2012–2024
43. Jeong JY, Chung YC, Jin BK (2019) Interleukin-4 and interleukin-13 exacerbate neurotoxicity of prothrombin Kringle-2 in cortex in vivo via oxidative stress. Int J Mol Sci 20(8). pii: E1927
44. Jianhai L, Qian W, Huanhuan H, Xin S, Guodong L, Shuai W et al (2019) NLRP3 inflammasome activation is involved in trimethyltin-induced neuroinflammation. Brain Res 1718:186–193
45. Kashyap D, Garg VK, Tuli HS, Yerer MB, Sak K, Sharma AK et al (2019) Fisetin and quercetin: promising flavonoids with chemopreventive potential. Biomolecules 9(5). pii: E174
46. Khan M, Rutten BPF, Kim MO (2019) MST1 regulates neuronal cell death via JNK/Casp3 signaling pathway in HFD mouse brain and HT22 cells. Int J Mol Sci 20(10). pii: E2504
47. Kim M, Jung J, Jeong NY, Chung HJ (2019) The natural plant flavonoid apigenin is a strong antioxidant that effectively delays peripheral neurodegenerative processes. Anat Sci Int 94 (4):285–294
48. Koch W (2019) Dietary polyphenols-important non-nutrients in the prevention of chronic noncommunicable diseases. a systematic review. Nutrients 11(5). pii: E1039
49. Kreiner G, Sonmez A, Liss B, Parlato R (2019) Integration of the deacetylase SIRT1 in the response to nucleolar stress: metabolic implications for neurodegenerative diseases. Front Mol Neurosci 12:106
50. Kruk J, Aboul-Enein HY, Kladna A, Bowser JE (2019) Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis. Free Radic Res 53:497–521
51. Kumar R, Jain V, Kushwah N, Dheer A, Mishra KP, Prasad D et al (2018) Role of DNA methylation in hypobaric hypoxia-induced neurodegeneration and spatial memory impairment. Ann Neurosci 25(4):191–200

52. Lee ES, Kim JS, Lee H, Ryu JY, Lee HJ, Sonn JK et al (2019) Auranofin, an anti-rheumatic gold drug, aggravates the radiation-induced acute intestinal injury in mice. Front Pharmacol 10:417
53. Lee Y, Kim S, Oh Y, Kim YM, Chin YW, Cho J (2019) Inhibition of oxidative neurotoxicity and scopolamine-induced memory impairment by gamma-mangostin: in vitro and in vivo evidence. Oxid Med Cell Longev 2019:3640753
54. Levy D, Reichert CO, Bydlowski SP (2019) Paraoxonases activities and polymorphisms in elderly and old-age diseases: an overview. Antioxidants (Basel) 8(5). pii: E118
55. Li H, Yahaya BH, Ng WH, Yusoff NM, Lin J (2019) Conditioned medium of human menstrual blood-derived endometrial stem cells protects against MPP(+)-induced cytotoxicity in vitro. Front Mol Neurosci 12:80
56. Limanaqi F, Biagioni F, Gaglione A, Busceti CL, Fornai F (2019) A sentinel in the crosstalk between the nervous and immune system: the (immuno)-proteasome. Front Immunol 10:628
57. Lin CY, Tsai CH, Feng LY, Chai WY, Lin CJ, Huang CY et al (2019) Focused ultrasound-induced blood brain-barrier opening enhanced vascular permeability for GDNF delivery in Huntington's disease mouse model. Brain Stimul 12(5):1143–1150
58. Liou CJ, Tong M, Vonsattel JP, de la Monte SM (2019) Altered Brain expression of insulin and insulin-like growth factors in frontotemporal lobar degeneration: another degenerative disease linked to dysregulation of insulin metabolic pathways. ASN Neuro 11:1759091419839515
59. Liu P, Li Y, Yang W, Liu D, Ji X, Chi T et al (2019) Prevention of Huntington's disease-like behavioral deficits in R6/1 mouse by tolfenamic acid is associated with decreases in mutant huntingtin and oxidative stress. Oxid Med Cell Longev 2019:4032428
60. Liu X, Zhang Y, Yang X (2019) PM2.5 induced neurodegenerative-like changes in mice and the antagonistic effects of vitamin E. Toxicol Res 8(2):172–179
61. Maguire G, Paler L, Green L, Mella R, Valcarcel M, Villace P (2019) Rescue of degenerating neurons and cells by stem cell released molecules: using a physiological renormalization strategy. Physiol Rep 7(9):e14072
62. Maki RA, Holzer M, Motamedchaboki K, Malle E, Masliah E, Marsche G et al (2019) Human myeloperoxidase (hMPO) is expressed in neurons in the substantia nigra in Parkinson's disease and in the hMPO-alpha-synuclein-A53T mouse model, correlating with increased nitration and aggregation of alpha-synuclein and exacerbation of motor impairment. Free Radic Biol Med 141:115–140
63. Marc G, Stana A, Oniga SD, Pirnau A, Vlase L, Oniga O (2019) New phenolic derivatives of thiazolidine-2,4-dione with antioxidant and antiradical properties: synthesis, characterization, in vitro evaluation, and quantum studies. Molecules 24(11). pii: E2060
64. Martin-Montanez E, Pavia J, Valverde N, Boraldi F, Lara E, Oliver B et al (2019) The S1P mimetic fingolimod phosphate regulates mitochondrial oxidative stress in neuronal cells. Free Radic Biol Med 137:116–130
65. Massaccesi L, Galliera E, Galimberti D, Fenoglio C, Arcaro M, Goi G et al (2019) Lag-time in Alzheimer's disease patients: a potential plasmatic oxidative stress marker associated with ApoE4 isoform. Immun Ageing 16:7
66. Moslemi M, Motamedi F, Asadi S, Khodagholi F (2019) Peroxisomal malfunction caused by mitochondrial toxin 3-NP: protective role of oxytocin. Iran J Pharm Res 18(1):296–307
67. Naoi M, Wu Y, Shamoto-Nagai M, Maruyama W (2019) Mitochondria in neuroprotection by phytochemicals: bioactive polyphenols modulate mitochondrial apoptosis system, function and structure. Int J Mol Sci 20(10). pii: E2451
68. Nesari A, Mansouri MT, Khodayar MJ, Rezaei M (2019) Preadministration of high-dose alpha-tocopherol improved memory impairment and mitochondrial dysfunction induced by proteasome inhibition in rat hippocampus. Nutr Neurosci 14:1–11
69. Qi G, Mi Y, Fan R, Li R, Liu Z, Liu X (2019) Nobiletin protects against systemic inflammation-stimulated memory impairment via MAPK and NF-kappaB signaling pathways. J Agric Food Chem 67(18):5122–5134

70. Radovanovic V, Vlainic J, Hanzic N, Ukic P, Orsolic N, Baranovic G et al (2019) Neurotoxic effect of ethanolic extract of propolis in the presence of copper ions is mediated through enhanced production of ROS and stimulation of caspase-3/7 activity. Toxins (Basel) 11(5). pii: E273
71. Rajendran P, Alzahrani AM, Hanieh HN, Kumar SA, Ben Ammar R, Rengarajan T et al (2019) Autophagy and senescence: a new insight in selected human diseases. J Cell Physiol 234 (12):21485–21492
72. Rathnayake D, Chang T, Udagama P (2019) Selected serum cytokines and nitric oxide as potential multi-marker biosignature panels for Parkinson disease of varying durations: a case-control study. BMC Neurol 19(1):56
73. Raza C, Anjum R, Shakeel NUA (2019) Parkinson's disease: Mechanisms, translational models and management strategies. Life Sci 226:77–90
74. Sadegh Malvajerd S, Izadi Z, Azadi A, Kurd M, Derakhshankhah H, Sharif Zadeh M et al (2019) Neuroprotective potential of curcumin-loaded nanostructured lipid carrier in an animal model of Alzheimer's disease: behavioral and biochemical evidence. J Alzheimer's Dis 69 (3):671–686
75. Schubert D, Kepchia D, Liang Z, Dargusch R, Goldberg J, Maher P (2019) Efficacy of cannabinoids in a pre-clinical drug-screening platform for Alzheimer's disease. Mol Neurobiol 56(11):7719–7730
76. Sghaier R, Zarrouk A, Nury T, Ilham B, O'Brien N, Mackrill JJ et al (2019) Biotin attenuation of oxidative stress, mitochondrial dysfunction, lipid metabolism alteration and 7beta-hydroxycholesterol-induced cell death in 158N murine oligodendrocytes. Free Radic Res 53:535–561
77. Shafi S, Ansari HR, Bahitham W, Aouabdi S (2019) The impact of natural antioxidants on the regenerative potential of vascular cells. Front Cardiovasc Med 6:28
78. Shah A, Chao J, Legido-Quigley C, Chang RC (2019) Oxyresveratrol exerts ATF4- and Grp78-mediated neuroprotection against endoplasmic reticulum stress in experimental Parkinson's disease. Nutr Neurosci 17:1–16
79. Sieteiglesias V, Gonzalez-Burgos E, Bermejo-Bescos P, Divakar PK, Gomez-Serranillos MP (2019) Lichens of parmelioid clade as promising multi-target neuroprotective agents. Chem Res Toxicol 32(6):1165–1177
80. Silva AR, Grosso C, Delerue-Matos C, Rocha JM (2019) Comprehensive review on the interaction between natural compounds and brain receptors: benefits and toxicity. Eur J Med Chem 174:87–115
81. Singh A, Kukreti R, Saso L, Kukreti S (2019) Oxidative stress: a key modulator in neurodegenerative diseases. Molecules 24(8). pii: E1583
82. Solingapuram Sai KK, Bashetti N, Chen X, Norman S, Hines JW, Meka O et al (2019) Initial biological evaluations of (18)F-KS1, a novel ascorbate derivative to image oxidative stress in cancer. EJNMMI Res 9(1):43
83. Sottero B, Rossin D, Staurenghi E, Gamba P, Poli G, Testa G (2019) Omics analysis of oxysterols to better understand their pathophysiological role. Free Radic Biol Med 144:55–71
84. Spiers JG, Breda C, Robinson S, Giorgini F, Steinert JR (2019) Drosophila Nrf2/Keap1 mediated redox signaling supports synaptic function and longevity and impacts on circadian activity. Front Mol Neurosci 12:86
85. Tang BL (2019) Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. J Cell Biochem 120(9):14285–14295
86. Telkoparan-Akillilar P, Suzen S, Saso L (2019) Pharmacological applications of Nrf2 inhibitors as potential antineoplastic drugs. Int J Mol Sci 20(8). pii: E2025
87. Tu W, Wang H, Li S, Liu Q, Sha H (2019) The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases. Aging Dis 10(3):637–651
88. Ullah R, Khan M, Shah SA, Saeed K, Kim MO (2019) Natural antioxidant anthocyanins-a hidden therapeutic candidate in metabolic disorders with major focus in neurodegeneration. Nutrients 11(6). pii: E1195

89. Vallee A, Lecarpentier Y, Vallee JN (2019) Circadian rhythms and energy metabolism reprogramming in Parkinson's disease. Curr Issues Mol Biol 31:21–44
90. Vegh C, Stokes K, Ma D, Wear D, Cohen J, Ray SD et al (2019) A bird's-eye view of the multiple biochemical mechanisms that propel pathology of Alzheimer's disease: recent advances and mechanistic perspectives on how to halt the disease progression targeting multiple pathways. J Alzheimers Dis 69(3):631–649
91. Voyer LE, Alvarado C (2019) [Maillard reaction. Pathogenic effects]. Medicina 79(2):137–143
92. Wang H, Zhao P, Huang Q, Chi Y, Dong S, Fan J (2019) Bisphenol-A induces neurodegeneration through disturbance of intracellular calcium homeostasis in human embryonic stem cells-derived cortical neurons. Chemosphere 229:618–630
93. Wang YJ, Wang XY, Hao XY, Yan YM, Hong M, Wei SF et al (2019) Ethanol extract of centipeda minima exerts antioxidant and neuroprotective effects via activation of the Nrf2 signaling pathway. Oxid Med Cell Longev 2019:9421037
94. Wu H, Dunnett S, Ho YS, Chang RC (2019) The role of sleep deprivation and circadian rhythm disruption as risk factors of Alzheimer's disease. Front Neuroendocrinol 54:100764
95. Zhao X, Fang J, Li S, Gaur U, Xing X, Wang H et al (2019) Artemisinin attenuated hydrogen peroxide (H2O2)-induced oxidative injury in SH-SY5Y and hippocampal neurons via the activation of AMPK pathway. Int J Mol Sci 20(11). pii: E2680
96. Zhao X, Jin Y, Li L, Xu L, Tang Z, Qi Y et al (2019) MicroRNA-128-3p aggravates doxorubicin-induced liver injury by promoting oxidative stress via targeting Sirtuin-1. Pharmacol Res 146:104276
97. Zheng Y, Zhu G, He J, Wang G, Li D, Zhang F (2019) Icariin targets Nrf2 signaling to inhibit microglia-mediated neuroinflammation. Int Immunopharmacol 73:304–311
98. Zhong J, Wang F, Wang Z, Shen C, Zheng Y, Ma F et al (2019) Aloin attenuates cognitive impairment and inflammation induced by d-galactose via down-regulating ERK, p38 and NF-kappaB signaling pathway. Int Immunopharmacol 72:48–54
99. Xia S, Wang L, Fu TM, Wu H (2019) Mechanism of TRPM2 channel gating revealed by cryo-EM. FEBS J 286(17):3333–3339

Role of Oxidative Stress in Complexity of Respiratory Diseases

5

Maliheh Ghadiri, Amanda Eugenia Yung, and Mehra Haghi

Abstract

Oxidative stress can arise from excess production of endogenous reactive oxygen species (ROS) or from exposure to exogenous ROS. In the lungs, oxidative stress causes lipid, protein and DNA oxidation, changes to histone acetylation, and inflammation. These pathways are thought to underlay the pathophysiology of respiratory diseases such as asthma, chronic obstructive pulmonary disease, cystic fibrosis, acute respiratory distress syndrome, pneumonia, lung cancer, and obstructive sleep apnoea. This chapter discusses the risk factors for respiratory disease, the evidence for the role of oxidative stress in these diseases, and proposed antioxidant therapies for the treatment of respiratory disease.

Keywords

Inflammation · Asthma · COPD · Lung cancer · Acute respiratory distress syndrome · Obstructive sleep apnoea · *N*-Acetyl-L-cysteine · Malondialdehyde · 8-Isoprostane

Maliheh Ghadiri and Amanda Eugenia Yung contributed equally to the work.

M. Ghadiri
Woolcock Institute of Medical Research, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia

School of Biomedical Engineering, Faculty of Engineering and Internet Technology, University of Technology Sydney, Ultimo, NSW, Australia

A. E. Yung
School of Medicine, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia

M. Haghi (✉)
Graduate School of Health, University of Technology Sydney, Ultimo, NSW, Australia
e-mail: mehra.haghi@uts.edu.au

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_5

5.1 Introduction

Reactive organic molecules are produced in low concentrations during normal cellular metabolism for use in signal transduction [1]. At high concentrations, these molecules can cause oxidative damage to cells [1]. Most reactive organic molecules may be categorised into reactive oxygen species (ROS) or reactive nitrogen species (RNS). ROS are by far the most common reactive organic species and include free oxygen radicals such as superoxide anions and hydroxyl radicals, as well as oxygen derivatives such as hydrogen peroxide and ozone [1]. The most abundant RNS is nitrogen oxide (NO), which may react with superoxide radicals to produce the ROS peroxynitrite and may also be converted into hydroxyl radicals [2].

Under normal physiological conditions, a homeostasis between ROS/RNS production and defence mechanisms that remove ROS/RNS is maintained. This redox balance is necessary to enable the proper functioning of signalling pathways that control cell proliferation, differentiation, and apoptosis. Excesses of ROS/RNS or deficiencies in antioxidant mechanisms can disrupt this delicate balance in favour of oxidants, resulting in oxidative stress [3]. Oxidative stress is now recognised to be a key part of the pathophysiology of many diseases. In particular, the lung is especially prone to oxidative stress owing to its large surface area, high vascularity, exposure to high levels of oxygen, and exposure to environmental oxidants [1]. Many respiratory diseases involve oxidative stress as a cause or as part of disease progression (Fig. 5.1).

5.1.1 Sources of Oxidative Stress

Oxidative stress can be the result of excess production of endogenous ROS or environmental factors.

ROS in lung cells may be endogenous or exogenous. Endogenous ROS are produced in all cells by electron leakage at complex I and III in the electron transport chain (ETC) [3] and by enzymes, primarily NADPH oxidase (Nox) [1]. An ETC is a series of complexes that transfer electrons from electron donors to electron acceptors via redox (both reduction and oxidation occurring simultaneously) reactions and couples this electron transfer with the transfer of protons (H^+ ions) across a membrane. In addition, NO is produced by nitric oxide synthase (NOS), but in the absence of oxygen or co-factors, NOS uncoupling results in production of superoxide anions instead of NO [3]. Thus, during metabolic dysfunction where oxidant-producing enzymes are upregulated, excess endogenous ROS may be produced, resulting in oxidative stress.

As an example, for endogenous ROS activity, it can occur as a result of inflammation. Factors that induce pulmonary inflammation, such as infection or inhaled substances, activate alveolar macrophages and neutrophil recruitment resulting in

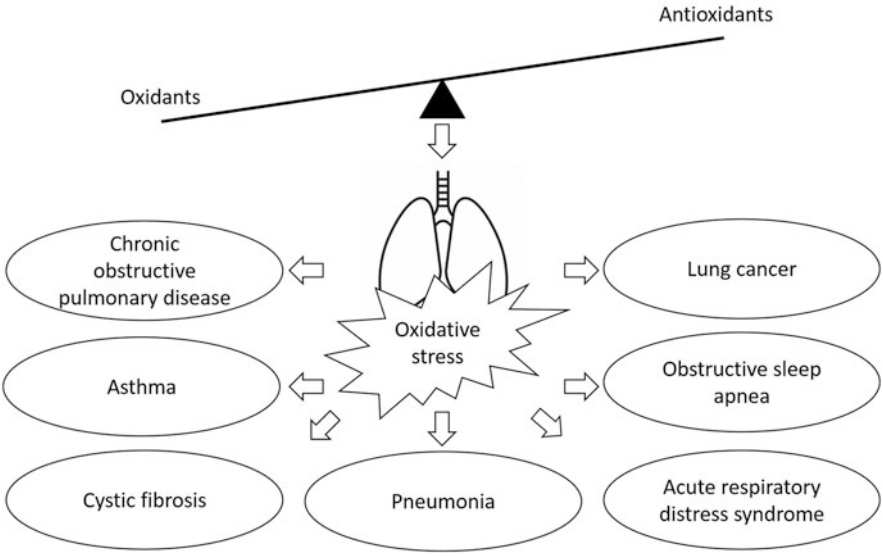


Fig. 5.1 Respiratory diseases associated with an imbalance in the oxidant/antioxidant ratio in favour of oxidants

ROS overproduction and oxidative stress. In the site of inflammation, phagocytic cells contain more ROS-producing enzymes such as haem peroxidase, myeloperoxidase, and eosinophil peroxidase and produce large amounts of ROS in a reaction known as the respiratory burst [1].

Exogenous sources of ROS include both tobacco and e-cigarette smoke, airborne pollutants, and particulate matter [1]. Radicals in these inhaled substances, such as superoxide anions in the gas phase of cigarette smoke, directly damage the respiratory epithelium or induce inflammation to further contribute to oxidative stress [4].

5.1.2 Pathways from Oxidative Stress to Pathology

There are few known pathways for OS to cause respiratory diseases (Fig. 5.2). These pathways include the following:

Pathway 1: Lipid peroxidation. ROS can oxidise membrane phospholipids and free fatty acids. Lipid peroxidation products include malondialdehyde, thiobarbituric acid reactive substances, oxidised low-density lipoprotein [3], and F2-isoprostanes (8-isoprostanes). F2-isoprostanes can further act to cause smooth muscle constriction and hyperplasia [4], while malondialdehyde can modify proteins, nucleotides, and other lipids and induce inflammation [5]. In addition,

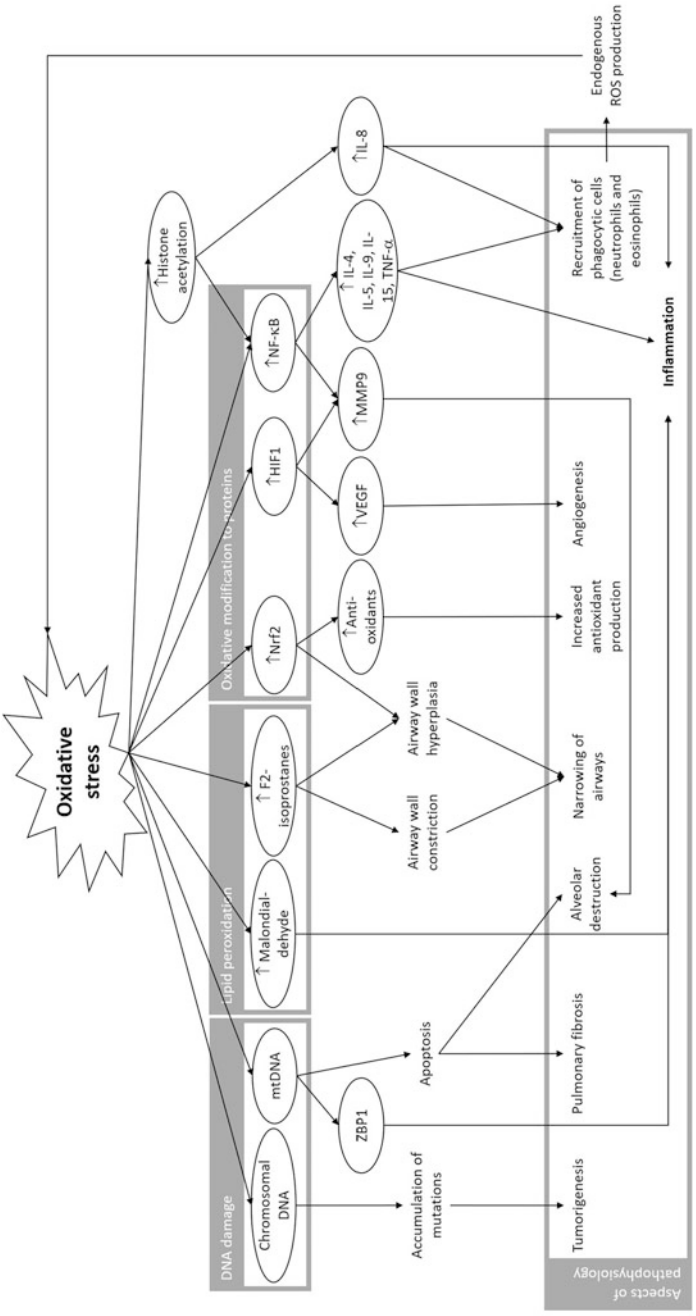


Fig. 5.2 Cellular pathways affected by oxidative stress and their contributions to pathophysiology in the respiratory system

damage to the membrane may lead to loss of integrity of lung epithelium, increasing permeability and contributing to greater mucus secretion [6].

Pathway 2: Protein oxidation and modulation of signalling pathways. Oxidative alteration of proteins may be reversible or irreversible [4]. Irreversible changes are typically in the form of carbonylation of protein residues, while reversible changes involve disulphide formation, S-glutathionylation, or S-nitrosylation of cysteine residues. These modifications are used physiologically to regulate protein function, and thus oxidative stress results in overactivation or underactivation of proteins and disruption to several signalling pathways [7]. As pathways modulated by ROS are mainly inflammatory signalling pathways, the effect of ROS on these pathways will be further discussed in Pathway 5.

Pathway 3: DNA oxidation. The reaction of ROS with DNA results mainly in 8-oxo-2′-deoxyguanosine (8-oxodG) adducts, as well as double- or single-stranded breaks [2]. These lesions can cause abnormal gene expression that can be pro-inflammatory, impair cellular function, or result in tumorigenesis [8]. Mitochondrial DNA (mtDNA) is especially predisposed to oxidative damage due to its proximity to the ROS-producing electron transport chain, limited DNA repair mechanisms, and lack of histones [2]. Damaged mtDNA interacts with Z binding protein-1 (ZBP1) to trigger an inflammatory response in the cell and is released from the cell to induce inflammatory responses in neighbouring cells [9]. Excessive damage may also result in apoptosis of alveolar epithelial cells, impairing lung function and promoting pulmonary fibrosis [10].

Pathway 4: Effects on histone acetylation. Levels of gene transcription are directly related to levels of histone acetylation, which is promoted by histone acetyltransferases (HATs) and inhibited by histone deacetylases (HDACs). While the exact pathways are still unclear, oxidative stress is associated with changes in histone acetylation and chromatin remodelling, resulting in increased expression of pro-inflammatory genes such as IL-8 [11].

Pathway 5: Inflammation. Several of the pathways above share a common endpoint: the recruitment of inflammatory cells and release of inflammatory mediators. These mediators increase vascular permeability and oedema and enable extravasation of phagocytic cells that further contribute to increased oxidative stress [12]. Inflammation in the respiratory system is an important step in the pathogenesis and exacerbation of several respiratory diseases including chronic obstructive pulmonary disease (COPD) and asthma [13].

In addition, as mentioned above, oxidative stress disrupts several inflammatory signalling pathways. Some of the key pathways include NF-κB, HIF1, and Nrf2 which will be discussed in details.

NF-κB: Nuclear factor-kappa B (NF-κB) is a key transcription factor involved in oxidative stress-induced damage and controls the expression of genes encoding adhesion molecules and inflammatory cytokines such IL-4, IL-5, IL-9, IL-15, TNF-α, and vascular cell adhesion molecule-1 [1]. In the absence of stimulation,

NF-κB is bound to inhibitor of κB (IκB) and remains inactive in the cytoplasm. Phosphorylation of IκB results in release of NF-κB, which translocates to the nucleus to produce its effects [4]. In alveolar macrophages, ROS may directly activate NF-κB by oxidation of its cysteine-SH group or cause the ubiquitination and proteolysis of IκB to release NF-κB, resulting in greater production of inflammatory mediators. Moreover, activation of NF-κB in alveolar macrophages and neutrophils results in the production of elastolytic enzymes such as metalloproteinase (MMP) 9, which contribute to alveolar destruction and mucus secretion [14].

HIF1: Hypoxia-induced factor 1 (HIF1) is an oncogene that activates transcription of hypoxia response genes [1] such as MMP2 and MMP9, which cause alveolar destruction, vascular endothelial growth factor (VEGF) contributing to abnormal angiogenesis, and B cell lymphoma-2 (Bcl-2), which induces apoptosis [15]. The α subunit of HIF is stabilised by ROS, and thus HIF1 activity is elevated in oxidative stress.

Nrf2: Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor responsible for the activation of antioxidant response enzymes. ROS-mediated modification of protein thiols results in activation of Nrf2. While this is initially protective against oxidative damage, during chronic oxidative stress, oxidative damage exceeds the protective effects of Nrf2 [4]. Overactivation of Nrf2 also contributes to epithelial cell hyperplasia and carcinogenesis [16].

5.1.3 Exogenous Risk Factors

Multiple risk factors predispose an individual to oxidative stress in the respiratory system, the most common of which is smoking. A meta-analysis of 36 studies showed that smoking was associated with a systemic increase in oxidative stress, as measured by urinary markers of oxidative stress [17]. Tobacco smoke contains gaseous radicals that can directly damage cells in the respiratory epithelium. For example, gaseous radicals inactivate human alpha-1-proteinase inhibitor, leading to the development of emphysema in smokers [18]. Tobacco smoke also contains compounds that induce inflammation and endogenous ROS generation in lung epithelial cells [19] and cause oxidative DNA damage [8]. These compounds include benzo[a]pyrene, which induces chromosomal mutation in lung cells and macrophage apoptosis [20], and acrolein, which induces ROS generation in lung epithelial cells and degranulation of airway mast cells, leading to airway inflammation [21]. In addition, acrolein induces DNA adduct formation and damages rRNA, which activates tumour protein p53 and results in apoptosis [22]. Such mechanisms may explain why smoking is the most significant risk factor associated with both COPD [23] and lung cancer [8].

This problem has perhaps been further confounded with the rise of e-cigarettes marketed as a ‘safe’ alternative to conventional cigarettes. Use of e-cigarettes has been shown to induce production of ROS in human bronchial [24] and lung epithelial cells [25] in vitro, as well as in healthy human subjects [26].

Chronic ethanol consumption may also increase risk of oxidative stress in the respiratory system. A large amount of ingested alcohol reaches the lungs, where it is metabolised through the cytochrome P-450 system, generating ROS as side products [27]. Alcohol that diffuses and vaporises into the airways is deposited back into the airway lining, increasing exposure of the airway epithelium to alcohol [28]. As such, alcohol abuse is associated with increased risk of acute respiratory distress syndrome (ARDS) and susceptibility to respiratory infections [29]. While few studies have investigated the mechanisms by which this occurs in human subjects, ethanol has been shown to increase oxidative stress in human airway smooth muscle cell cultures [27]. In rat models, ethanol ingestion increased oxidant levels and decreased antioxidant levels in lung tissue, leading to alveolar degeneration and leukocyte infiltration [28, 30].

Air pollution is another risk factor for oxidative stress. Air pollutants comprise a wide range of gases such as nitrogen oxides, sulphur dioxide and ozone, aerosols, as well as particulate matter (PM), particularly fine PMs (diameter < 2.5 μm; PM2.5). While the mechanisms for each pollutant differ, air pollutants generally produce ROS directly within airway or lung tissue or induce inflammatory reactions that in turn produce ROS [31]. For example, polycyclic aromatic hydrocarbons are converted by the P450 system to quinones that act as oxidants [2], and ozone exposure is associated with greater oxidant status in bronchiolar epithelium [32] resulting in increased respiratory mortality, decreased lung function, and COPD hospitalization [33]. PMs containing transition metals can produce hydroxyl radicals via Fenton-type reactions, while silica PMs induce apoptosis in alveolar macrophages [34]. Exposure to such PMs is linked to increased risk of lung cancer and respiratory death [35].

Finally, occupational hazards can increase workers' risk of oxidative stress and respiratory pathology. Occupations that increase exposure to the air pollutants discussed above will inherently increase risk of oxidative stress. Studies have reported higher oxidant status in sewage workers due to inhalation of dust and gaseous pollutants [36], welders exposed to respirable dust in welding fumes [37], sugarcane workers during burnt sugarcane harvesting [38], and ceramic workers exposed to silica dust [39]. Asbestos exposure in many professions is also well established as a cause of pulmonary fibrosis (asbestosis) and lung cancer [40]. It has been suggested that inhaled asbestos fibres trigger a strong inflammatory response and also complex with iron to catalyse ROS formation, thus increasing oxidative stress in alveolar epithelial cells [41].

5.1.4 Genetic Risk Factors

The genetic contribution to risk of disease is most well established in lung cancer. In the gene encoding paraoxonase-1, an antioxidant enzyme, the R662S GG genotype [42] and Q192R QR and RR genotypes [43] are associated with increased risk of lung cancer. In addition, the R662S GG genotype was associated with increased level of 8-hydroxy-2′-deoxyguanosine (8-OHdG), predominant form of free radical-

induced oxidative lesions detected in urine, demonstrating a genetic predisposition to increased oxidative stress [42]. Other polymorphisms associated with increased risk of lung cancer include the C47T polymorphism in superoxide dismutase 2 (SOD2) [23] and the m1 CC and m2 AG genotypes in the gene encoding CYP1A1, an ROS-producing enzyme [44]. In patients with COPD, single nucleotide polymorphisms in the genes encoding IDH3B and SOD3, both antioxidant enzymes, are also associated with greater rate of decline in forced expiratory volume [45]. It is thus apparent that polymorphisms in genes related to oxidative stress may predispose individuals to developing oxidative stress-induced respiratory disease.

5.2 Oxidative Stress in Respiratory Diseases

5.2.1 Asthma

Asthma is a chronic condition characterised by airway inflammation, remodelling, and hyperresponsiveness, leading to recurrent exacerbations with symptoms such as wheezing and breathlessness [46]. In allergic asthma, the most common form of asthma, exacerbations are triggered by allergens, which activate IgE-dependent mechanisms to release histamine, and inflammatory mediators and recruit high numbers of eosinophils, degranulated mast cells, and Th2 lymphocytes to the airways [47].

In asthma, oxidative stress may induce production of inflammatory mediators and contribute to the chronic inflammation [47] (*pathway 5*). In addition, oxidative stress is associated with bronchoconstriction and airway hyperresponsiveness [48]. While the pathways here have not been entirely characterised, studies in human airway smooth muscle cells suggest that ROS activate calcium channels to increase airway contractility [49]. In addition, in vivo animal studies suggest that ROS cause hyperactivity of muscarinic receptors and beta-adrenergic receptors [50] and may increase Ca^{2+} transport in pulmonary microsomes resulting in increased smooth muscle contractility [51] and sustained airway constriction [52].

There is strong evidence for an association between oxidative stress and asthma. Studies on childhood-onset asthma have focused on oxidant and antioxidant statuses in plasma, urine, and exhaled breath condensate (EBC) in children. In plasma, asthmatic children have been shown to have higher total oxidant status [53], higher levels of malondialdehyde as a biomarker of oxidative stress, and decreased levels of reduced glutathione [54]. These findings suggest a systemic oxidant-antioxidant imbalance in asthmatic children. Moreover, in EBC, multiple studies have consistently found elevated malondialdehyde levels and decreased levels of reduced glutathione in asthmatic children, demonstrating oxidative stress localised to the airways [55, 56].

Similarly, in adults, plasma biomarkers of oxidative stress such as malondialdehyde, myeloperoxidase [47], clusterin [57], and 8-isoprostane [58] have been shown to be elevated in asthmatic patients. These levels were positively correlated with poor asthma control [58] and decreased pulmonary function

[57]. Total antioxidant capacity, which is measured by challenging the ability of a sample of plasma to reduce an oxidant substrate, has also been found to be decreased in such patients [59]. In EBC, asthmatic patients have increased concentrations of hydrogen peroxide [60, 61] and 8-isoprostane [62], as well as decreased levels of reduced glutathione [63].

Lastly, multiple factors that induce oxidative stress have been linked to higher rates of asthma in children and adults. These factors include chronic exposure to irritants in the workplace [64], decreased dietary intake of antioxidants [65], and exposure to traffic-associated air pollution [66].

5.2.2 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease characterised by chronic bronchitis, inflammation of the airways and emphysema, and destruction of lung parenchyma, resulting in irreversible limitation of airflow [67]. In patients with COPD, lung cells enter a senescent state and secrete pro-inflammatory cytokines, similar to what is observed in natural lung ageing [68]. COPD is predominantly caused by cigarette smoking, with active smoking being associated with COPD severity [69], but COPD may also be caused or exacerbated by environmental factors such as exposure to coal dust [70], aromatic solvents, and biomass fuels [71]. Genetic factors such as alpha-1 antitrypsin deficiency can also lead to early-onset COPD [72].

The role of oxidative stress has been well established in the pathogenesis of COPD. In susceptible individuals, exposure to risk factors such as dusts and gaseous irritants induces local inflammation. In particular, cigarette smoke, which is the main risk factor for developing COPD, contains ROS which react with the plasma membranes of airway epithelial cells, activating Toll-like receptor (TLR) signalling and NF-κB [73]. This induces expression of pro-inflammatory genes and production of profibrotic mediators such as TGF-β [74]. Expression of epithelial growth factor receptors is also upregulated, resulting in mucus hypersecretion, to obstruct the lumen of small airways [75].

Inflammatory mediators released by ROS exposure stimulate neutrophils and alveolar macrophages (*pathway 5*), which release proteolytic enzymes such as MMPs, resulting in the destruction of pulmonary parenchyma [76]. Moreover, in COPD patients, alveolar macrophages secrete more inflammatory proteins with greater elastolytic activity, suggesting that oxidative stress induces greater activation of macrophages compared to other physiological stimuli [74].

Cigarette smoke also induces production of endogenous ROS from the electron transfer chain in the mitochondria, resulting in an excess of ROS in mitochondria and elevated oxidative stress. This causes oxidative damage to mtDNA and impaired mitochondrial function [76] associated with lung cell apoptosis as seen in emphysema and cellular senescence characteristic of COPD [77].

Finally, ROS may have a significant effect on DNA in airway tissue. Increased oxidative stress impairs telomerase activity, leading to telomere shortening. COPD

patients have been shown to have significantly shorter telomere length, associated with lower levels of the antioxidant enzyme superoxide dismutase [78]. Telomere shortening activates p21 (a major target of p53 activity and a regulator of cell cycle progression), resulting in cellular senescence and further release of inflammatory mediators [68]. ROS also decrease levels and activities of histone deacetylases (HDACs), which regulate DNA expression. Decreased activity of HDAC2 results in cellular senescence, while decreased activity of Sirtuin 1 (HDAC3) is associated with increased p53 acetylation and apoptosis [79] (*pathway 4*). This may contribute to emphysema characteristic of COPD.

Multiple studies have demonstrated elevated oxidative stress in COPD patients. COPD patients exhibit higher concentrations of hydrogen peroxide in EBC [80], higher levels of malondialdehyde in sputum [81], and lower levels of glutathione in sputum [82] and epithelial lining fluid, correlating with the degree of airflow limitation [83]. Lung tissue biopsies also showed significant 8-OHdG formation in COPD patients, confirming a large increase in oxidative stress localised to the respiratory system in COPD [84]. In plasma and serum, total oxidant status [85], ischemia-modified albumin, oxidised low-density lipoprotein [86], 8-OHdG [87], and malondialdehyde [88] were significantly elevated in COPD patients, while levels of antioxidants such as ascorbic acid, lycopene, carotenoids [89], reduced glutathione, superoxide dismutase [90], and paraoxonase-1 [91] were decreased. Oxidative stress markers also negatively correlated with forced expiratory volume, indicating an association between oxidative stress and severity of COPD [92]. Overall, these findings point to an elevation in oxidative stress both locally in pulmonary tissue and throughout the body in COPD.

5.2.3 Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder, caused by mutations in a gene on chromosome 7 encoding cystic fibrosis transmembrane regulator (CFTR), a membrane chloride channel [93]. The lungs are the most severely affected by these mutations. In airway epithelial cells, CFTR mutations result in chloride impermeability leading to production of thick, sticky mucus which obstructs the airways. This enables bacterial colonization of the airways by pathogens such as *Pseudomonas*, *Haemophilus influenzae*, and *Staphylococcus aureus,* which in turn induces local inflammation [94].

Oxidative stress plays an important role in the pathogenesis of CF. Chronic inflammation in CF results in increased infiltration of neutrophils into lung tissue [95], which then produce ROS and oxidised proteins. This results in elevated oxidative stress associated with greater lipid peroxidation and cell damage [96], as well as activation of signalling pathways that culminate in induction of inflammatory genes, thus further contributing to inflammation in the respiratory system [97] (*pathway 2*). In children with CF, analysis of bronchoalveolar lavage fluid showed higher protein carbonyl levels correlating positively with neutrophil count and

inversely to lung function, suggesting that neutrophils may be responsible for elevated oxidative stress and damage to the airway epithelium in CF [98].

In addition, CFTR is involved in the transmembrane transport of reduced glutathione (GSH), a key antioxidant [97]. CFTR mutations directly impair GSH transport into extracellular fluid. As GSH catalyses cleavage of disulphide bonds in mucus, GSH deficiency may impair mucolysis and contribute to the build-up of mucus characteristic of CF [96]. Studies of CFTR-deficient cell lines have shown lower GSH content in apical fluid despite similar intracellular GSH levels compared to controls, suggesting a decrease in GSH efflux [99].

Elevated oxidative stress has been demonstrated in CF patients in several studies. CF patients show higher malondialdehyde levels in sputum [100], higher levels of CO [101] and 8-isoprostane [102] in EBC, and lower concentrations of glutathione in bronchoalveolar lavage [103] and EBC [104]. In plasma, levels of 8-isoprostane [105] and malondialdehyde are also raised [100], and malondialdehyde levels increased with respiratory exacerbations [106], reduced pulmonary function, and CF severity [107]. Levels of antioxidants such as vitamin C, beta-carotene [105], and superoxide dismutase [108] were also decreased in plasma [109].

5.2.4 Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is an illness with a high morbidity and mortality burden, characterised by severe pulmonary oedema leading to progressive hypoxemia [110]. Unlike the diseases discussed in prior sections, the causes of ARDS are less defined. It may result from acute lung injury such as pneumonia and inhalation of harmful substances or indirect causes such as sepsis and pancreatitis, resulting in alveolar capillary injury that then triggers inflammation and increased alveolar capillary permeability [111]. As a result of inflammation, large numbers of neutrophils infiltrate lung tissue [112].

Oxidative stress is thought to be an important mediator of alveolar capillary injury in ARDS. ROS may be produced as a result of hypoxemia or activated neutrophils and can damage the phospholipid membrane and increase permeability (*pathway 1*). In one study, healthy plasma incubated with stimulated polymorphonuclear leukocytes showed a similar pattern of decreased antioxidant levels compared to plasma from ARDS patients, as well as increased levels of lipid peroxides suggesting that the immune response plays a key role in generating oxidative stress and lipid damage [113]. ROS may also downregulate expression of the sodium-potassium pump and epithelial sodium channel, which decreases the clearance of alveolar liquid across the epithelium and contributes to pulmonary oedema [114].

At the nuclear level, ROS such as hydrogen peroxide can decrease transcription of Nrf2 [115], which regulates antioxidant production, apoptosis, and autophagy [116]. In addition, ROS signalling is involved in the regulation of HIF-1, and increased ROS production may upregulate hypoxia-inducible factor (HIF-1), resulting in the release of vascular endothelial growth factor (VEGF) (*pathway 2*).

This has been suggested to be an important mediator of endothelial barrier dysfunction and increased fluid entry into the alveoli [117].

Development of oxidative stress may provide the mechanism underlying the association between chronic alcohol abuse and ARDS. Chronic alcohol consumption has been shown to impair alveolar macrophage function, resulting in greater ROS production via increased activity of the ROS-producing enzymes NADPH oxidase-2 (Nox2) [118] and xanthine oxidoreductase [119]. In addition, alcohol exposure decreases levels of glutathione in alveolar epithelial lining fluid, resulting in greater susceptibility to oxidative stress [120].

There is strong evidence for the presence of oxidative stress during ARDS. In ARDS patients, levels of malondialdehyde and hydrogen peroxide are elevated, and levels of superoxide dismutase are lower in plasma and EBC [121]. Plasma levels of the antioxidants beta-carotene [122] and citrulline are also decreased [123]. Moreover, restoration of glutathione peroxidase via selenium administration improved airway resistance in ARDS patients, suggesting an association between oxidative stress due to antioxidant deficiency and development of ARDS [124].

5.2.5 Pneumonia

Pneumonia refers to an infection of the lung parenchyma when pathogens reach the alveoli and are not sufficiently controlled by host defences. Pneumonia may be divided into community-acquired pneumonia (CAP), acquired in a non-hospitalised population, or hospital-acquired pneumonia, most commonly ventilator-associated pneumonia (VAP), associated with mechanical ventilation [125]. The severity of pneumonia is dependent on both immune resistance, the ability of host defences to remove pathogens, and tissue resilience, the ability of lung tissues to tolerate the stress of infection [126].

Based on the close relationship between inflammation and oxidative stress and the inflammation that occurs in pneumonia, it is not unreasonable to expect that elevated oxidative stress also occurs in pneumonia. A study of pneumonia induced by lipopolysaccharide injection in rats showed higher ROS generation in the blood and lung tissue associated with greater neutrophil activation, suggesting that inflammation in pneumonia can induce local oxidative stress [127]. In addition, mouse models of viral pneumonia due to influenza A (H1N1) show higher levels of xanthine oxidase and 8-OHdG in the lung tissue [128].

Elevated oxidative stress may also occur due to factors released by pneumonia-causing pathogens. *Streptococcus pneumoniae*, the most common causal pathogen of pneumonia, has been shown to release hydrogen peroxide, resulting in lower reduced glutathione levels, Nrf2 activation, and increased oxidative stress in airway epithelial cells [129].

Susceptibility to pneumonia may also occur as a result of elevated oxidative stress. Welders exposed to ROS-inducing metal fumes have been shown to have higher risk of pneumonia. Studies in mice suggest that this association is mediated by an increase in lung platelet-activating factor receptor (PAFR) mRNA. As

S. pneumoniae adheres to PAFR to infect cells, upregulation of PAFR may result in greater susceptibility to pneumonia [130].

Patients with CAP have been shown to have increased ROS production due to upregulation of Nox2 and subsequent activation of polymorphonuclear leukocytes [131]. A separate study found higher serum isoprostanes associated with higher levels of bacterial endotoxins in serum, suggesting an association between pneumonia severity and level of oxidative stress [132]. Higher levels of malondialdehyde and lower levels of reduced glutathione, beta-carotene, vitamin C, vitamin E, and superoxide dismutase have also been observed in patients with CAP [133, 134]. Finally, higher levels of thiobarbituric acid reactant substances, a byproduct of lipid peroxidation and a measure of oxidative stress, were found in plasma and bronchoalveolar lavage samples from patients with VAP [135].

5.2.6 Lung Cancer

Lung cancer is the most common cancer and the leading cause of cancer-related death globally. Lung cancer can be divided into non-small cell lung cancer (NSCLC), which comprises 80–85% of cases, and small cell lung cancer [136].

It has been well established that oxidative stress is a major factor in carcinogenesis. Excessive ROS in cells can cause point mutations, single- or double-stranded DNA breaks, and DNA cross-linking (*pathway 3*). When such mutations occur in proto-oncogenes, this can act as a driving force for tumorigenesis [137, 138]. Significantly higher DNA oxidative modifications have been observed in lung tissue from patients with lung cancer [139], and levels of oxidatively damaged DNA in the blood show high sensitivity and specificity for lung cancer [140]. Hence, the effect of oxidative stress on changes at the genetic level cannot be understated.

As endogenous ROS usually act as intracellular signals, excessive ROS can also indirectly influence expression of genes by overstimulation of signalling pathways. Mitogen-activated protein kinase (MAPK) pathways can be overactivated in this fashion, leading to cellular proliferation [137]. Similarly, ROS-mediated overactivation of NF-κB leads to the production of inflammatory mediators and cell cycle proteins such as cyclins D1, D2, D3, and E1 and cyclin-dependent kinases, further contributing to proliferation, and upregulation of VEGF can lead to angiogenesis, a hallmark of cancer (*pathway 2*). In lung cancer patients, VEGF levels have been shown to be elevated in serum and correlated with serum markers of oxidative stress [141].

Other ROS-mediated changes at the genetic level include telomere shortening, which results in genomic instability and susceptibility to chromosomal breaks and mutation [2], and epigenetic changes in the form of DNA methylation and histone modification. For example, oxidative stress may downregulate the HDAC SIRT1 (from the histone deacetylase superfamily, Sirtuins) in the lung tissue, which results in upregulation of NF-κB, and downregulation of the oxidative stress sensor KEAP1 by CpG methylation, which results in cellular susceptibility to stress [142] (*pathway*

4). Ultimately, the net effect of oxidative stress in lung cells is a continuous accumulation of genetic changes that drive tumorigenesis [143].

Finally, oxidative stress can damage and alter cellular proteins. ROS have been shown to damage tumour suppressor proteins, resulting in decreased apoptosis and increased proliferation [137] (*pathway 2*). Recently, it has also been reported that ROS can alter proteins in lung tumour cells to produce novel epitopes to avoid phagocytosis, promoting survival of the tumour [144].

No discussion of lung cancer would be complete without consideration of its risk factors. Multiple environmental factors are associated with significantly increased risk of lung cancer, and these risk factors are also associated with increased oxidative stress. Notably, tobacco smoking, which is one of the most significant risk factors for lung cancer [145], is associated with higher carbonyl levels, lower levels of superoxide dismutase in plasma of lung cancer patients [146], and higher levels of 8-OHdG levels in airways of lung cancer patients correlating with smoking index [8]. Inhalation of organophosphate pesticides, another risk factor, is also associated with higher ROS generation and impaired DNA repair pathways in lung cancer cell lines [147].

Innumerable studies have demonstrated the presence of oxidative stress in lung cancer. In plasma, malondialdehyde levels and catalase activity are elevated and correspond with advancing stage of lung cancer, while glutathione peroxidase activity, superoxide dismutase activity, and levels of beta-carotene, ascorbic acid, and glutathione are decreased [148–150]. Urine levels of 8-OHdG are also elevated in primary or metastatic lung cancer [151, 152].

5.2.7 Obstructive Sleep Apnoea

Obstructive sleep apnoea (OSA) is characterised by recurrent collapses of the upper airway during sleep. This may be due to reduced airway dimensions, lack of the pharyngeal protective reflex, increased airway resistance, increased pharyngeal collapsibility, or a combination thereof [153]. Partial or complete collapse of the airway results in intermittent episodes of hypoxia, which plays a significant role in the development of comorbidities such as cardiovascular disease, excessive daytime sleepiness [153], endocrine dysfunction, and increased risk of vehicle accidents [154].

Oxidative stress may play both the roles of cause and consequence of OSA. ROS in the upper airway can activate neutrophils and induce release of inflammatory mediators (*pathway 4*), resulting in vasodilation, squamous cell hyperplasia, and blunting of the physiological dilator reflex [155], leading to narrowing of the upper airway and increased risk of upper airway collapse. The intermittent hypoxia caused by recurrent airway collapse is analogous to repeated ischemia and reperfusion of tissues [3]. This is thought to result in systemic production of ROS [156], thus further contributing to oxidative stress.

Oxidative stress caused by intermittent hypoxia activates NF-κB (*pathway 2*), which induces inflammation in the arterial wall and endothelial dysfunction

[156]. Endothelial dysfunction is considered a precursor lesion of atherosclerosis [156] and may also lead to vasoconstriction and smooth muscle hyperplasia, manifesting as hypertension [157]. Moreover, ROS produced in the aorta may lead to aortic remodelling [158], which is a risk factor for stroke and aortic dissection [159].

There is strong evidence for systemic oxidative stress in OSA. In serum, patients with OSA have been shown to have higher levels of malondialdehyde [160], thioredoxin [161], superoxide radical [162], hypoxanthine [163], advanced oxidation protein products, and total oxidant status, with lower total antioxidative capacity [164] and serum native thiol levels [165]. Urinary isoprostanes [166] and 8-hydroxy-2′-deoxyguanosine (8-OHdG) [167] were also elevated in patients with OSA. Moreover, thioredoxin [161] and hypoxanthine [163] were significantly and positively correlated with apnoea hypopnea index (AHI), an indicator of OSA severity, and plasma markers of oxidative stress were lowered after treatment of OSA [168]. These studies indicate a strong association between OSA pathophysiology and systemic oxidative stress. What remain less clear, perhaps, are the level of oxidative stress localised to the upper airway and its role in causing upper airway collapse. Patients with OSA have been shown to have higher levels of exhaled malondialdehyde [169] and 8-isoprostane [170], but it is not known whether this was due to local oxidative stress in the upper airways or simply a reflection of systemic oxidative stress.

5.3 Biomarkers of Oxidative Stress in Respiratory Diseases

A clinically useful biomarker is a substance, measurement, or process that shows specificity for a certain disease, has prognostic value, and/or correlates with disease activity. Numerous molecules have been investigated as putative biomarkers of oxidative stress in respiratory disease.

Firstly, the reaction of ROS with lipids produces aldehydes, which further react with proteins to produce advanced lipoxidation end products. One such aldehyde is malondialdehyde, which is one of the most commonly investigated markers of oxidative stress. While multiple studies have demonstrated an increase in serum malondialdehyde in COPD [88, 171], OSA [160], CF [100], and ARDS [121], it has been suggested that malondialdehyde lacks sensitivity as it is generally protein-bound and often undetected [172]. Other oxidation products include isoprostanes formed by oxidation of arachidonic acid, among which F2-isoprostanes are considered the most reliable markers of oxidative stress [172]. 8-Isoprostane, an F2-isoprostane, has been shown to be increased in EBC in COPD [173], CF [104], and asthma [174, 175]. In COPD, 8-isoprostane levels did not correlate with forced expiration volume and thus did not reflect disease severity [173], but in asthma, levels correlated with cysteinyl leukotrienes, markers of allergic inflammation [175]. In addition, in urine, 8-isoprostane levels were elevated in patients with lung cancer and correlated with higher risk of lung cancer [151]. Thus,

8-isoprostane in EBC or urine is a promising biomarker in respiratory disease and warrants greater investigation into its correlation with disease severity or treatment.

8-OHdG, the main result of ROS-induced DNA damage [176], is also used as a marker of oxidative stress. 8-OHdG levels in peripheral blood mononuclear cells have been shown to be elevated in COPD and negatively correlate with lung function, with good correlation with oxidative DNA damage in lung tissue [84]. Moreover, 8-OHdG levels demonstrate high sensitivity and specificity for lung cancer [140]. 8-OHdG levels are also elevated in urine following oxidative damage due to asbestos exposure [177]. As such, 8-OHdG may prove to be a useful biomarker in respiratory disease. Its use as a clinical biomarker is presently limited by the need for chromatography with mass spectrometry to reliably quantify 8-OHdG levels, but this may change with the advent of new technologies [176].

Protein products of genes regulated by ROS can also reflect changes in the cellular oxidative state. As discussed in previous sections, NF-κB and HIF-1α are signalling factors involved in inflammation, and oxidative stress is known to change expression of these factors. In patients with OSA, NF-κB and HIF-1α protein levels were shown to be elevated and correlated with disease severity [158].

Finally, as oxidative stress inherently causes an imbalance in the oxidant/antioxidant ratio, decreased levels of antioxidants may act as markers for oxidative stress. GSH, the main antioxidant in lung epithelial lining fluid, is sensitive to oxidative stress [178]. GSH levels are decreased in patients with CF, especially in lobes with structural disease, and negatively correlate with disease scores [179]. In patients with lung cancer, low levels of GSH have been suggested to reflect tumour aggressiveness [136]. GSH thus shows potential as a clinical biomarker of respiratory disease, but care should be taken during interpretation of GSH levels as they may be influenced by nutritional deficiencies in cysteine [172].

5.4 Therapeutics Targeting Oxidative Stress

Oxidative stress plays an important role in the pathophysiology of many respiratory diseases, in which case antioxidant therapies may be of use. Most attention has been focused on the small molecule thiol drug *N*-acetyl-L-cysteine (NAC). NAC reduces disulphide bonds in proteins, resulting in a mucolytic action [180]. While NAC has been in use as a mucolytic for many decades, it is also thought to have antioxidative activity by scavenging free radicals [181]. In addition, NAC is a precursor to GSH and thus promotes GSH synthesis [182]. It may also scavenge free radicals and reduce disulphide bonds in proteins, resulting in a mucolytic action [180]. In the BRONCUS (Bronchitis Randomised on NAC Cost-Utility Study), 423 COPD patients were randomly assigned to NAC (600 mg oral daily) or placebo. NAC was not shown to affect the rate of decline of forced expiratory volume [183]. However, in the PANTHEON study with 1006 COPD patients randomly allocated to receive NAC (600 mg oral twice daily) or placebo, the rate of exacerbations was reduced [184]. Similarly, in the HIACE study, COPD patients given NAC (600 mg twice daily) showed increased forced expiratory volume and decreased exacerbation

frequency [185]. A meta-analysis of the evidence suggested that a high dose of NAC (>600 mg daily) may benefit COPD patients with confirmed airway obstruction, whereas a low dose of NAC may benefit patients with bronchitis but not airway obstruction [180]. In CF, a phase II randomised controlled trial demonstrated that oral NAC decreased the rate of lung function decline [186]. It remains to be seen whether NAC does play a role in the treatment of other respiratory diseases and whether its therapeutic effects are due to its antioxidant properties or its mucolytic effect. Other thiol drugs under investigation include procysteine, erdosteine [187], and carbocysteine, which were shown to decrease the exacerbation rate of COPD in a randomised controlled trial [188].

Direct administration of GSH by oral, intravenous, or inhalation routes has also been investigated. A randomised, single-blind controlled trial suggested that inhaled GSH increases forced expiratory volume over 12 months in patients with CF compared to placebo, but markers of oxidative stress in serum were not decreased [189]. A meta-analysis showed that evidence on the effectiveness of GSH is conflicting, and it remains uncertain whether improvements in patients are due to the antioxidant effects of GSH, other effects of GSH, or other concurrent treatment [190].

Another approach to antioxidant therapies is pharmacological activation of Nrf2 to promote endogenous antioxidant production. The Nrf2 activator sulforaphane has been shown to improve phagocytosis in vitro in alveolar macrophages taken from COPD patients [191] but was not shown to have any effect on Nrf2 expression or antioxidant levels during phase II clinical trials [192].

Finally, some studies have suggested dietary modification and supplementation to increase antioxidant levels in the body. Fruits and vegetables are rich in naturally occurring dietary antioxidants including polyphenols such as resveratrol, beta-carotene, and bioflavonoids. Resveratrol, found in grapes, has been the centre of much research as it has been shown to improve mitochondrial function, downregulate NF-κB, and activate Nrf2 [193] resulting in restoration of GSH levels in human airway epithelial cell lines exposed to cigarette smoke [194]. Generally, dietary antioxidants do not appear to be beneficial [182] due to their low bioavailability [187], and thus their use is limited to patients in whom dietary intake of antioxidants has been proven to be lacking. COPD patients who increased their intake of bioflavonoid-rich foods did not improve over 12 weeks but demonstrated increased forced expiratory volume over 3 years [195]. In addition, a pilot study of flaxseed supplementation in CF patients showed that patients with low lignan (a flaxseed metabolite) levels experienced a significant decrease in oxidative stress [196]. This suggests that the use of dietary antioxidants as a therapeutic may be limited to patients in whom disease pathology is a direct result of antioxidant deficiency.

Ultimately, the use of antioxidant therapy is still a relatively unexplored area, and much more research is needed to determine the role of antioxidants in treating lung disease.

5.5 Conclusion

The role of oxidative stress in the pathophysiology of respiratory disease cannot be understated. In COPD, and lung cancer, there is strong evidence for oxidative stress being the cause of pathology. In diseases such as OSA, asthma, and CF, while oxidative stress may not be the root cause, disease progression generally involves oxidative stress as a key step. Thus, it is important to recognise and target oxidative stress in the treatment of such diseases. While some antioxidant-based therapeutics such as NAC and resveratrol have some evidence for efficacy in treating respiratory disease, there is still a dearth of evidence, and this remains an avenue for further research.

References

1. Park HS, Kim SR, Lee YC (2009) Impact of oxidative stress on lung diseases. Respirology 14 (1):27–38
2. Valavanidis A et al (2013) Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health 10(9):3886–3907
3. Lavie L (2015) Oxidative stress in obstructive sleep apnea and intermittent hypoxia—revisited—the bad ugly and good: implications to the heart and brain. Sleep Med Rev 20:27–45
4. Rahman I, Adcock IM (2006) Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J 28(1):219–242
5. Papac-Milicevic N, Busch CJ, Binder CJ (2016) Malondialdehyde epitopes as targets of immunity and the implications for atherosclerosis. Adv Immunol 131:1–59
6. Santus P et al (2014) Oxidative stress and respiratory system: pharmacological and clinical reappraisal of N-acetylcysteine. COPD 11(6):705–717
7. Cai Z, Yan LJ (2013) Protein oxidative modifications: beneficial roles in disease and health. J Biochem Pharmacol Res 1(1):15–26
8. Cao C et al (2016) Smoking-promoted oxidative DNA damage response is highly correlated to lung carcinogenesis. Oncotarget 7(14):18919–18926
9. Szczesny B et al (2018) Mitochondrial DNA damage and subsequent activation of Z-DNA binding protein 1 links oxidative stress to inflammation in epithelial cells. Sci Rep 8(1):914
10. Liu X, Chen Z (2017) The pathophysiological role of mitochondrial oxidative stress in lung diseases. J Transl Med 15(1):207
11. Rahman I (2002) Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. Biochem Pharmacol 64(5–6):935–942
12. Mittal M et al (2014) Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 20(7):1126–1167
13. Racanelli AC et al (2018) Autophagy and inflammation in chronic respiratory disease. Autophagy 14(2):221–232
14. Barnes PJ (2014) Cellular and molecular mechanisms of chronic obstructive pulmonary disease. Clin Chest Med 35(1):71–86
15. Liu W et al (2012) Targeted genes and interacting proteins of hypoxia inducible factor-1. Int J Biochem Mol Biol 3(2):165–178
16. Tebay LE et al (2015) Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radic Biol Med 88(Pt B):108–146

17. Ellegaard PK, Poulsen HE (2016) Tobacco smoking and oxidative stress to DNA: a meta-analysis of studies using chromatographic and immunological methods. Scand J Clin Lab Invest 76(2):151–158
18. Pryor WA, Stone K (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann N Y Acad Sci 686:12–27; discussion 27–28
19. Goldkorn T, Filosto S, Chung S (2014) Lung injury and lung cancer caused by cigarette smoke-induced oxidative stress: molecular mechanisms and therapeutic opportunities involving the ceramide-generating machinery and epidermal growth factor receptor. Antioxid Redox Signal 21(15):2149–2174
20. Chen JH et al (2005) Gaseous nitrogen oxide repressed benzo[a]pyrene-induced human lung fibroblast cell apoptosis via inhibiting JNK1 signals. Arch Toxicol 79(12):694–704
21. Sun Y et al (2015) Enhancement of the acrolein-induced production of reactive oxygen species and lung injury by GADD34. Oxidative Med Cell Longev 2015:170309
22. Wang HT et al (2016) Acrolein preferentially damages nucleolus eliciting ribosomal stress and apoptosis in human cancer cells. Oncotarget 7(49):80450–80464
23. Li N, Huang HQ, Zhang GS (2014) Association between SOD2 C47T polymorphism and lung cancer susceptibility: a meta-analysis. Tumour Biol 35(2):955–959
24. Scheffler S et al (2015) Evaluation of E-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells. Int J Environ Res Public Health 12(4):3915–3925
25. Lerner CA et al (2015) Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PLoS One 10(2):e0116732
26. Cai H, Wang C (2017) Graphical review: the redox dark side of e-cigarettes; exposure to oxidants and public health concerns. Redox Biol 13:402–406
27. Kaphalia L et al (2016) Effects of acute ethanol exposure on cytokine production by primary airway smooth muscle cells. Toxicol Appl Pharmacol 292:85–93
28. Das SK, Mukherjee S (2010) Long-term ethanol consumption leads to lung tissue oxidative stress and injury. Oxidative Med Cell Longev 3(6):414–420
29. Yeligar SM et al (2016) Alcohol and lung injury and immunity. Alcohol 55:51–59
30. Aytacoglu BN et al (2006) Alcohol-induced lung damage and increased oxidative stress. Respiration 73(1):100–104
31. Holguin F (2013) Oxidative stress in airway diseases. Ann Am Thorac Soc 10(Suppl):S150–S157
32. Sunil VR et al (2013) Ozone-induced injury and oxidative stress in bronchiolar epithelium are associated with altered pulmonary mechanics. Toxicol Sci 133(2):309–319
33. Pirozzi C et al (2015) Effect of naturally occurring ozone air pollution episodes on pulmonary oxidative stress and inflammation. Int J Environ Res Public Health 12(5):5061–5075
34. Gawda A et al (2017) Air pollution, oxidative stress, and exacerbation of autoimmune diseases. Cent Eur J Immunol 42(3):305–312
35. Deng X et al (2013) PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. Toxicol In Vitro 27(6):1762–1770
36. Shadab M et al (2014) Occupational health hazards among sewage workers: oxidative stress and deranged lung functions. J Clin Diagn Res 8(4):BC11–BC12
37. Li N et al (2015) Influenza infection induces host DNA damage and dynamic DNA damage responses during tissue regeneration. Cell Mol Life Sci 72(15):2973–2988
38. Prado GF et al (2012) Burnt sugarcane harvesting: particulate matter exposure and the effects on lung function, oxidative stress, and urinary 1-hydroxypyrene. Sci Total Environ 437:200–208
39. Anlar HG et al (2017) Effects of occupational silica exposure on OXIDATIVE stress and immune system parameters in ceramic workers in TURKEY. J Toxicol Environ Health A 80 (13–15):688–696

40. Gilham C et al (2016) Pleural mesothelioma and lung cancer risks in relation to occupational history and asbestos lung burden. Occup Environ Med 73(5):290–299
41. Liu G, Cheresh P, Kamp DW (2013) Molecular basis of asbestos-induced lung disease. Annu Rev Pathol 8:161–187
42. Eom SY et al (2015) Interactions between paraoxonase 1 genetic polymorphisms and smoking and their effects on oxidative stress and lung cancer risk in a Korean population. PLoS One 10 (3):e0119100
43. Wang H et al (2012) Association of genetic polymorphisms in the paraoxonase 1 gene with the risk and prognosis of non-small cell lung cancer in Chinese Han population. J Investig Med 60 (3):592–597
44. Peddireddy V et al (2016) Association of CYP1A1, GSTM1 and GSTT1 gene polymorphisms with risk of non-small cell lung cancer in Andhra Pradesh region of South India. Eur J Med Res 21:17
45. Tang W et al (2013) Genetic variation in antioxidant enzymes, cigarette smoking, and longitudinal change in lung function. Free Radic Biol Med 63:304–312
46. James AL, Wenzel S (2007) Clinical relevance of airway remodelling in airway diseases. Eur Respir J 30(1):134–155
47. Kleniewska P, Pawliczak R (2017) The participation of oxidative stress in the pathogenesis of bronchial asthma. Biomed Pharmacother 94:100–108
48. Lazrak A et al (2015) Hyaluronan mediates airway hyperresponsiveness in oxidative lung injury. Am J Physiol Lung Cell Mol Physiol 308(9):L891–L903
49. Gorlach A et al (2015) Calcium and ROS: a mutual interplay. Redox Biol 6:260–271
50. van Hoof IH, van Bree L, Bast A (1996) Changes in receptor function by oxidative stress in Guinea pig tracheal smooth muscle. Cent Eur J Public Health 4(Suppl):3–5
51. Menshikova EV et al (1995) Pulmonary microsomes contain a Ca^{2+}-transport system sensitive to oxidative stress. Biochim Biophys Acta Bioenergetics 1228(2–3):165–174
52. Lai Y-L, Huang P-C (2002) Reactive oxygen species in sustained airway constriction induced by citric acid aerosol inhalation. Eur J Pharmacol 452(2):229–233
53. Emin O, Hasan A, Rusen DM (2015) Plasma paraoxonase, oxidative status level, and their relationship with asthma control test in children with asthma. Allergol Immunopathol (Madr) 43(4):346–352
54. Ercan H et al (2006) Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. J Allergy Clin Immunol 118(5):1097–1104
55. Dut R et al (2008) Oxidative stress and its determinants in the airways of children with asthma. Allergy 63(12):1605–1609
56. Celik M et al (2012) Oxidative stress in the airways of children with asthma and allergic rhinitis. Pediatr Allergy Immunol 23(6):556–561
57. Kwon HS et al (2014) Clusterin expression level correlates with increased oxidative stress in asthmatics. Ann Allergy Asthma Immunol 112(3):217–221
58. Andrianjafimasy M et al (2017) Oxidative stress biomarkers and asthma characteristics in adults of the EGEA study. Eur Respir J 50(6):1701193
59. Fatani SH (2014) Biomarkers of oxidative stress in acute and chronic bronchial asthma. J Asthma 51(6):578–584
60. Emelyanov A et al (2001) Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients. Chest 120(4):1136–1139
61. Ganas K et al (2001) Total nitrite/nitrate in expired breath condensate of patients with asthma. Respir Med 95(8):649–654
62. Montuschi P et al (1999) Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. Am J Respir Crit Care Med 160(1):216–220
63. Kelly FJ et al (1999) Altered lung antioxidant status in patients with mild asthma. Lancet 354 (9177):482–483
64. Dumas O, Le Moual N (2016) Do chronic workplace irritant exposures cause asthma? Curr Opin Allergy Clin Immunol 16(2):75–85

65. Moreno-Macias H, Romieu I (2014) Effects of antioxidant supplements and nutrients on patients with asthma and allergies. J Allergy Clin Immunol 133(5):1237–44; quiz 1245
66. Delfino RJ et al (2013) Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. J Expo Sci Environ Epidemiol 23(5):466–473
67. Cosio Piqueras MG, Cosio MG (2001) Disease of the airways in chronic obstructive pulmonary disease. Eur Respir J 18(1):41–49
68. MacNee W (2016) Is chronic obstructive pulmonary disease an accelerated aging disease? Ann Am Thorac Soc 13(Suppl 5):S429–S437
69. Riesco JA et al (2017) Active smoking and COPD phenotype: distribution and impact on prognostic factors. Int J Chron Obstruct Pulmon Dis 12:1989–1999
70. Santo Tomas LH (2011) Emphysema and chronic obstructive pulmonary disease in coal miners. Curr Opin Pulm Med 17(2):123–125
71. Kim V, Criner GJ (2015) The chronic bronchitis phenotype in chronic obstructive pulmonary disease: features and implications. Curr Opin Pulm Med 21(2):133–141
72. Anzueto A (2015) Alpha-1 antitrypsin deficiency-associated chronic obstructive pulmonary disease: a family perspective. COPD 12(4):462–467
73. Bialas AJ et al (2016) The role of mitochondria and oxidative/antioxidative imbalance in pathobiology of chronic obstructive pulmonary disease. Oxidative Med Cell Longev 2016:7808576
74. Barnes PJ (2016) Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 138(1):16–27
75. Gao W et al (2015) Bronchial epithelial cells: the key effector cells in the pathogenesis of chronic obstructive pulmonary disease? Respirology 20(5):722–729
76. Jiang Y, Wang X, Hu D (2017) Mitochondrial alterations during oxidative stress in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 12:1153–1162
77. Michaeloudes C et al (2017) Dealing with stress: defective metabolic adaptation in chronic obstructive pulmonary disease pathogenesis. Ann Am Thorac Soc 14(Suppl_5):S374–S382
78. Houben JM et al (2009) Telomere shortening in chronic obstructive pulmonary disease. Respir Med 103(2):230–236
79. Sundar IK, Yao H, Rahman I (2013) Oxidative stress and chromatin remodeling in chronic obstructive pulmonary disease and smoking-related diseases. Antioxid Redox Signal 18 (15):1956–1971
80. Dekhuijzen PN et al (1996) Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 154(3 Pt 1):813–816
81. Antus B et al (2014) Monitoring oxidative stress during chronic obstructive pulmonary disease exacerbations using malondialdehyde. Respirology 19(1):74–79
82. Zeng M et al (2013) Local and systemic oxidative stress status in chronic obstructive pulmonary disease patients. Can Respir J 20(1):35–41
83. Numakura T et al (2017) Production of reactive persulfide species in chronic obstructive pulmonary disease. Thorax 72(12):1074–1083
84. Yang S et al (2014) Feasibility of 8-OHdG formation and hOGG1 induction in PBMCs for assessing oxidative DNA damage in the lung of COPD patients. Respirology 19 (8):1183–1190
85. Ekin S et al (2017) The relationships among the levels of oxidative and antioxidative parameters, FEV1 and prolidase activity in COPD. Redox Rep 22(2):74–77
86. Can U, Yerlikaya FH, Yosunkaya S (2015) Role of oxidative stress and serum lipid levels in stable chronic obstructive pulmonary disease. J Chin Med Assoc 78(12):702–708
87. Liu X et al (2019) 8-Hydroxy-2′-deoxyguanosine as a biomarker of oxidative stress in acute exacerbation of chronic obstructive pulmonary disease. Turk J Med Sci 49(1):93–100
88. Cristovao C et al (2013) Evaluation of the oxidant and antioxidant balance in the pathogenesis of chronic obstructive pulmonary disease. Rev Port Pneumol 19(2):70–75

89. Kodama Y et al (2017) Antioxidant nutrients in plasma of Japanese patients with chronic obstructive pulmonary disease, asthma-COPD overlap syndrome and bronchial asthma. Clin Respir J 11(6):915–924
90. Arja C et al (2013) Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease. Respirology 18(7):1069–1075
91. Rumora L et al (2014) Paraoxonase 1 activity in patients with chronic obstructive pulmonary disease. COPD 11(5):539–545
92. Ahmad A, Shameem M, Husain Q (2013) Altered oxidant-antioxidant levels in the disease prognosis of chronic obstructive pulmonary disease. Int J Tuberc Lung Dis 17(8):1104–1109
93. Ratjen F, Döring G (2003) Cystic fibrosis. Lancet 361(9358):681–689
94. Brown SD, White R, Tobin P (2017) Keep them breathing: cystic fibrosis pathophysiology, diagnosis, and treatment. JAAPA 30(5):23–27
95. Rafeeq MM, Murad HAS (2017) Cystic fibrosis: current therapeutic targets and future approaches. J Transl Med 15(1):84
96. Hudson VM (2001) Rethinking cystic fibrosis pathology: the critical role of abnormal reduced glutathione (GSH) transport caused by CFTR mutation. Free Radic Biol Med 30 (12):1440–1461
97. Kleme ML, Levy E (2015) Cystic fibrosis-related oxidative stress and intestinal lipid disorders. Antioxid Redox Signal 22(7):614–631
98. Starosta V et al (2006) Oxidative changes of bronchoalveolar proteins in cystic fibrosis. Chest 129(2):431–437
99. Gao L et al (1999) Abnormal glutathione transport in cystic fibrosis airway epithelia. Am J Phys 277(1):L113–L118
100. Antus B et al (2015) Comparison of airway and systemic malondialdehyde levels for assessment of oxidative stress in cystic fibrosis. Lung 193(4):597–604
101. Paredi P et al (1999) Increased carbon monoxide in exhaled air of patients with cystic fibrosis. Thorax 54(10):917–920
102. Montuschi P et al (2000) Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. Thorax 55(3):205–209
103. Kettle AJ et al (2014) Oxidation contributes to low glutathione in the airways of children with cystic fibrosis. Eur Respir J 44(1):122–129
104. Spicuzza L et al (2018) Exhaled markers of antioxidant activity and oxidative stress in stable cystic fibrosis patients with moderate lung disease. J Breath Res 12(2):026010
105. Wood LG et al (2001) Oxidative stress in cystic fibrosis: dietary and metabolic factors. J Am Coll Nutr 20(2):157–165
106. McGrath LT et al (1999) Oxidative stress during acute respiratory exacerbations in cystic fibrosis. Thorax 54(6):518–523
107. Brown RK, Wyatt H, Price FJ, Kelly FJ (1996) Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress. Eur Respir J 9:334–339
108. Olveira C et al (2017) Inflammation and oxidation biomarkers in patients with cystic fibrosis: the influence of azithromycin. Eurasian J Med 49(2):118–123
109. Lagrange-Puget M et al (2004) Longitudinal study of oxidative status in 312 cystic fibrosis patients in stable state and during bronchial exacerbation. Pediatr Pulmonol 38(1):43–49
110. Tobin M, Manthous C (2017) What is acute respiratory distress syndrome? Am J Respir Crit Care Med 196(9):P17–P18
111. Yadam S, Bihler E, Balaan M (2016) Acute respiratory distress syndrome. Crit Care Nurs Q 39 (2):190–195
112. Ware LB, Matthay MA (2000) The acute respiratory distress syndrome. N Engl J Med 342 (18):1334–1349
113. Cross CE et al (1990) Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. J Lab Clin Med 115(4):396–404
114. Lucas R et al (2009) Regulators of endothelial and epithelial barrier integrity and function in acute lung injury. Biochem Pharmacol 77(12):1763–1772

115. Ning JL, Mo LW, Lai XN (2010) Low- and high-dose hydrogen peroxide regulation of transcription factor NF-E2-related factor 2. Chin Med J 123(8):1063–1069
116. Rojo de la Vega M et al (2016) Role of Nrf2 and autophagy in acute lung injury. Curr Pharmacol Rep 2(2):91–101
117. Tasaka S et al (2008) Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. Antioxid Redox Signal 10(4):739–753
118. Downs CA et al (2013) Chronic alcohol ingestion changes the landscape of the alveolar epithelium. Biomed Res Int 2013:470217
119. Fini MA et al (2017) Alcohol abuse is associated with enhanced pulmonary and systemic xanthine oxidoreductase activity. Am J Physiol Lung Cell Mol Physiol 313(6):L1047–L1057
120. Liang Y, Yeligar SM, Brown LA (2012) Chronic-alcohol-abuse-induced oxidative stress in the development of acute respiratory distress syndrome. ScientificWorldJournal 2012:740308
121. Liu D et al (2015) Changes in the concentrations of mediators of inflammation and oxidative stress in exhaled breath condensate during liver transplantation and their relations with postoperative ARDS. Respir Care 60(5):679–688
122. Nelson JL et al (2003) Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants on antioxidant status in patients with acute respiratory distress syndrome. JPEN J Parenter Enteral Nutr 27(2):98–104
123. Ware LB et al (2013) Low plasma citrulline levels are associated with acute respiratory distress syndrome in patients with severe sepsis. Crit Care 17(1):R10
124. Mahmoodpoor A et al (2019) The effect of intravenous selenium on oxidative stress in critically ill patients with acute respiratory distress syndrome. Immunol Investig 48 (2):147–159
125. Alcon A, Fabregas N, Torres A (2005) Pathophysiology of pneumonia. Clin Chest Med 26 (1):39–46
126. Mizgerd JP (2017) Pathogenesis of severe pneumonia: advances and knowledge gaps. Curr Opin Pulm Med 23(3):193–197
127. Serebrovska Z et al (2017) Anti-inflammatory and antioxidant effect of cerium dioxide nanoparticles immobilized on the surface of silica nanoparticles in rat experimental pneumonia. Biomed Pharmacother 92:69–77
128. Li H et al (2015) Oxidative stress, telomere shortening, and DNA methylation in relation to low-to-moderate occupational exposure to welding fumes. Environ Mol Mutagen 56 (8):684–693
129. Zahlten J et al (2015) Streptococcus pneumoniae-induced oxidative stress in lung epithelial cells depends on pneumococcal autolysis and is reversible by resveratrol. J Infect Dis 211 (11):1822–1830
130. Suri R et al (2016) Exposure to welding fumes and lower airway infection with Streptococcus pneumoniae. J Allergy Clin Immunol 137(2):527–534.e7
131. Violi F et al (2015) Nox2 up-regulation is associated with an enhanced risk of atrial fibrillation in patients with pneumonia. Thorax 70(10):961–966
132. Loffredo L et al (2016) Impaired flow-mediated dilation in hospitalized patients with community-acquired pneumonia. Eur J Intern Med 36:74–80
133. Cemek M et al (2006) Oxidative stress and enzymic-non-enzymic antioxidant responses in children with acute pneumonia. Cell Biochem Funct 24(3):269–273
134. Chen Y et al (2014) Vitamin C mitigates oxidative stress and tumor necrosis factor-alpha in severe community-acquired pneumonia and LPS-induced macrophages. Mediat Inflamm 2014:426740
135. Duflo F et al (2002) Alveolar and serum oxidative stress in ventilator-associated pneumonia. Br J Anaesth 89(2):231–236
136. Dirican N et al (2016) Thiol/disulfide homeostasis: a prognostic biomarker for patients with advanced non-small cell lung cancer? Redox Rep 21(5):197–203
137. Durham AL, Adcock IM (2015) The relationship between COPD and lung cancer. Lung Cancer 90(2):121–127

138. Ng Kee Kwong F et al (2017) Is mitochondrial dysfunction a driving mechanism linking COPD to nonsmall cell lung carcinoma? Eur Respir Rev 26(146). https://doi.org/10.1183/16000617.0040-2017
139. Gegotek A et al (2016) Lipid mediators involved in the oxidative stress and antioxidant defence of human lung cancer cells. Redox Biol 9:210–219
140. Barreiro E et al (2013) Oxidative stress and inflammation in the normal airways and blood of patients with lung cancer and COPD. Free Radic Biol Med 65:859–871
141. Katsabeki-Katsafli A et al (2008) Serum vascular endothelial growth factor is related to systemic oxidative stress in patients with lung cancer. Lung Cancer 60(2):271–276
142. Lawless MW, O'Byrne KJ, Gray SG (2009) Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy. J Cell Mol Med 13(9A):2800–2821
143. Ahmad S et al (2018) Oxidation, glycation and glycoxidation-the vicious cycle and lung cancer. Semin Cancer Biol 49:29–36
144. Upadhyay S, Vaish S, Dhiman M (2019) Hydrogen peroxide-induced oxidative stress and its impact on innate immune responses in lung carcinoma A549 cells. Mol Cell Biochem 450 (1–2):135–147
145. Bialous SA, Sarna L (2017) Lung cancer and tobacco: what is new? Nurs Clin North Am 52 (1):53–63
146. Margaret AL, Syahruddin E, Wanandi SI (2011) Low activity of manganese superoxide dismutase (MnSOD) in blood of lung cancer patients with smoking history: relationship to oxidative stress. Asian Pac J Cancer Prev 12(11):3049–3053
147. Thakur S, Dhiman M, Mantha AK (2018) APE1 modulates cellular responses to organophosphate pesticide-induced oxidative damage in non-small cell lung carcinoma A549 cells. Mol Cell Biochem 441(1–2):201–216
148. Peddireddy V et al (2012) Assessment of 8-oxo-7,8-dihydro-2′-deoxyguanosine and malondialdehyde levels as oxidative stress markers and antioxidant status in non-small cell lung cancer. Biomarkers 17(3):261–268
149. Zablocka-Slowinska K et al (2018) Serum and whole blood Zn, Cu and Mn profiles and their relation to redox status in lung cancer patients. J Trace Elem Med Biol 45:78–84
150. Esme H et al (2008) High levels of oxidative stress in patients with advanced lung cancer. Respirology 13(1):112–116
151. Yuan JM et al (2018) Relationship of the oxidative damage biomarker 8-epi-prostaglandin F2alpha to risk of lung cancer development in the Shanghai Cohort Study. Carcinogenesis 39 (7):948–954
152. Yano T et al (2009) Significance of the urinary 8-OHdG level as an oxidative stress marker in lung cancer patients. Lung Cancer 63(1):111–114
153. Levy P et al (2015) Obstructive sleep apnoea syndrome. Nat Rev Dis Primers 1:15015
154. Davies CR, Harrington JJ (2016) Impact of obstructive sleep apnea on neurocognitive function and impact of continuous positive air pressure. Sleep Med Clin 11(3):287–298
155. Bikov A, Losonczy G, Kunos L (2017) Role of lung volume and airway inflammation in obstructive sleep apnea. Respir Investig 55(6):326–333
156. Yamauchi M, Kimura H (2008) Oxidative stress in obstructive sleep apnea: putative pathways to the cardiovascular complications. Antioxid Redox Signal 10(4):755–768
157. Stiefel P et al (2013) Obstructive sleep apnea syndrome, vascular pathology, endothelial function and endothelial cells and circulating microparticles. Arch Med Res 44(6):409–414
158. Toraldo DM et al (2016) Obstructive sleep apnea syndrome: coagulation anomalies and treatment with continuous positive airway pressure. Sleep Breath 20(2):457–465
159. Cicco S et al (2018) Analysis of aortic remodeling and stiffness in patients with obstructive sleep apnea syndrome: preliminary results. Adv Exp Med Biol 1072:251–255
160. Sunnetcioglu A et al (2016) Evaluation of oxidative damage and antioxidant mechanisms in COPD, lung Cancer, and obstructive sleep apnea syndrome. Respir Care 61(2):205–211
161. Guo Q et al (2013) Levels of thioredoxin are related to the severity of obstructive sleep apnea: based on oxidative stress concept. Sleep Breath 17(1):311–316

162. Franco CM et al (2012) Obstructive sleep apnea severity correlates with cellular and plasma oxidative stress parameters and affective symptoms. J Mol Neurosci 47(2):300–310
163. Hira HS et al (2014) Plasma level of hypoxanthine/xanthine as markers of oxidative stress with different stages of obstructive sleep apnea syndrome. Ann Saudi Med 34(4):308–313
164. Ozben S et al (2014) Advanced oxidation protein products and ischaemia-modified albumin in obstructive sleep apnea. Eur J Clin Investig 44(11):1045–1052
165. Dinc ME et al (2017) Thiol/disulfide homeostasis as a novel indicator of oxidative stress in obstructive sleep apnea patients. Laryngoscope 127(7):E244–E250
166. Cherneva RV et al (2017) 8-Isoprostanes and resistin as markers of vascular damage in non-hypersomnolent obstructive sleep apnoea patients. Clin Physiol Funct Imaging 37 (6):695–702
167. Yamauchi M et al (2005) Oxidative stress in obstructive sleep apnea. Chest 127(5):1674–1679
168. Celec P et al (2012) Oxidative and carbonyl stress in patients with obstructive sleep apnea treated with continuous positive airway pressure. Sleep Breath 16(2):393–398
169. Tichanon P et al (2016) Effect of continuous positive airway pressure on airway inflammation and oxidative stress in patients with obstructive sleep apnea. Can Respir J 2016:3107324
170. Carpagnano GE et al (2003) 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. Chest 124(4):1386–1392
171. Zinellu E et al (2016) Circulating biomarkers of oxidative stress in chronic obstructive pulmonary disease: a systematic review. Respir Res 17(1):150
172. Frijhoff J et al (2015) Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal 23(14):1144–1170
173. Montuschi P et al (2000) Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. Am J Respir Crit Care Med 162(3 Pt 1):1175–1177
174. Rahman I, Biswas SK (2004) Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues. Redox Rep 9(3):125–143
175. Brussino L et al (2010) Oxidative stress and airway inflammation after allergen challenge evaluated by exhaled breath condensate analysis. Clin Exp Allergy 40(11):1642–1647
176. Marrocco I, Altieri F, Peluso I (2017) Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxidative Med Cell Longev 2017:6501046
177. Mesaros C et al (2015) Bioanalytical techniques for detecting biomarkers of response to human asbestos exposure. Bioanalysis 7(9):1157–1173
178. Lin JL, Thomas PS (2010) Current perspectives of oxidative stress and its measurement in chronic obstructive pulmonary disease. COPD 7(4):291–306
179. Esther CR Jr et al (2016) Metabolomic biomarkers predictive of early structural lung disease in cystic fibrosis. Eur Respir J 48(6):1612–1621
180. Matera MG, Calzetta L, Cazzola M (2016) Oxidation pathway and exacerbations in COPD: the role of NAC. Expert Rev Respir Med 10(1):89–97
181. Samuni Y et al (2013) The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta 1830(8):4117–4129
182. Thomson NC (2018) Targeting oxidant-dependent mechanisms for the treatment of respiratory diseases and their comorbidities. Curr Opin Pharmacol 40:1–8
183. Decramer M et al (2005) Effects of N-acetylcysteine on outcomes in chronic obstructive pulmonary disease (Bronchitis Randomized on NAC Cost-Utility Study, BRONCUS): a randomised placebo-controlled trial. Lancet 365(9470):1552–1560
184. Zheng J-P et al (2014) Twice daily N-acetylcysteine 600 mg for exacerbations of chronic obstructive pulmonary disease (PANTHEON): a randomised, double-blind placebo-controlled trial. Lancet Respir Med 2(3):187–194
185. Tse HN et al (2013) High-dose N-acetylcysteine in stable COPD: the 1-year, double-blind, randomized, placebo-controlled HIACE study. Chest 144(1):106–118

186. Conrad C et al (2015) Long-term treatment with oral N-acetylcysteine: affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial. J Cyst Fibros 14(2):219–227
187. Domej W, Oettl K, Renner W (2014) Oxidative stress and free radicals in COPD—implications and relevance for treatment. Int J Chron Obstruct Pulmon Dis 9:1207–1224
188. Zheng J-P et al (2008) Effect of carbocisteine on acute exacerbation of chronic obstructive pulmonary disease (PEACE Study): a randomised placebo-controlled study. Lancet 371 (9629):2013–2018
189. Calabrese C et al (2015) Randomized, single blind, controlled trial of inhaled glutathione vs placebo in patients with cystic fibrosis. J Cyst Fibros 14(2):203–210
190. Ciofu O, Lykkesfeldt J (2014) Antioxidant supplementation for lung disease in cystic fibrosis. Cochrane Database Syst Rev 8:CD007020
191. Harvey CJ et al (2011) Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. Sci Transl Med 3(78):78ra32
192. Wise RA et al (2016) Lack of effect of oral sulforaphane administration on Nrf2 expression in COPD: a randomized, double-blind, placebo controlled trial. PLoS One 11(11):e0163716
193. Beijers R, Gosker HR, Schols A (2018) Resveratrol for patients with chronic obstructive pulmonary disease: hype or hope? Curr Opin Clin Nutr Metab Care 21(2):138–144
194. Kode A et al (2008) Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am J Physiol Lung Cell Mol Physiol 294(3):L478–L488
195. Baldrick FR et al (2012) Effect of fruit and vegetable intake on oxidative stress and inflammation in COPD: a randomised controlled trial. Eur Respir J 39(6):1377–1384
196. Turowski JB et al (2015) Flaxseed modulates inflammatory and oxidative stress biomarkers in cystic fibrosis: a pilot study. BMC Complement Altern Med 15:148

The Role of Synbiotics in Alleviating Oxidative Stress in Colorectal Cancer

6

Monica Gulati, Sachin Kumar Singh, and Apoorva Singh

Abstract

Colorectal cancer (CRC) is one of the leading malignancies that result in high morbidity and mortality among both the genders, though the incidence is higher in females. It is a multifactorial and multistage disease. One of the major progenitors of colorectal cancer is the reactive oxygen species. A disturbance in the prooxidant-antioxidant balance in the cells leads to compromise in DNA repair mechanism, protein denaturation, lipid peroxidation, and apoptosis leading finally to tumor proliferation. Dysbiosis in the colon is a known cause of oxidative stress. This chapter aims to elucidate the role played by oxidative stress in colorectal oncogenesis. The potential palliative role of probiotics against CRC is also discussed. Probiotics have been known for possessing potent antioxidant attributes and preventing colorectal carcinogenesis. However, mechanism of action of probiotics in amelioration of oxidative stress is not very clear. Thus, the aim of the present chapter is also to review the antioxidant mechanisms of probiotics, choice of species specific to colorectal disease, and role of prebiotics in potentiating the effect of probiotics by balancing the microbiota and physicochemical conditions of colorectal milieu, in the production of anti-cancer compounds and in decreasing oxidative stress. Synbiotics, i.e., the combination of probiotics and prebiotics, are known to be an effective potential strategy in combating CRC.

M. Gulati (✉) · S. K. Singh
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

A. Singh
Department of Anaesthesiology and Intensive Care, Postgraduate Institute of Medical Education and Research, Chandigarh, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_6

Keywords

Oxidative stress · Probiotic · Synbiotic · Gut microbiota · Colorectal cancer · Colitis-associated cancer

6.1 Colorectal Cancer

Worldwide, CRC is reported as the third most prevalent malignancy in men and the second most prevalent malignancy in women. The incidence of CRC over various regions is reported to vary over tenfolds. Highest incidence rates are reported in Australia, New Zealand, Europe, and North America, while Africa and South Central Asia report the lowest incidence [1]. Apart from the geographic disparity that could to be attributed to variation in diet, environmental factors as well as genetic susceptibility, age, socioeconomic status, physical inactivity, smoking, alcohol consumption, and obesity are the common risk factors.

Molecular mechanisms of pathogenesis of CRC are not completely deciphered hitherto despite significant efforts by a number of groups working in this direction. A number of factors are involved in the etiology of CRC. These include genetic mutations, diet, inflammatory processes, and an imbalance in the gut microflora. Most of the colorectal cancers (more than 95%) are reported to be sporadic, i.e., occurring in patients who do not possess genetic predisposition toward the disease [2]. Approximately 3% of the cases are attributed to Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC), while 1% are attributed to certain other hereditary conditions including familial adenomatous polyposis (FAP) and MYH-associated polyposis (MAP) [3].

An imbalance in the optimum composition of gut microbiota has been largely considered as a significant factor for the development of CRC. However, the exact mechanism through which the gut microbiota influences the development of CRC is not very clear. It is generally believed that many factors like inflammation, lifestyle, and genetics play a role to increase the gut carcinogenicity of altered gut microbial picture [4].

6.2 Role of Oxidative Stress

Majority of factors involved in colon carcinogenesis involve intracellular redox imbalance in the colon cells. There is an excessive production of reactive oxygen species (ROS) with concomitant compromise in anti-oxidative protection. It is widely known that ROS induces DNA damages and genetic mutations, which, in turn, are a critical cause of CRC. The main causative factors for CRC are reported to be inflammation, fat metabolism, consumption of meat and alcohol, and tobacco smoking. The only similarity in the outcomes of all these contributing factors is the production of ROS and lipid peroxidation. The dichotomy in roles of these ROSs is very significant. While at optimum concentrations, they are indispensable as

mediators of critical physiological phenomena of cell survival and proliferation, at high concentrations, the same reactive species become cytotoxic. At high concentrations, they lead to pathological oxidation of DNA, lipids, and proteins.

There are a number of causative factors leading to oxidative damage that may ultimately lead to CRCs. These include:

6.2.1 Inflammation

Inflammation was acknowledged as a cofactor in carcinogenesis in 1863 when Rudolf Virchow observed leucocytes in the cancer tissues and suggested an association between inflammation and cancer [5]. This is particularly applicable in case of sporadic colitis-associated cancer (CAC) and CRC. During inflammation, the activated macrophages produce reactive oxygen species (ROS) and reactive nitrogen species (RON) that can induce oxidation of nucleic acids, proteins, and lipids. Oxidation of polyunsaturated fatty acids generates reactive aldehydes that can diffuse throughout the cell [6]. Cytokines, and other inflammatory modulators, on the other hand, lead to generation of ROS inside the epithelial cells, which, in turn, induces mutations and epigenetic silencing of tumor suppressor genes.

Chronic inflammation in CAC has been reported to cause p53 mutations observed in tumor cells, inactivation of mismatch repair (MMR) genes, and inactivation of mismatch repair enzymes at protein level [7]. Production of proteases that are responsible for disruption of extracellular matrix is enhanced by inflammatory signals. These, in turn, facilitate the invasion and extravasation of cancer cells.

6.2.2 Consumption of Alcohol

A strong correlation has been established between alcohol consumption and occurrence of colorectal cancer [8]. Alcohol consumption has been reported to increase the incidence of CRC by a factor of 60% [9]. This correlation, however, is reported to vary according to race, lifestyle including cigarette smoking, body weight, type and quantity of alcohol consumed, and tumor site.

This epidemiological correlation has been attributed to many potential mechanisms for the effect of alcohol on risk of development of colorectal cancers. The evidence points in several directions. First, alcohol is metabolized by alcohol dehydrogenases (ADH), catalase, or cytochrome P450 2E1 (CYP2E1) to acetaldehyde. Acetaldehyde is a well-known oxidative stressor [10]. It is widely reported to interfere with DNA methylation, synthesis, and repair. Moreover, it binds to protein molecules as well as deoxynucleotides of DNA, which, in turn, leads to damage to the DNA and eventually to cell proliferation. Second, the high levels of acetaldehyde in the colon are reported to degrade folate, which is known to reduce the occurrence of CRC. Third, alcohol is a known antagonist of metabolism of methyl-group, which, in turn, leads to formation of aberrant DNA methylation profiles and eventually to cancer-related gene expression. Fourth, alcohol use is known to lead to

immunosuppression. Impairment of host defense due to alcohol consumption is attributed to a plethora of factors including a compromise in inflammatory response, decrease in cytokine formation, formation of abnormal ROS, and decrease in T lymphocyte and antigen-presenting cell functions. Apart from these, there are a number of factors associated with alcohol consumption that may lead to increased risk of CRC that include disruption of intestinal epithelial barrier, alteration in normal microbiota, increased uptake of carcinogens, activation of liver pro-carcinogens by induction of cytochrome P-450 enzymes, and changes in bile acid composition [11].

6.2.3 Consumption of Red Meat

In a recent prospective study with a follow-up of 5.7 years, a strong correlation has been established between red and processed meat consumption and occurrence of CRC [12]. Carcinogenicity associated with red meat is largely attributed to the presence of heme (iron-protoporphyrin IX) which is an essential cofactor involved in a number of biological processes. On the other hand, free heme (not bound to proteins) is known to catalyze the production of ROS, which, in turn, leads to oxidative stress. This free heme contains iron with strong redox properties that can participate in all four types of lipid peroxidation, i.e., Fenton reaction, iron(III)/iron (IV) mechanism, pseudoperoxidase mechanism, and iron(II)/iron(IV) mechanism. These lead to the formation of free hydroxyl, alkoxyl, and peroxyl radicals. The generated ROS damage is cytotoxic and genotoxic as they damage lipid membranes, proteins, and nucleic acids. As the heme molecules are lipophilic in nature, they get well accommodated among the phospholipid molecules and intercalate between the phospholipid bilayers of cell membranes. As phospholipids are highly susceptible to oxidation, the heme iron catalyzes the oxidation reaction and enhances lipid peroxide formation [13]. Increased intracellular levels of iron were observed on incubation of colon adenocarcinoma, SW480 cells with Hb, leading to DNA oxidation. Hb from both red meat and colonic bleeding was found to lead to an increase in fatty acid hydroperoxide genotoxicity, which, in turn, results in DNA lesions in colon cells. The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). While MDA reacts with DNA to form adducts with deoxyguanosine, deoxyadenosine, and deoxycytidine, 4-HNE affects the signal transduction pathways and is known to induce apoptosis [14].

6.2.4 Tobacco Smoking

A number of epidemiological studies have linked tobacco smoking with the risk for occurrence of CRC [15]. Though the molecular mechanism is still regarded as uncertain, the carcinogenic potential is largely attributed to the presence of free radicals and oxidants in cigarette smoke. These result in oxidative damage to DNA and modified products such as 8-oxodeoxyguanosine, thymine glycol, thymidine

glycol, and 5-hydroxymethyluracil. Cessation of smoking has been reported to decrease the formation of 8-oxodeoxyguanosine substantially [16]. A strong correlation has been reported between smoking and urinary excretion of 3,*N*4-ethenodeoxycytidine, the generation of which has been attributed to lipid peroxidation.

Though smoking of E-cigarettes, also known as vaping, is generally considered as safe by smokers, recent reports indicate otherwise. A detailed report by Tommasi et al. demonstrates a deregulation of critically important genes and associated molecular pathways in the oral epithelium of vapers that bears both resemblances and differences with that of smokers [17].

6.2.5 Psychological Stress

In the 1980s, an epidemiological trend was observed that indicated an increased susceptibility to cancer after bereavement, more specifically widowhood. The association was attributed to psychological trauma of losing a spouse [18]. In a cohort study on the survival rates of married, divorced, never married, or widowed patients diagnosed with colon and rectal cancer, the widowed group was found to show the minimum survival rate [19]. These studies lead to a number of systematic studies that clearly indicated a relationship between stress and susceptibility to CRC [20]. In order to find the causative factor of this increased susceptibility, the levels of 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative stress, were studied in relation to tension, anxiety, depression, rejection, anger, hostility, fatigue, and confusion scores of the Profile of Mood States. The levels were found to be significantly increased in response to stress conditions [21]. 8-Hydroxy-dG is one of the most significant products of DNA oxidation and important predictor of carcinogenesis. The oxidative stress caused by the perceived psychological stress was, therefore, considered to lead to carcinogenesis by oxidative DNA damage. A vicious cycle has been observed with depression and anxiety leading to CRC. Depression in CRC patients further accelerates oxidative damage, hinders the repair of DNA, and aggravates the condition [22].

6.2.6 Radiation

Several studies indicate that patients who have undergone radiotherapy to treat the cancers located in the pelvic region have a higher risk factor to develop CRC as the colorectal region gets exposed to some radiation inadvertently. Radiotherapy administered for ureteral, urinary bladder, ovarian, uterine, endometrial, cervical, vaginal, testicular, and prostate cancer leads to generation of oxygen free radicals by the ionizing nature of the radiation, which in turn causes damage to DNA. The oxidative damage of DNA leads to apoptosis, finally leading to development of CRC [23]. The susceptibility to the development of CRC and the latency period, i.e., the interval between the radiation exposure to the diagnosis of the secondary cancer, is

reported to vary according to the strength of radiation, mode of administration, and the duration of radiotherapy [24].

6.2.7 Overweight

Obesity, also termed as adiposity, has been considered as a strong risk factor for CRCs. Earlier, the risk of CRC due to obesity and physical inactivity was considered to be more pronounced in men as compared to women. But in some recent studies, not only the risk has been ascertained in women, but an early onset of CRC has also been associated with obesity among women [25]. Higher levels of estrogens along with lower levels of insulin sensitivity are thought to be the attributing factors for CRCs resulting from high adiposity. Risk has been more significantly related to abdominal adiposity than to overall obesity. As an extension of obesity, lack of physical activity has also been considered as a significant risk factor [26]. Increase in physical activity is also reported to reduce the risk of recurrence of CRCs [27].

Body mass index (BMI) has been the conventional measure of obesity. Recently, however, newer indices/ratios have been in trend for the measurement of body fat, particularly the fat located in the intestines. The risk of CRCs due to higher adiposity is attributed to the generation of pro-inflammatory cytokines like IL-2, IL-6, and IL-8 and enzymes like LDH and TNFα. These, as discussed in Sect. 6.2.1, lead to high risk of CRC. Also, fatty acid peroxidation leads to generation of metabolites with oncogenic properties. These are known to increase the levels of prostaglandin E2, which, in turn, is associated with CRC [28].

Insulin resistance related to obesity has been reported to increase the levels of free IGF-1, while a decrease in free IGF-1 is observed in the insulin-sensitive subjects. Increased levels of insulin also leads to dysregulation of IGF-1 signaling leading to higher levels of free IGF. Higher levels of IGF-1 have been associated with an increase in the CRC risk [29].

6.2.8 Antibiotics

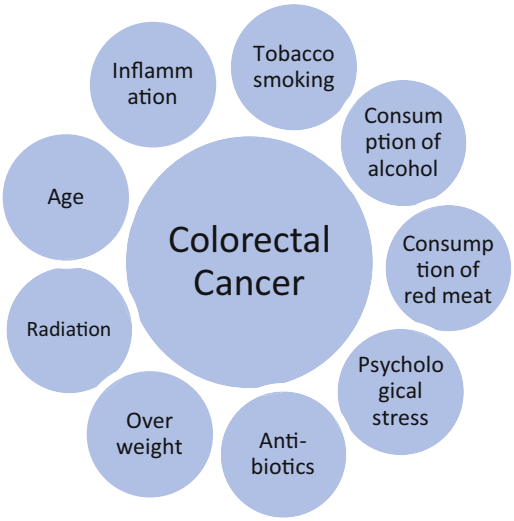
The use of antibiotics, particularly antianaerobic antibiotics, has been consistently associated with the higher risk of CRC. Antibiotic use compromises the natural colonic flora, thereby promoting colorectal colonization with enterobacteria. The ratio of bacteria like *Peptostreptococcus anaerobius* vis-à-vis the commensal bacteria increases in the colorectal milieu. Bacteria like *P. anaerobius* are known to lead to formation of ROS that have a definite oncogenic role by promoting cholesterol synthesis and cell proliferation [30]. The risk for development of CRC due to antibiotic therapy has been reported to be more with the long-term use rather than with shorter durations of therapy [31].

Surprisingly, the use of antibiotics has been shown to reduce the risk of colorectal oncogenesis in a recent study [32]. Interestingly, the seemingly opposite results in the study also are attributed to the suppression of the growth of the bacteria having

oncogenic potential due to antibiotic therapy. These bacterial subgroups have been identified as *Clostridium leptum* and *Bacteroides fragilis*. Antibiotic therapy, in this study, was found to inhibit the deviant methylation in the epithelial cells of colon, which, in turn, leads to a decrease in CRCs. This has been attributed to the anti-inflammatory role of the antibiotics. As discussed in Sect. 6.2.1, inflammation itself plays a significant role in colorectal oncogenesis.

6.2.9 Age

Aging is one of the important risk factors of CRC. The free radical theory of aging considers aging as a result of the failure of inherent defense system of the body to handle the reactive oxygen species (ROS)-induced damage. Moreover, on aging, the resident gut microflora like *Bifidobacteria* and *Lactobacillus* reduce, while opportunistic ones like *Clostridia* increase in ratio due to a decrease in physical activity and diet pattern. Only about 11% of diagnoses of CRC are made in patients below 50 years of age [33].



6.3 Colorectal Dysbiosis

All the abovementioned factors leading to oxidative damage have been clearly implicated in affecting the colonic microbiome locally and/or systemically. Apart from their individual effects, all these factors have a complicated interplay with the gut bacteria and thus exert a two pronged-effect on the CRC development. Almost every major modern lifestyle attribute described in Sect. 6.2 brings about a change in

the gut microflora to favor oncogenesis. The microflora of the gut helps to maintain a homeostasis in the body. An alteration in normal colonic microflora has been unequivocally reported to be an important causative factor in the development of CRC [34]. The colonic microbiota along with the mucosal epithelial cells, diet components that act as probiotics and prebiotics, enzymes, mucus, and bile salts are a part of a complex mutualistic microecosystem. Colon, which is the main colonization site in the body, houses about 10^{14} microbial cells with more than 1000 different bacterial species wherein *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* are the dominant species. Prevalent conditions in colonic milieu such as near-neutral pH, low concentration of bile salts, anaerobic conditions, long transit time, high viscosity, and very weak peristalsis are favorable for the proliferation of this anaerobic microbiota. Some facultative anaerobes/aerotolerant bacteria such as enterobacteria, enterococci, lactobacilli, and streptococci that constitute a critical component of microbial homeostasis are also present, though to a lesser extent. Because of the action of the bacterial enzymes on the undigested dietary residues as well as endogenous mucins in the colon, the colonic microbiota acts like a metabolic organ [35]. This leads to the production of energy through the formation of short-chain fatty acids from these residues.

An optimum balance among the constituents of the colonic ecosystem results in the production of essential nutrients, absorption of nutrients and strengthens the immune. It also prevents the colonization of pathogen and dysbiosis which leads to inflammation, damage of tissue mucosa, compromise in barrier integrity and function, eventually leading to colon cancer [36]. The process of oncogenesis is a complex one, involving a gradual change in the gut microflora, as well as their biological milieu, which, in turn, leads to dysbiosis, and the altered ratio of resident to potential oncopathogenic microbes leads to oncogenesis. The presence of certain non-commensal bacterial species like *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis*, and *Enterococcus faecalis* has been linked to the occurrence of CRC [37].

These bacteria with carcinogenic potential exert their effect by different mechanisms. *F. nucleatum*, the epithelial adherent bacteria, act through different routes leading to tumorigenesis. While acting through their FadA adhesion, they modify the E-cadherin/beta-catenin signaling, through their nuclear factor-kappa b (NF-κb) signaling pathway, they lead to inflammation [38]. *E. coli*, on the other hand, produce enterobacterial genotoxins, which are a potential tumorigens [39]. Tumorigenic mechanism of *B. fragilis* is somewhat similar to that of *F. nucleatum* involving toxin production and E-cadherin cleavage and resulting in inflammation through Th17/IL-17 pathway [40]. *E. faecalis*, on the other hand, produces extracellular superoxide which leads to DNA breaks [41].

6.4 Role of Synbiotics

Apart from playing a well-documented role in the prevention and treatment of CRC, another area of protagonistic contribution of synbiotics is that as an adjunct therapy for conventional therapies of CRCs for mitigation of their adverse effects.

Individually, the use of probiotics defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" and prebiotics as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon that have the potential to improve host health" has long been recognized as helpful in the prevention of CRC [42]. It is interesting to note that both probiotics and prebiotics help in replenishing the balance of gut microecosystem. Prebiotics constitute the food for probiotics and allow them to flourish. Therefore, combining the two leads to an increased efficiency of the process, thereby culminating in the formulation of "synbiotics." Probiotic strains like *Lactobacillus*, *Bifidobacteria* and *Pediococcus*, *Leuconostoc*, and *Enterococcus* and yeast such as *Saccharomyces boulardii* are reported to reduce the incidence of colon cancer. Synbiotics are known to act through a number of ways to prevent CRC. These include:

6.4.1 Effect on Carcinogenic Metabolic Products

The bacterial enzymes β-glucuronidase, azoreductase, and nitroreductases have the ability to hydrolyze many xenobiotics and mutagens in cooked meat. These enzymes convert procarcinogens to carcinogenic molecules, e.g., aromatic amines, hydrogen sulfide, acetaldehydes, secondary bile salts, aglycones, and ROS, and release them in the colorectal milieu. Probiotics like *L. rhamnosus*, *L. acidophilus*, and *L. casei* were found to decrease the levels of these enzymes and thus inhibit carcinogenesis. On discontinuing the probiotic therapy, the effect was found to be reversed [43]. Intestinal metabolic products of proteins like *N*-nitroso compounds, indoles, etc. which are known to possess tumorigenic properties are deactivated, thus reducing their detrimental effect on mesenchymal cells and epithelial cells. Many species of *L. rhamnosus*, *B. longum*, *L. acidophilus*, and *S. salivarius* are reported to bind the mutagenic heterocyclic amines and release them into feces. Certain *Lactobacillus* species have the capability of scavenging reactive intermediates of carcinogenic compounds like *N*-methyl-*N*9-nitro-*N*-nitrosoguanidine (MNNG) and dimethylhydrazine (DMH). This is accomplished by generating certain chemoprotective enzymes like glutathione-*S*-transferase, glutathione reductase, glutathione peroxidase, and superoxide dismutase and catalase which deactivate carcinogens [44]. Many *Lactobacillus* species like *L. casei* and *L. helveticus* are reported to have produce chemicals having a capability to chelate transition metal ions, which are instrumental in catalyzing oxidation leading to the formation of peroxyl and alkoxyl radicals by the decomposition of hydroperoxides.

6.4.2 Alteration of Physicochemical Conditions in the Colon

The pH in the human colon varies from about 5 to 7 with the values in ascending and transverse colon being lower than that in distal colon. During CRC, the colorectal pH shifts from normal to a higher value. This, in turn, alters the composition of the

microflora. Certain bacterial fermentation processes like butyrogenic reactions occur at mildly acidic pH, while reactions leading to the formation of propionate occur at neutral pH. Butyrogenic bacteria like *Faecali bacterium* and *Roseburia* grow better at mildly acidic pH which protects against DNA damage caused by hydrogen peroxide-induced oxidative stress. Propionate, generally produced by *Bacteroides*, shows anti-inflammatory properties which, per se, are protective against CRC as discussed in Sect. 6.2.1 [45]. Even prebiotic inulin has been reported to lead to decrease in colonic pH, shifting the microbial ecosystem toward higher concentration of *lactobacilli*. Levels of procarcinogenic markers like aberrant crypt foci were found to be reduced [46]. Another mechanism by which synbiotics act is by regularizing the bowel movement [47]. Though there is no significant correlation between bowel movement frequency and CRC risk reported, loose stools and the use of non-fiber laxatives are considered as a risk for CRC development [48].

6.4.3 Short-Chain Fatty Acid (SCFA) Production

SCFAs produced by probiotics and prebiotics exert their protective effects by different routes. In in vitro studies, butyrate, one of the most significant SCFAs, has been reported to exhibit antiproliferative effect against cancer cells and promote apoptosis.

A high butyrate-producing strain of *Butyrivibrio fibrisolvens* was found to reduce the glucuronidase activity in the colorectal milieu, increase the numbers of natural killer cells, and reduce the number of aberrant crypts in mouse model of colon cancer. SCFAs increased the immune response, indicated by an increase in NK cell numbers. Apart from this, SCFAs are known to improve barrier function and intestinal mucus synthesis, stimulate immunosuppressive cytokines such as interleukin 10 (IL-10), and decrease the concentration of pro-inflammatory mediators. Moreover, they lead to preferential growth of protective bacteria over pathogenic strains. Propionate- and acetate-producing probiotics, *Propionibacterium acidipropionici*, have also been reported to have similar protective effects against CRC [49].

6.4.4 Apoptosis Induction

Apoptosis, a programmed cell death, is a genetic suicidal process for the maintenance of tissue homeostasis and safeguards against the process of tumorigenesis. While CRC oncogenesis processes take place, the cancer cells become resistant to apoptosis due to certain genetic and epigenetic alterations. A number of reports indicate that probiotics play a significant role in the regulation of cell apoptosis via both intrinsic and extrinsic pathways. The proapoptotic effect of probiotics has been attributed to their ability to bring about differentiation, arrest cell cycle, and enhance the apoptotic mechanisms in cancer cells. These effects, however, vary greatly with the phenotypic state of cells as well as the probiotic strain used. In a recent study,

ferrichrome has been identified as the molecule produced by probiotics that induces apoptosis. Ferrichrome produced by *L. casei* exerts a tumoricidal effect which is greater than that of cisplatin and 5-fluorouracil while having less effect on noncancerous cells than both the established anticancer drugs [50].

6.4.5 Elevation of the Host's Immune Response

Probiotics are reported to enhance both the adaptive and innate immune responses of the hosts. While the adaptive immune response depends on B and T lymphocytes, the innate immune response depends on the pathogen-associated molecular patterns (PAMPs) shared by the vast majority of pathogens. These PAMPs bind to the pattern recognition receptors (PPRs) like Toll-like receptors (TLRs) to trigger the primary response. TLRs are transmembrane receptors located on cell surface of a number of cells including B cells, NK cells, dendritic cells, macrophages, fibroblasts, epithelial cells, and endothelial cells. Probiotics are reported to downregulate the TLR expression, inhibit TNF-α from entering blood mononuclear cells, and inhibit NF-κB signaling. A strain of *Lactobacillus casei* has been reported to enhance the innate immune response by phosphorylation of NF-κB, p65, p3, MAPK, and MAPKAPK-2 signaling pathway. In another study, probiotic mixture was found to inhibit IL-8 and TNF-α production. Moreover, probiotics have a capability to combat dendritic cells that play a significant role in both innate and adaptive immunity [51].

6.5 Conclusion

Synbiotics have shown substantial palliative as well as curative effects in CRC. Probiotics, prebiotics, as well as synbiotics have demonstrated potential against CRC through a number of in vitro and in vivo studies. Nevertheless, before prescribing them for CRC in a routine manner, proper deliberations must be done for the selection of the probiotic and its supportive prebiotic and standardized dose. Most of the studies point to the fact that symbiotic combinations with their synergistic effect produce maximum beneficial effects during initiation or early stage of colon cancer. Though results from animal studies have proven that synbiotics form an effective strategy for tumor preventive effects, substantial data is lacking in human trials and a number of clinical trials are still underway. Fixation of a standard treatment regimen in all CRC patients seems to be unconvincing as a large number of variables are associated with the colonic microflora picture, which may be decisive factor for the selection of the probiotics and prebiotics. In order to overcome the limitations associated with synbiotics like allergy, gut discomfort or chances of infection, platelet aggregation, and aggravation of hemolytic uremic syndrome, the new concept of metabiotics has emerged recently. These are metabolites produced by probiotics that help in the maintenance of gut homeostasis, which, in turn, leads to increase in the growth of bacterial species that inhibit the conversion of procarcinogens into carcinogens by decreasing the levels of enzymes like

nitroreductase, β-glucuronidase, and β-glucosidase [52].As these possess an exact chemical structure, precisely dosage, safety, and longer shelf life as compared to classic probiotics, they may be positioned to replace all three options, i.e., probiotics, prebiotics, and synbiotics.

References

1. Gandomani HS, Aghajani M, Mohammadian-Hafshejani A, Tarazoj AA, Pouyesh V, Salehiniya H (2017) Colorectal cancer in the world: incidence, mortality and risk factors. Biomed Res Ther 4(10):1656–1675
2. Watson AJ, Collins PD (2011) Colon cancer: a civilization disorder. Dig Dis 29(2):222–228
3. Pačínková A, Popovici V (2019) Cross-platform data analysis reveals a generic gene expression signature for microsatellite instability in colorectal cancer. Biomed Res Int 2019:6763596
4. Vipperla K, O'Keefe SJ (2016) Diet, microbiota, and dysbiosis: a 'recipe' for colorectal cancer. Food Funct 7(4):1731–1740
5. Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357 (9255):539–545
6. Bartsch H, Nair J (2004) Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Cancer Detect Prev 28(6):385–391
7. Terzić J, Grivennikov S, Karin E, Karin M (2010) Inflammation and colon cancer. Gastroenterology 138(6):2101–2114
8. Park S-Y, Wilkens LR, Setiawan VW, Monroe KR, Haiman CA, Le Marchand L (2018) Alcohol intake and colorectal cancer risk in the multiethnic cohort study. Am J Epidemiol 188(1):67–76
9. Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma K-N et al (1997) Energy balance and colon cancer—beyond physical activity. Cancer Res 57(1):75–80
10. Tamura M, Ito H, Matsui H, Hyodo I (2014) Acetaldehyde is an oxidative stressor for gastric epithelial cells. J Clin Biochem Nutr 55:26–31
11. Na H-K, Lee J (2017) Molecular basis of alcohol-related gastric and colon cancer. Int J Mol Sci 18(6):1116
12. Bradbury KE, Murphy N, Key TJ (2019) Diet and colorectal cancer in UK Biobank: a prospective study. Int J Epidemiol. https://doi.org/10.1093/ije/dyz064
13. Cascella M, Bimonte S, Barbieri A, Del Vecchio V, Caliendo D, Schiavone V et al (2018) Dissecting the mechanisms and molecules underlying the potential carcinogenicity of red and processed meat in colorectal cancer (CRC): an overview on the current state of knowledge. Infectious Agents Cancer 13(1):3
14. Bastide NM, Pierre FH, Corpet DE (2011) Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. Cancer Prev Res 4(2):177–184
15. Hamada T, Nowak JA, Masugi Y, Drew DA, Song M, Cao Y et al (2018) Smoking and risk of colorectal cancer sub-classified by tumor-infiltrating T cells. J Natl Cancer Inst 111(1):42–51
16. Priemé H, Loft S, Klarlund M, Grønbæk K, Tønnesen P, Poulsen HE (1998) Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7, 8-dihydro-2′-deoxyguanosine excretion. Carcinogenesis 19(2):347–351
17. Tommasi S, Caliri AW, Caceres A, Moreno DE, Li M, Chen Y et al (2019) Deregulation of biologically significant genes and associated molecular pathways in the oral epithelium of electronic cigarette users. Int J Mol Sci 20(3):738
18. Burgoa M, Regidor E, Rodríguez C, Gutiérrez-Fisac JL (1998) Mortality by cause of death and marital status in Spain. Eur J Public Health 8(1):37–42
19. Johansen C, Schou G, Soll-Johanning H, Mellemgaard A, Lynge E (1996) Influence of marital status on survival from colon and rectal cancer in Denmark. Br J Cancer 74(6):985

20. Kikuchi N, Nishiyama T, Sawada T, Wang C, Lin Y, Watanabe Y et al (2017) Perceived stress and colorectal cancer incidence: the Japan Collaborative Cohort Study. Sci Rep 7:40363
21. Yoshihara K, Hiramoto T, Sudo N, Kubo C (2011) Profile of mood states and stress-related biochemical indices in long-term yoga practitioners. BioPsychoSoc Med 5(1):6
22. Wei Y-C, Zhou F-L, He D-L, Bai J-R, Hui L-Y, Wang X-Y et al (2009) The level of oxidative stress and the expression of genes involved in DNA-damage signaling pathways in depressive patients with colorectal carcinoma. J Psychosom Res 66(3):259–266
23. Rombouts A, Hugen N, van Beek J, Poortmans P, de Wilt J, Nagtegaal I (2018) Does pelvic radiation increase rectal cancer incidence?–A systematic review and meta-analysis. Cancer Treat Rev 68:136–144
24. Boice JD Jr, Engholm G, Kleinerman RA, Blettner M, Stovall M, Lisco H et al (1988) Radiation dose and second cancer risk in patients treated for cancer of the cervix. Radiat Res 116(1):3–55
25. Campbell PT, Cotterchio M, Dicks E, Parfrey P, Gallinger S, McLaughlin JR (2007) Excess body weight and colorectal cancer risk in Canada: associations in subgroups of clinically defined familial risk of cancer. Cancer Epidemiol Prev Biomarkers 16(9):1735–1744
26. Shaw E, Farris MS, Stone CR, Derksen JW, Johnson R, Hilsden RJ et al (2018) Effects of physical activity on colorectal cancer risk among family history and body mass index subgroups: a systematic review and meta-analysis. BMC Cancer 18(1):71
27. Meyerhardt JA, Heseltine D, Niedzwiecki D, Hollis D, Saltz LB, Mayer RJ et al (2006) Impact of physical activity on cancer recurrence and survival in patients with stage III colon cancer: findings from CALGB 89803. J Clin Oncol 24(22):3535–3541
28. Martinez-Useros J, Garcia-Foncillas J (2016) Obesity and colorectal cancer: molecular features of adipose tissue. J Transl Med 14(1):21
29. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH et al (1999) Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 91(7):620–625
30. Tsoi H, Chu ES, Zhang X, Sheng J, Nakatsu G, Ng SC et al (2017) Peptostreptococcus anaerobius induces intracellular cholesterol biosynthesis in colon cells to induce proliferation and causes dysplasia in mice. Gastroenterology 152(6):1419–33.e5
31. Cao Y, Wu K, Mehta R, Drew DA, Song M, Lochhead P et al (2018) Long-term use of antibiotics and risk of colorectal adenoma. Gut 67(4):672–678
32. Hattori N, Niwa T, Ishida T, Kobayashi K, Imai T, Mori A et al (2019) Antibiotics suppress colon tumorigenesis through inhibition of aberrant DNA methylation in an azoxymethane and dextran sulfate sodium colitis model. Cancer Sci 110(1):147
33. Majumdar AP, Basson MD (2006) Effect of aging on the gastrointestinal tract. Physiol Gastrointest Tract 4:405–433
34. Han S, Gao J, Zhou Q, Liu S, Wen C, Yang X (2018) Role of intestinal flora in colorectal cancer from the metabolite perspective: a systematic review. Cancer Manag Res 10:199
35. Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489(7415):242
36. Kosumi K, Mima K, Baba H, Ogino S (2018) Dysbiosis of the gut microbiota and colorectal cancer: the key target of molecular pathological epidemiology. J Lab Precis Med 3:76
37. Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E et al (2016) Gut microbiota imbalance and colorectal cancer. World J Gastroenterol 22(2):501
38. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW (2013) Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe 14(2):195–206
39. Taieb F, Petit C, Nougayrède J-P, Oswald E (2016) The enterobacterial genotoxins: cytolethal distending toxin and colibactin. EcoSal Plus 7(1). https://doi.org/10.1128/ecosalplus.ESP-0008-2016
40. Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, Lazarev MG et al (2014) The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. Clin Infect Dis 60(2):208–215

41. Boonanantanasarn K, Gill AL, Yap Y, Jayaprakash V, Sullivan MA, Gill SR (2012) Enterococcus faecalis enhances cell proliferation through hydrogen peroxide-mediated epidermal growth factor receptor activation. Infect Immun 80(10):3545–3558
42. Ohara T, Yoshino K, Kitajima M (2010) Possibility of preventing colorectal carcinogenesis with probiotics. Hepatogastroenterology 57(104):1411–1415
43. Nagpal R, Kumar A, Kumar M, Behare PV, Jain S, Yadav H (2012) Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett 334 (1):1–15
44. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D et al (2017) Antioxidant properties of probiotic bacteria. Nutrients 9(5):521
45. Ilhan ZE, Marcus AK, Kang D-W, Rittmann BE, Krajmalnik-Brown R (2017) pH-mediated microbial and metabolic interactions in fecal enrichment cultures. Msphere 2(3):e00047–e00017
46. Shukla G, Verma A, Singh J, Yadav H (2014) Prebiotic inulin alters the colonic mass, pH, microflora and short chain fatty acids in 1,2-dimethylhydrazine dihydrochloride induced early colon carcinogenesis in male Laca mice. J Probiotics Health 2:1–6
47. Zhao Y, Yu Y-B (2016) Intestinal microbiota and chronic constipation. Springerplus 5(1):1130
48. Zhang X, Wu K, Cho E, Ma J, Chan AT, Gao X et al (2013) Prospective cohort studies of bowel movement frequency and laxative use and colorectal cancer incidence in US women and men. Cancer Causes Control 24(5):1015–1024
49. Raman M, Ambalam P, Kondepudi KK, Pithva S, Kothari C, Patel AT et al (2013) Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes 4 (3):181–192
50. Konishi H, Fujiya M, Tanaka H, Ueno N, Moriichi K, Sasajima J et al (2016) Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. Nat Commun 7:12365
51. Ashraf R, Shah NP (2014) Immune system stimulation by probiotic microorganisms. Crit Rev Food Sci Nutr 54(7):938–956
52. Sharma M, Shukla G (2016) Metabiotics: one step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01940

7 Oxidative Stress and Immunological Complexities in Multidrug-Resistant Tuberculosis

Ridhima Wadhwa, Nikita Sehgal, Naresh G, Taru Aggarwal, Saurabh Satija, Meenu Mehta, Gaurav Gupta, Dinesh K. Chellappan, Murtaza M. Tambuwala, Brain Oliver, Trudi Collet, Pawan K. Maurya, Philip M. Hansbro, and Kamal Dua

Abstract

Mycobacterium tuberculosis (Mtb), the causitive agent of Tuberculosis (TB) is the leading cause of infection. The infection is caused by aerosols and infects the alveolar macrophage. The lungs counteract against the infection by the antioxidant system in response to the oxidative stress (OS) caused. *Mycobacterium*

The original online version of this chapter was revised due to the error in the last name of the chapter author. A correction to this chapter is available at: https://doi.org/10.1007/978-981-15-1568-2_17.

R. Wadhwa
Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi, India

Centre for Inflammation, Centenary Institute, Sydney, NSW, Australia

Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW, Australia

N. Sehgal · Naresh G · T. Aggarwal
Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

S. Satija
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW, Australia

M. Mehta
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Centre for Inflammation, Centenary Institute, Sydney, NSW, Australia

Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW, Australia

G. Gupta
School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_7

stimulates the lung macrophage to produce reactive oxygen species (ROS). Furthermore, the treatment involves long term multiple drug regimens resulting in poor patient compliance leading to multidrug resistance (MDR-TB). The different first-line and second-line antibiotics are administered in an inactive form which gets converted into the active form by the OS response system of the host and the Mtb. Mtb alters the redox balance via mycolic acid, NADH/NAD^+ ratio, and antioxidant enzymes in response to stress. In the following chapter, we have discussed the role of oxidative stress in the host and the pathogen along with the immunological complexities and the genetic modifications resulting in multidrug-resistant tuberculosis. It also discusses the different strategies to target the Mtb infection.

Keywords

Mycobacterium tuberculosis · Multidrug-resistant tuberculosis · Drug-susceptible tuberculosis · Oxidative stress

D. K. Chellappan
Department of Life Sciences, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia

M. M. Tambuwala
School of Pharmacy and Pharmaceutical Science, Ulster University, County Londonderry, Northern Ireland, UK

B. Oliver
School of Life Sciences, Faculty of Science, University of Technology Sydney (UTS), Ultimo, NSW, Australia

T. Collet
Innovative Medicines Group, Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia

P. K. Maurya
Department of Biochemistry, Central University of Haryana, Mahendragarh, Haryana, India

P. M. Hansbro
School of Life Sciences, Faculty of Science, University of Technology Sydney (UTS), Ultimo, NSW, Australia

Priority Research Centre for Healthy Lungs, University of Newcastle & Hunter Medical Research Institute, Newcastle, NSW, Australia

Centre for Inflammation, Centenary Institute, Sydney, NSW, Australia

K. Dua (✉)
Priority Research Centre for Healthy Lungs, University of Newcastle & Hunter Medical Research Institute, Newcastle, NSW, Australia

Centre for Inflammation, Centenary Institute, Sydney, NSW, Australia

Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW, Australia
e-mail: Kamal.Dua@uts.edu.au

7.1 Introduction

Mycobacterium tuberculosis (Mtb) is the major cause of tuberculosis, however it can also be caused by other members of family Mycobacteriacea, including *M. africanum* and *M. bovis*. Tuberculosis (TB) is the leading cause of death because of a single infectious agent causing 1.3 million deaths in 2017 itself [1]. It was estimated that 10.0 million people developed TB in 2017 globally, out of which 5.8 million were males, 3.2 million females, and 1.0 million children. Development of drug-resistant TB has been one of the crucial concerns worldwide. In 2017, 558,000 cases were rifampicin resistant (RR-TB), and 82% of the TB account for multidrug resistance (MDR) [1].

It is predominantly spread by sneezing, spitting, or coughing as aerosols from an active TB patient. When inhaled these pathogens by the new host, it leads to the infection. So, the infection starts in the new host by the inhalation of these pathogen carrying droplets deep into the lungs and its internalization in the alveolar macrophages [2]. Mtb fuses with lysosome by preventing the maturation of phagosome that allows it to reside in the macrophages [3]. This stage of the disease is inevitable. A latent infection is controlled by maintaining the low viability of Mtb with minimal symptoms. When Mtb does not replicate in infected macrophages, it results in granulomatous inflammatory disease. Further, infected macrophages are surrounded by other macrophages which fuse to form multinucleated giant cells. The lesions caused are composed of central necrotic tissue composed of macrophages, bound by T lymphocytes and B lymphocytes. This leads to fibrosis, cavitations, lung parenchyma destruction, and bronchiectasis [4, 5]. The additional symptoms of infection are fever, weight loss, and night sweats. However, even after successful treatment of Mtb infection, individuals may develop chronic airway obstruction and loss of pulmonary function [6]. So, it is essential to identify the specific pathophysiology behind the lung tissue damage in TB patients to reduce the impact of the disease.

7.2 Oxidative Stress by Mtb on the Lungs

Oxidative stress (OS) occurs via imbalance between reactive oxygen species (ROS) and antioxidants in the body. Lungs are exposed to various exogenous sources of oxidative stress including pollutants, pathogens, cigarette smoke, and allergens [7–9]. These exogenous factors induce inflammatory cells to produce free radicals. Along with different enzyme-mediated pathways such as Nicotinamide adenine dinucleotide phosphate (NADP) oxidases, eosinophil peroxidase produces endogenous ROS such as superoxide, hydrogen peroxide, etc. ROS not only damages the host cell but also destroys the pathogens that enter the human body by binding on amino acid side chains like histidine, proline, arginine, lysine for oxidation, modifying tyrosine to nitrotyrosine, causing modification to the structure and function of proteins [10, 11]. It also causes oxidation of polyunsaturated fatty

acids (PUFA) of the microbial cell membrane via process known as lipid peroxidation. ROS directly or indirectly damages the DNA, protein, and lipids [11].

Mtb invades and replicates in the alveolar macrophage of the host. These macrophages cause a respiratory burst to produce a high level of ROS against Mtb [12]. It is evident by elevated levels of serum malondialdehyde, a biomarker for lipid peroxidation in TB patients [12]. Mtb antioxidant system is stronger than host ROS allowing it to survive and multiply. NO (Nitric oxide) is another important OS biomarker and is an anti-TB agent [13]. A study reports that NO synthase-deficient mice are highly susceptible to Mtb infection because NO is produced in macrophage. Therefore, high levels of NO have been observed in active TB patients [14]. Mtb has been reported to act in dose-dependent manner against ROS production. High concentration has been reported to be lethal for Mtb. Whereas, in low ROS production, Mtb stimulates the production of DNA damage-responsive genes for repair within the Mycobacteria, while the high concentration of ROS is lethal for the bacteria [15, 16].

7.3 Oxidative Stress Endurance and Emergence of Multidrug-Resistant Tuberculosis (MDR-TB)

Mtb acts against redox defense of the host through several mechanisms. The cell wall-associated lipid mycolic acid acts as a physical barrier against the host-associated OS. The difference in the mycolic acid production in phylogenetically linked Mtb affecting the interaction with the host has been demonstrated by Portevien and coworkers [17]. Various cellular mechanisms act against the redox imbalance and affect the efficiency of the specific antibiotic. A point mutation in *ndh* gene encoding for NADH II dehydrogenase results in elevated $NADH/NAD^+$, causing co-resistance to ethionamide and isoniazid. Such strains are resistant to acidified nitrites and peroxides [18]. Some of the Mtb have a specific protein called enhanced intracellular survival (Eis) which identifies and counteracts the ROS [19]. Mtb has peroxiredoxin composed of thioredoxin reductase (TrxR) and thioredoxin that can repair and alter oxidative stress through disulfide reductase activity [20, 21].

It produces antioxidant enzymes during OS, such as KatG, a catalase peroxidase and SOD (Superoxide dismutase). KatG gene is responsible for catalase, peroxidase, and peroxynitrite activity. KatG is also responsible for metalloenzymes, iron-containing SOD (SodA), and copper-zinc-containing SOD (SodC) [22]. SodA is responsible for the protection against superoxide, while SodC encodes superoxide dismutase [23]. Further analysis shows the presence of DosS and DosT as redox sensors activate the transcription factor DosR to aid anaerobic survival of Mtb and leads to a latent phase of infection [24, 25].

7.4 The Role of Immune Factors in the Development of MDR-TB

The association of multiple factors like patient age, previous anti-TB therapies, educational status, employment status, income level, socio-economic status, family history, alcohol abuse, etc. has been considered to be posing a risk for the development of MDR-TB. Recently, other independent risk factors like human immunodeficiency virus (HIV) infection, nutritional status, etc. have been linked to MDR-TB fuelling the need for the investigation of immune factors associated with the disease. Various researchers around the globe have then conducted individual studies to reveal the parallelism among immunological factors and MDR-TB.

In order to ascertain the relation between immune components and drug-resistant TB, a study was conducted on drug-resistant pulmonary TB and drug-susceptible TB pulmonary patients by En-too Sun et al. The analysis in their study revealed that the re-treatment was related to drug-resistant TB. Patients having a previous treatment history are more susceptible to multidrug-resistant TB (MDR-TB) than the ones previously untreated [26]. The first-line anti-TB drugs also seems to have little effect on re-treatment. In addition, one-third of the cases falls under the category of MDR-TB. To maintain a safe limit of the pathogen *Mycobacterium tuberculosis*, T cells and their subsets, i.e., CD4+ and CD8+, have a significant role as they produce cytokines for the containment of *Mycobacterium tuberculosis*. CD4 and CD8 together can accurately differentiate between the active TB and inactive or dormant TB [27–29]. This study has produced data to support its finding that the CD3 and CD4 are associated with drug-resistant TB and MDR-TB patients had increased levels of CD3 and CD4. Recently, the role of CD4+ T-cell subset called as "Treg" has come into play and was found to be expressed in higher concentrations in MDR-TB patients as compared to drug-susceptible patients. However, the levels of Treg dropped to healthy control level following a 6-month anti-TB treatment.

T-regulatory cells (Treg) have also been studied by another group of researchers where they compared the relative frequencies of Treg in drug-susceptible and MDR-TB, both before and after anti-TB treatment. Flow cytometry was used to measure circulatory Treg by utilizing the CD4 cell surface marker and FoxP3 intracellular marker. Treg was found to be in almost the same concentrations in DS-TB and MDR-TB patients and was much higher than the control patients. Prior to treatment, it was present in sufficiently high amounts in active infection stage. Posttreatment, the frequency of Treg normalized to the level of control in both MDR-TB and DS-TB patients [30].

In accordance with the reports of L. Geffner et al. [31] the immune response to different strains of MDR-TB is dependent upon the equilibrium between the ability of the strains to drive Th1 and Th2 profiles and the individual response of the host [31]. They considered two strains of *Mycobacterium tuberculosis*, i.e., Ra and M, and evaluated the produced immune response in peripheral blood mononuclear cells. The patients studied were both with active MDR-TB or DS-TB and healthy controls. Through their study, they unveiled that strain M was a weaker IFN-γ (gamma-interferon) inducer as compared to H37Rv for control group, but it could provoke the

highest IL-4 (interleukin-4) expression in CD4+ and CD8+ T-cells from MDR-TB and DS-TB coupled with an extremely low CTL (cytotoxic T lymphocytes) activity in diseased and control groups. This CTL impairment is a characteristic feature of strain M, and in this way it evades the effectors of macrophages by M-specific CTL effectors. Also, they revealed an increase in Treg cells upon stimulation by *M. tuberculosis*.

Achkar et al. and J. Chan et al. independently suggested the roles of B cells and antibodies of humoral immune response and the mechanisms by which they provide protection against the infections caused by *Mycobacterium tuberculosis* [32, 33].

IgM is also studied in association with DR-TB with IgM concentration being directly proportional to the magnitude of infection. The reason for the elevation of only IgM and not any other antibodies in DR-TB patients became a topic of interest of many researchers. Further studies explained the reason for these differences in antibodies. According to them, IgM is the first Ab of the humoral immunity which is synthesized and secreted, and the patients were possibly in that stage of humoral immunity when they were under consideration. Other factors regulating the IgM response includes the state of infection, *i.e.*, active/dormant, bacillary load affecting IgG response and subsequently IgM response, TB recurrence, individual differences at the genetic level, nutritional status, etc. [34].

At the time of chronic or advanced-stage DR-TB, the bacillary load is very high which stimulates the production of antigens leading to a dysregulation of T-cell homeostasis. Hence, the bacillary count is indicative of MDR-TB [35].

7.5 Genetic Complexities in the Development of MDR-TB

Mtb is an obligate pathogen existing in nature for millions of years now. Its metabolism and physiology have adapted itself so well to the environment and in the human host all through these years that it now becomes extremely challenging to propose a therapy against them [36, 37]. Mtb uses a wide range of mechanisms to evade the drug treatment complicating the possibility to design new therapeutics against MDR-TB. In Mtb, the acquisition of drug resistance is related to SNPs (Single nucleotide polymorphisms), multinucleotide polymorphism, rearrangement, insertion, and deletion in the genes coding for the drug targets, drug efflux systems, and the enzymes that are responsible for metabolizing prodrugs into active drugs [38–40].

In context to antibiotic resistance in the bacteria, the role of DNA repair systems has emerged since they seem to have a direct influence on the mutations occurring in the bacteria. Any sort of impairment in DNA repair system alters the repair mechanisms of damaged DNA, thereby enhancing the rate of mutation [41, 42] and the survivability of the bacteria under stressful conditions. M. Ebrahimi-Rad et al. [43] studied the correlation among mutations in the repair mechanism, hypermutator phenotypes, and the tendency to develop antibiotic resistance in the

Beijing lineage [43]. Unique polymorphisms in three anti-mutator genes (mut) were observed due in part to missense mutations in the mut genes. Confirmatory studies from the whole-genome sequencing have also hypothesized that Beijing lineages are more variable in comparison with the non-Beijing ones. In this parameter, some genes of the DNA repair proteins were found to be variable too fuelling the requirement for further study.

Chromosomal mutations in certain genes are a prominent cause of drug-resistant TB [44]. This mutation-mediated resistance can be validated by tests like Genotype MTBDR-plus v2 [45], AID-TB resistance line probe assay [46], etc., but these tests are less sensitive for hetero-resistant strains when the mutant frequency drops below 5–50% [47, 48]. Accurate identification of chromosomal mutation can be done using whole-genome sequencing (WGS) of the isolates. The predictive power of WGS is so high that it differentiates the strains on the basis of the resistor types [49].

Exposure to certain antibiotics like fluoroquinolones (primary mode of action: damage to DNA) can significantly raise the risk of mutation. Fluoroquinolones, in particular, generate ds-DNA breaks by targeting DNA gyrase and generating transcriptional modifications in the genes involved in DNA repair and in preserving the integrity of genome [50]. The stimulation of DNA repair clusters in response to fluoroquinolones has been studied in *Mtb* using genome-wide expression studies [51, 52].

Another genetic factor, an SOS-induced DNA polymerase DnaE2, does not have its own proofreading activity causing mutations. DnaE2 mediates induced mutagenesis rate up to 20–50 times and is a major factor for the drug resistance stage in *Mtb*. Mutations in the genes gyrA and gyrB have shown to produce cross-resistance to multiple fluoroquinolones [53]. Also, rpoB mutations confer cross-resistance to other rifamycins. In another such example, a mutation in RV0678, a transcriptional regulator results in cross-resistance to clofazimine, a leprosy drug widely used in the treatment of DR-TB, and to Bedaquiline, a newly accepted MDR-TB treatment drug.

Bacterial genetics alone cannot be blamed for the resistance, host variation has a major hand in it too. Gene polymorphisms which code for drug-metabolizing enzymes or transmembrane transporters too are attributed to be linked to resistance development to TB treatment [54]. The key transporters coded by multidrug-resistant MDR1 (ABCB1 gene) which are involved in drug uptake and efflux are named p-glycoproteins (P-gp). They are located in the kidney, colon, liver, and placenta leucocytes, and they also shield the tissues from cytotoxic agents, xenobiotics, and other physiologically active substances [55]. Various single nucleotide polymorphisms (SNPs) of the gene ABCB1 have been identified, mutations in amino acids of which can alter the mRNA stability and thereby the P-gp expression [56]. This has a direct influence on the drug pharmacology and hence on the bioavailability of the drug [57]. The expression levels and functionality of ABCB1 gene and its products exert an influence on the response to any treatment. This study conducted by Y. Pontual et al. [58] was the first in its own which correlated the combination of ABCB1 gene and SNPs to the drug resistance in TB patients [58, 59].

7.6 Targeting Virulence: A New Paradigm for Antimicrobial Therapy

With the advancement of every antibiotic drug, there is a significant antibiotic resistance associated with the clinical nature of the drug. Consequently, the need for treatment of antibiotic resistance has surpassed the production of new antibiotics. Researchers have been interested in the study of virulence factors responsible for the damage to the host and the progression of the disease [60]. An alternative approach for the treatment of resistance rather than conventional antibiotics is to target the salient functions essential for causing infection. This approach would be beneficial in preserving the endogenous microbiome of the host, enhancing the repository of bacterial targets and imposing less selective pressure that would lead to decreased resistance. Since the last three decades, there has been a widespread enhancement in the production of novel antibiotics for the treatment of diseases such as tuberculosis [60]. Scientists are in the race to discover novel antimicrobial targets against antibiotic-resistant strains. Antibiotics have been widely classified according to their ability to inhibit growth (bacteriostatic) and to kill bacteria (bactericidal). Essential bacterial functions such as DNA replication, RNA transcription, cell wall synthesis, and protein synthesis are inhibited by these drugs [61]. However, the action of these drugs leads to selective pressure that would facilitate the growth of antibiotic-resistant strains. Hence, there is a wide gap between the treatment of such strains and the production of new drugs posing a threat to post antibiotic era. Moreover, it is necessary to discover novel modes of action against such strains [62].

The tremendous effort of understanding the mechanism of bacteria causing diseases would lead to the study of pathogens that could be targeted to eradicate the infection in lieu of targeting in vitro bacterial viability. The paradigm of targeting virulence has helped to disrupt the interactions between the host and the pathogen. Virulence is defined as the ability of a pathogen to cause a particular disease with compliance to the conventional concept [61]. Bacterial factors such as toxins, proteases, and cytolysins and the mechanisms that actively cause damage to host tissues are defined as virulence determinants. Inhibition of these virulence factors by developing anti-virulence therapies is a new approach for disarming the pathogens that have direct harm on the host. Targeting virulence in vivo, compounds are created with live and attenuated strain. This therapeutic approach would lead to the clearance of harmful pathogens by the host immune response with limited impact on human microbiota. Inhibiting virulence by new antimicrobials poses a potential advantage rather than inhibiting growth; this would impose lesser selective pressure for the development of antibiotic resistance [60] (Fig. 7.1).

7.6.1 Strategies for the Paradigm of Targeting Virulence

7.6.1.1 Inhibition of Toxin Function

Damage to the host tissue by bacteria is caused by the release of toxins. These toxins are released in the form of proteins that disrupt the cellular functions of the host and

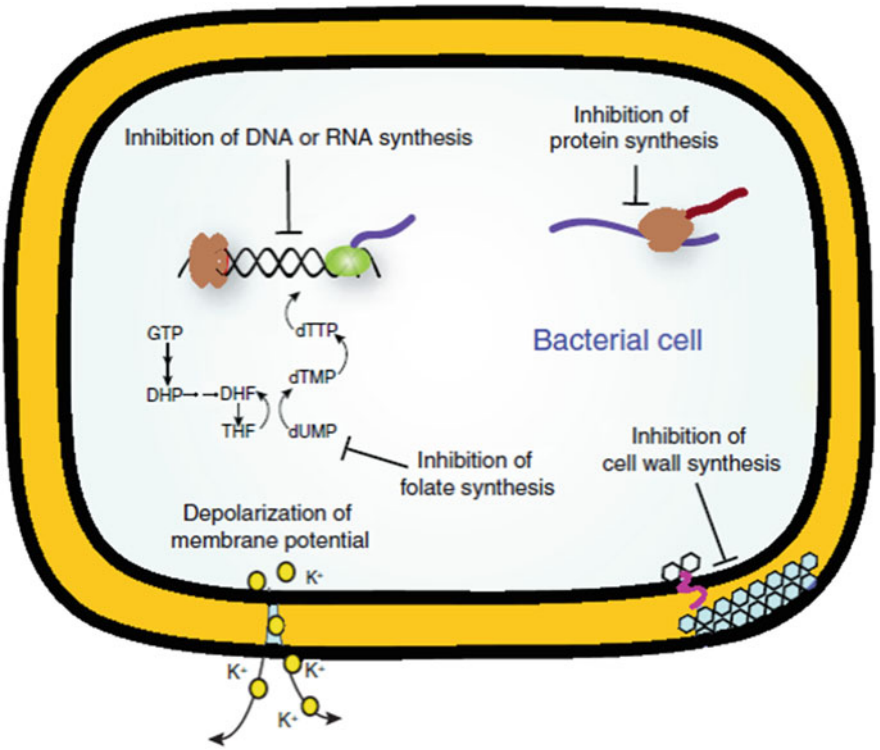


Fig. 7.1 Targets of antibacterial compounds. These antibiotic compounds function by inhibiting protein synthesis (e.g., aminoglycosides), cell wall synthesis (e.g., β-lactams), DNA or RNA synthesis (e.g., fluoroquinolones), folate synthesis (e.g., sulfa drugs), or depolarizing membrane potential (daptomycin) [60]

ultimately lead to cell death [60]. There could be various approaches for the inhibition of bacterial virulence that tends to disrupt the toxicity of the function incurred. In the direct approach, the inhibition of toxicity occurs by itself, whereas in indirect approach, modulation of cellular responses to the toxins is incurred. Antibodies against tuberculosis are examples of direct inhibition of toxins using a conventional method. Many efforts have been made to withstand the effects of three major proteins involved in tuberculosis infection such as edema factor (EF), lethal factor (LF), and protective antigen (PA) [60]. However, the presence of each component of the toxins would not cause any effect, but rather the synergism of any of the two out of three toxins are responsible for the disease pathology. The mechanism of toxins infusion into the mammalian cells occurs through the diffusion of PA monomers into the surface of the cells where proteolytic cleavage happens by host proteases [61]. Oligomerization of cleaved PA into heptamers occurs where PA binds to either LF or EF. Endocytosis over the cell surface occurs where the complexes such as

PA-EF and PA-LF are trafficked to the endosome. This results in the decrease in pH of the cell membrane which causes a conformational change in the PA to transfer to the transmembrane pore. Respective toxic effects of LF and EF are exerted when they are translocated through PA pore into the host cytosol [61] (Fig. 7.2).

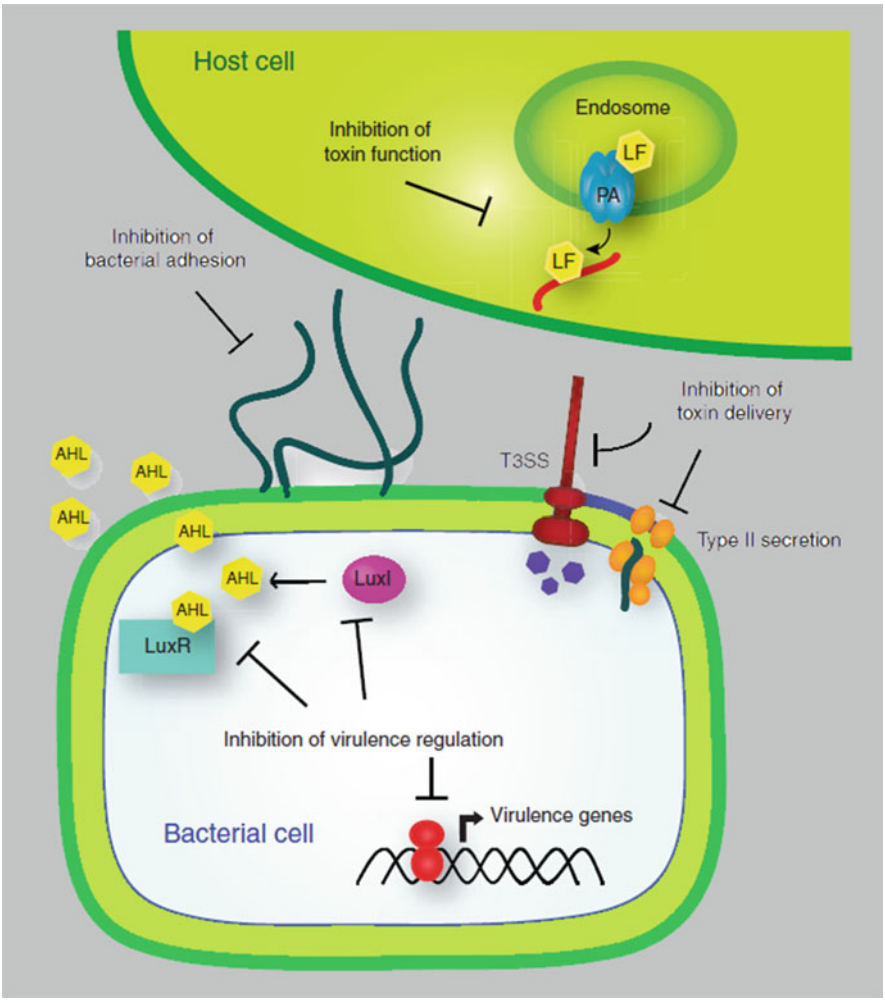


Fig. 7.2 Virulence inhibitors targeting toxic functions. The various functions that could be targeted to inhibit virulence include toxin delivery, targeting and inhibiting various bacterial systems such as type II or type III secretion (T3SS), inhibiting transcriptional regulators that control virulence gene expression, inhibiting the regulation of virulence gene (e.g., AHL-mediated quorum sensing circuitry (LuxI or LuxR homologs)), inhibiting toxin function (e.g., *B. anthracis* LF catalytic activity or translocation through PA), or bacterial adhesion to host cells (e.g., inhibition of the formation of pilicides) [60]

7.6.1.2 Targeting the Regulation of Virulence Expression or Inhibition of Quorum Signaling

It is preferred to disrupt the regulation of the mechanism of toxin action in regard to target toxin function or delivery that would prevent the formation of the toxin. Quorum signaling or sensing is a mode of bacterial communication found to be used by many bacterial species to regulate their essential processes such as biofilm formation, bioluminescence, virulence factor expression, etc. [62]. In order to sense their population density, bacteria utilize quorum sensing phenomenon. They release diffusible signaling molecules that get accumulated in the population and the surrounding environment. Up to a certain threshold concentration of these molecules, the bacterial population increases [61]. Various genes get expressed which are involved in the virulence at the critical threshold concentrations. In order to cause infection, the bacteria express certain virulence factors when they reach a significantly large population size. Therefore, bacterial virulence mechanism could be inhibited by interfering with the quorum sensing pathways [61].

In *M. tuberculosis*, this phenomenon is mediated by acyl homoserine lactone molecules. They are synthesized and recognized by quorum sensing circuits composed of Lux1 and LuxR homologs. Through the common metabolite intermediate *S*-adenosylmethionine, AHL molecules are synthesized by Lux1 homologs and acyl-acyl carrier protein. Genes associated with the virulence are triggered by the binding of AHL molecules with their respective transcriptional activator at critical threshold concentrations. Thus, by inhibiting the enzymes responsible for the synthesis of Lux1 homologs, one could inhibit the AHL-mediated quorum sensing [60]. Another way of inhibiting quorum sensing is that by altering the concentrations of AHL signaling molecules through degradation. Some species of *Mycobacterium* produce acyl homoserine lactonase enzyme which could hydrolyze the lactone ring of AHLs. This results in the alteration of signaling molecules [60].

7.6.1.3 Targeting Oxidative Phosphorylation in *Mycobacterium tuberculosis*

Using the oxidative phosphorylation pathway, bacteria can synthesize ATP molecules by substrate-level phosphorylation of fermentable carbon sources. They need oxidative phosphorylation for their growth [60]. A proton motive force is generated during oxidative phosphorylation across the biomembrane. This energy is utilized by the ATP synthase for the production of ATP. In *M. tuberculosis*, the production of ATP through the inflow of electrons and maintenance of proton motive force is essential for survival and growth [62]. As virulence target, one could employ small molecule inhibitors for oxidative phosphorylation which could block the production of ATP by preventing respiratory electron transport and breaking down of proton motive force. Some of the drugs which can act as small molecule inhibitors are discussed below [62].

In bacteria, type II NADH dehydrogenase acts as the entry point of electrons into the respiratory chain and can be targeted by phenothiazine drugs. Clofazimine is used as an anti-TB drug. Other drugs approved for the treatment of TB are antipsychotic drugs such as thioridazine. ATP synthase of *Mycobacterium* can be inhibited

by the small molecules of diarylquinoline class. Selective inhibition of oxidative phosphorylation may decrease the virulence. Targeting oxidative phosphorylation pathway has been regarded as a tool for eradicating virulence factors as this pathway is highly conserved between the eukaryotes and prokaryotes. Studies have shown that imidazopyridine causes significant inhibition of mycobacterial growth at non-molar concentrations [61] (Fig. 7.3).

7.6.1.4 Dismantling Bacterial Membrane Microdomains

In order to overcome the multidrug-resistant pathogens in *M. tuberculosis*, the suitable new therapeutic strategies are to design antimicrobial compounds that could target bacterial structures [62]. The bacterial cytoplasmic membrane acts as a protective barrier for the maintenance of intracellular physical chemical conditions for bacterial metabolism. It helps in the exchange of the information and substances with the extracellular domain. Hence, certain changes in the cellular membrane could disrupt its integrity and prevent the bacteria from causing virulence. Functional membrane microdomains are present in bacterial membranes which are lipid raft-like domains. They contain a characteristic lipid and protein content [62]. They are highly rich in polyisoprenoid lipids such as carotenoids and hopanoids imparting rigidity and compact features to the hydrophobic nature of FMM-associated proteins to prevent their diffusion away from them. Flotillins are essential FMM components that are present in the bacterial membrane [62]. They are involved in the regulation of membrane fluidity and are closely associated with microdomains and help in stabilizing and promoting the association of specific protein complexes via their scaffold activity. Some FMM-associated proteins play a vital role in protein secretion machinery signaling networks and proteolytic complexes [61].

7.6.1.5 Preventing Biofilm Formation

Biofilm is a structure formed by bacteria using extracellular polymeric substances such as protein, DNA, and polysaccharides. Colonization and dispersion of bacterial colonies take place through biofilm formation [61]. This helps in protecting bacteria against environmental stresses such as antibiotics and host defense components. *Mycobacterium tuberculosis* form biofilm over clinical devices such as medical devices, implants, endotracheal tubes, catheter, and others. Formation of biofilm on these surfaces acts as a source of infection. These biofilms can cause chronic infection and limit the success of antibiotics [61]. Many of the antibiotics available today fail to affect biofilm formation. New therapies to treat biofilm consist of organic and inorganic chemical compounds. Some anti-virulence compounds are used to inhibit the formation of biofilms in order to limit the bacterial adhesion on surfaces [61]. This affects the production of the extracellular matrix. In this process, it interferes with compounds that form flagella like structures that ensures the mobility of the bacteria and interaction with the surfaces through which the bacterial adhesion occurs. Coumarins from many medicinal plants have shown to reduce the biofilm formation. TCC or tetrachlorosalicylanilide is a chemical compound that inhibits the biofilm formation in *Mycobacterium* by interfering with the SinR protein that is involved in extracellular matrix production [61].

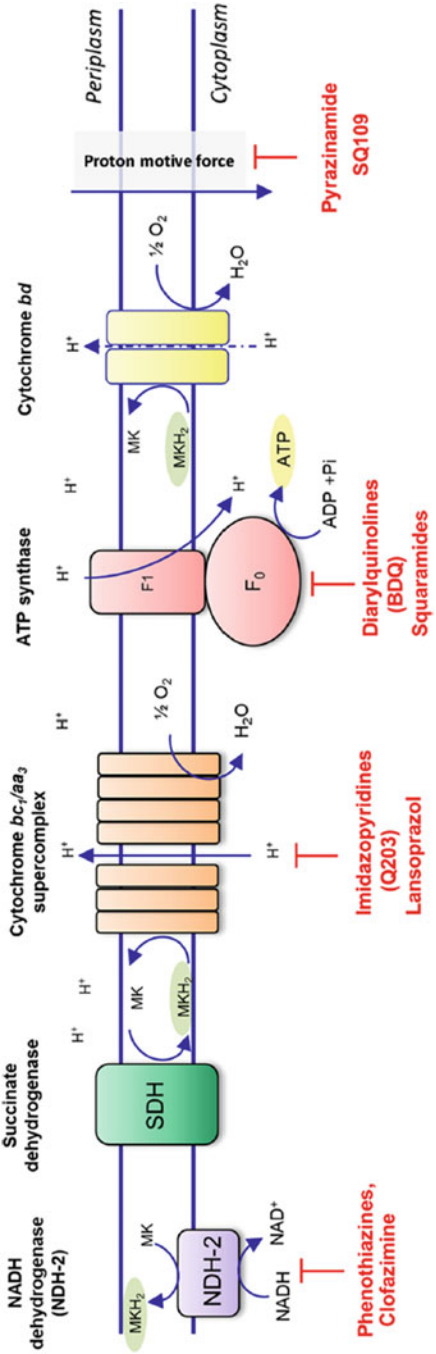


Fig. 7.3 Oxidative phosphorylation in *Mycobacterium tuberculosis*. Electrons that are derived from NADH are transferred into the electron transport chain by NADH dehydrogenase, leading to the reduction of the menaquinone pool (MK/MKH2). Type 1 NADH dehydrogenase is a homolog of complex I in mitochondria in *M. tuberculosis* which is dispensable for growth. Type II NADH dehydrogenase (NDH-2) is present in two copies in *M. tuberculosis*. Alternative electron donors reduce menaquinone via succinate dehydrogenase (SDH). There are two succinate dehydrogenase enzymes (Sdh-1 and Sdh-2) and one fumarate reductase present in *M. tuberculosis* which catalyze the reverse reaction. Electrons can be transferred to cytochrome bc1 complex from the menaquinone pool. With the aid of cytochrome aa3-type terminal oxidase, the cytochrome bc1 in mycobacteria transfers the electrons onto oxygen. In the alternative case, cytochrome bd-type terminal oxidase reduces the oxygen which directly accepts electrons from the menaquinone pool. A proton motive force is generated when protons are pumped across the membrane during electron transport along the respiratory chain. The energy of the PMF can be used by ATP synthase for the synthesis of ATP [62]

7.7 Future Prospects

The burden of MDR-TB/XDR-TB is increasing due to inadequate monitoring and lack of proper diagnosis and treatment [63]. The statistics of MDR-TB cases around the globe is worrisome, and the number of new cases is exceeding. The current treatment strategies are expensive and more toxic in comparison with the therapies for drug-susceptible TB. Hence, the introduction of a novel, reliable, nontoxic, easy-to-implement treatment strategies is the need of the hour.

Gene therapy is an emerging and promising tool for the treatment of various diseases. CRISPR (clustered regulatory interspaced short palindromic repeats) is one of the most widely accepted genome editing tool owing to its simplicity, robustness, and ease of implementation. CRISPR was used to decrease the expression of *Mycobacterium tuberculosis* up to >80% and to inhibit the expression of many other related genes. This was proven to accelerate the development of new therapies for *Mycobacterium tuberculosis* [64, 65].

According to D. Sharma and Bisht [66], iron is required as a functional element for the drug resistance in *Mtb*. Bacterioferritin and ferritin, the iron storage proteins, were shown to be overexpressed in the drug resistance stage [66]. These proteins along with their binding partners like Rv1877, trigger factor, MT3948 (transcription regulatory protein), MT1928, MT3947, glnA3 (glutamine synthetase), etc. regulate the growth, antibiotic resistance (particularly aminoglycoside), homeostasis, etc. in *Mtb*. Hence, these proteins can be a good source of information to produce novel drugs for the drug-resistant TB. Another beneficial approach could be nano-formulations with specific ligands which would attack a specific target to produce a therapeutic response. An example of it is the work done by Lemmer et al. [67] who used mycolic acid as a ligand and isoniazid PLGA nanoparticles for drug targeting [67]. The *Mycobacterium*-infected macrophages were observed to increasingly take up these mycolic acid NPs which makes mycolic acid a potential target ligand for further development of any effective formulation.

The effectiveness of a treatment can be determined using randomized controlled trials (RCTs). But, RCTs face a major challenge of the lack of accuracy of disease confirmation due to lesser site capacity and other diagnostic difficulties. This should be surmounted to maximize the effectiveness of a drug prepared after a certain diagnostic observation has been drawn. MODS (microscopic observation drug susceptibility) assay has been developed as an accurate, rapid diagnostic assay for TB detection in sputum. However, the policymakers still need to address the issue of affordability, scale-up, implementation to wider health groups, and the optimisation of the detection tests. This will help in enhancing the epidemiological footprints of disease prevention [68]. It has been a challenge to effectively treat MDR-TB since there is a dire requirement of at least four drugs which could keep up with the current magnitude of XDR/MDR-TB. The market is still deficient in the powerful third-line drugs to cure MDR-TB. Currently available third-line drugs include linezolid, clofazimine, imipenem plus cilastatin, amoxicillin plus clavulanate, and clarithromycin; however, their efficacy is still lagging and their exact roles are not clear [69]. Also, since the second-line drugs are costly for the majority of the

population of most of the middle- and low-income countries, to afford the third line drugs for the treatment seems an unattainable dream. Hence, this needs to be worked upon.

Scale-up, speed, and pragmatism remain underworked upon with respect to the treatment regimens. So, the policymakers, agencies, and different research groups are working on its applications in future regimens. Early and quick detection; allocation of safe, affordable, and accessible third-line drugs; and monitoring the potential patients who could transmit the disease are some of the pivotal issues to be addressed in order to decrease the prevalence of MDR-TB globally.

7.8 Conclusion

Oxidative stress caused by host macrophage plays an essential role in the prevention of the growth and development of the Mtb. Some drug-resistant strains of the *Mycobacterium* inhibit the oxidative stress burst via mycolic acid in the cell wall; antioxidant enzyme system including SOD, KatG, and NADH/NAD^+ ratio, etc. However, several mechanisms are involved in the emergence of drug resistance, which are not completely understood and need immediate attention. Drug resistance is attributed to growth deficits incurred by resistance mutations, strain genetic background, and compensatory evolution. The growing threat of MDR-TB promotes the need to study the complexities of drug resistance, diagnosis, and anti-TB drugs in order to provide a new direction to the tuberculosis clinics.

References

1. World Health Organization (2018) Global tuberculosis report 2018. WHO, Geneva. Available at: https://www.who.int/tb/publications/global_report/en/
2. Jordao L, Bleck CK, Mayorga L, Griffiths G, Anes E (2008) On the killing of mycobacteria by macrophages. Cell Microbiol 10(2):529–548
3. De Chastellier C (2009) The many niches and strategies used by pathogenic mycobacteria for survival within host macrophages. Immunobiology 214(7):526–542
4. Matteelli A, Sulis G, Capone S, D'Ambrosio L, Migliori GB, Getahun H (2017) Tuberculosis elimination and the challenge of latent tuberculosis. Presse Med 46(2):e13–e21
5. O'Toole R (2010) Experimental models used to study human tuberculosis. Adv Appl Microbiol 71:75–89
6. Shastri MD, Shukla SD, Chong WC, Dua K, Peterson GM, Patel RP et al (2018) Role of oxidative stress in the pathology and management of human tuberculosis. Oxid Med Cell Longev 2018:7695364
7. Dua K, Malyla V, Singhvi G, Wadhwa R, Krishna RV, Shukla SD et al (2019) Increasing complexity and interactions of oxidative stress in chronic respiratory diseases: an emerging need for novel drug delivery systems. Chem Biol Interact 299:168
8. Dua K, Rapalli VK, Shukla SD, Singhvi G, Shastri MD, Chellappan DK et al (2018) Multi-drug resistant Mycobacterium tuberculosis & oxidative stress complexity: emerging need for novel drug delivery approaches. Biomed Pharmacother 107:1218–1229

9. Mehta M, Sharma N, Vyas M, Khurana N, Maurya PK, Singh H et al (2019) Interactions with the macrophages: an emerging targeted approach using novel drug delivery systems in respiratory diseases. Chem Biol Interact 304:10
10. Chong W, Shastri M, Eri R (2017) Endoplasmic reticulum stress and oxidative stress: a vicious nexus implicated in bowel disease pathophysiology. Int J Mol Sci 18(4):771
11. Holguin F (2013) Oxidative stress in airway diseases. Ann Am Thorac Soc 10(Suppl):S150–S157
12. Goyal N, Kashyap B, Singh N, Kaur IR (2017) Neopterin and oxidative stress markers in the diagnosis of extrapulmonary tuberculosis. Biomarkers 22(7):648–653
13. Jamaati H, Mortaz E, Pajouhi Z, Folkerts G, Movassaghi M, Moloudizargari M et al (2017) Nitric oxide in the pathogenesis and treatment of tuberculosis. Front Microbiol 8:2008
14. Idh J, Andersson B, Lerm M, Raffetseder J, Eklund D, Woksepp H et al (2017) Reduced susceptibility of clinical strains of Mycobacterium tuberculosis to reactive nitrogen species promotes survival in activated macrophages. PLoS One 12(7):e0181221
15. Voskuil MI, Bartek I, Visconti K, Schoolnik GK (2011) The response of Mycobacterium tuberculosis to reactive oxygen and nitrogen species. Front Microbiol 2:105
16. Wu Y, Gulbins E, Grassmé H (2018) Crosstalk between sphingomyelinases and reactive oxygen species in mycobacterial infection. Antioxid Redox Signal 28(10):935–948
17. Portevin D, Sukumar S, Coscolla M, Shui G, Li B, Guan XL et al (2014) Lipidomics and genomics of Mycobacterium tuberculosis reveal lineage-specific trends in mycolic acid biosynthesis. Microbiologyopen 3(6):823–835
18. Vilcheze C, Weisbrod TR, Chen B, Kremer L, Hazbón MH, Wang F et al (2005) Altered NADH/NAD+ ratio mediates coresistance to isoniazid and ethionamide in mycobacteria. Antimicrob Agents Chemother 49(2):708–720
19. Awuh JA, Flo TH (2017) Molecular basis of mycobacterial survival in macrophages. Cell Mol Life Sci 74(9):1625–1648
20. Jaeger T (2007) Peroxiredoxin systems in mycobacteria. In: Peroxiredoxin systems. Springer, New York, NY, pp 207–217
21. Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66:75–87
22. Li Z, Kelley C, Collins F, Rouse D, Morris S (1998) Expression of katG in Mycobacterium tuberculosis is associated with its growth and persistence in mice and guinea pigs. J Infect Dis 177(4):1030–1035
23. Zhang Y, Lathigra R, Garbe T, Catty D, Young D (1991) Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of Mycobacterium tuberculosis. Mol Microbiol 5 (2):381–391
24. Piddington DL, Fang FC, Laessig T, Cooper AM, Orme IM, Buchmeier NA (2001) Cu, Zn superoxide dismutase of Mycobacterium tuberculosis contributes to survival in activated macrophages that are generating an oxidative burst. Infect Immun 69(8):4980–4987
25. Sivaramakrishnan S, Ortiz de Montellano P (2013) The DosS-DosT/DosR mycobacterial sensor system. Biosensors 3(3):259–282
26. Liu Q, Zhu L, Shao Y, Song H, Li G, Zhou Y et al (2013) Rates and risk factors for drug resistance tuberculosis in Northeastern China. BMC Public Health 13(1):1171
27. da Silva MV, Massaro Junior VJ, Machado JR, Silva DA, Castellano LR, Alexandre PB et al (2015) Expression pattern of transcription factors and intracellular cytokines reveals that clinically cured tuberculosis is accompanied by an increase in Mycobacterium-specific Th1, Th2, and Th17 cells. Biomed Res Int 2015:591237
28. Hermans SM, van Leth F, Kiragga AN, Hoepelman AI, Lange JM, Manabe YC (2012) Unrecognised tuberculosis at antiretroviral therapy initiation is associated with lower CD4+ T cell recovery. Trop Med Int Health 17(12):1527–1533
29. Rozot V, Patrizia A, Vigano S, Mazza-Stalder J, Idrizi E, Day CL et al (2014) Combined use of mycobacterium tuberculosis–specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. Clin Infect Dis 60(3):432–437

30. Lim H-J, Park JS, Cho Y-J, Yoon HI, Park KU, Lee C-T et al (2013) CD4+ FoxP3+ T regulatory cells in drug-susceptible and multidrug-resistant tuberculosis. Tuberculosis 93 (5):523–528
31. Geffner L, Yokobori N, Basile J, Schierloh P, Balboa L, Romero MM et al (2009) Patients with multidrug-resistant tuberculosis display impaired Th1 responses and enhanced regulatory T-cell levels in response to an outbreak of multidrug-resistant Mycobacterium tuberculosis M and Ra strains. Infect Immun 77(11):5025–5034
32. Achkar JM, Chan J, Casadevall A (2015) Role of B cells and antibodies in acquired immunity against Mycobacterium tuberculosis. Cold Spring Harb Perspect Med 5(3):a018432
33. Chan J, Mehta S, Bharrhan S, Chen Y, Achkar JM, Casadevall A et al (2014) The role of B cells and humoral immunity in Mycobacterium tuberculosis infection. Semin Immunol 26 (6):588–600
34. Hur Y-G, Kim A, Kang YA, Kim AS, Kim DY, Kim Y et al (2015) Evaluation of antigen-specific immunoglobulin g responses in pulmonary tuberculosis patients and contacts. J Clin Microbiol 53(3):904–909
35. Bose M, Gupta A, Banavalikar J, Saha K (1995) Dysregulation of homeostasis of blood T-lymphocyte subpopulations persists in chronic multibacillary pulmonary tuberculosis patients refractory to treatment. Tuber Lung Dis 76(1):59–64
36. Gagneux S (2018) Ecology and evolution of Mycobacterium tuberculosis. Nat Rev Microbiol 16(4):202
37. Hartman TE, Wang Z, Jansen RS, Gardete S, Rhee KY (2017) Metabolic perspectives on persistence. Microbiol Spectr 5(1):TBTB2-0026-2016
38. Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z et al (2014) Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis. Lancet Respir Med 2(4):321–338
39. Koch A, Mizrahi V, Warner DF (2014) The impact of drug resistance on Mycobacterium tuberculosis physiology: what can we learn from rifampicin? Emerg Microb Infect 3(1):1–11
40. Yadon AN, Maharaj K, Adamson JH, Lai Y-P, Sacchettini JC, Ioerger TR et al (2017) A comprehensive characterization of PncA polymorphisms that confer resistance to pyrazinamide. Nat Commun 8(1):588
41. Chopra I, O'Neill AJ, Miller K (2003) The role of mutators in the emergence of antibiotic-resistant bacteria. Drug Resist Updat 6(3):137–145
42. Martinez J, Baquero F (2000) Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother 44(7):1771–1777
43. Rad ME, Bifani P, Martin C, Kremer K, Samper S, Rauzier J et al (2003) Mutations in putative mutator genes of Mycobacterium tuberculosis strains of the W-Beijing family. Emerg Infect Dis 9(7):838
44. Gygli SM, Borrell S, Trauner A, Gagneux S (2017) Antimicrobial resistance in Mycobacterium tuberculosis: mechanistic and evolutionary perspectives. FEMS Microbiol Rev 41(3):354–373
45. Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Omar SV et al (2016) Multicenter noninferiority evaluation of Hain GenoType MTBDRplus version 2 and Nipro NTM+ MDRTB line probe assays for detection of rifampin and isoniazid resistance. J Clin Microbiol 54(6):1624–1630
46. Ritter C, Lucke K, Sirgel F, Warren R, van Helden P, Böttger E et al (2014) Evaluation of the AID TB resistance line probe assay for rapid detection of genetic alterations associated with drug resistance in Mycobacterium tuberculosis strains. J Clin Microbiol 52(3):940–946
47. Engström A (2016) Fighting an old disease with modern tools: characteristics and molecular detection methods of drug-resistant Mycobacterium tuberculosis. Infect Dis 48(1):1–17
48. Streicher E, Bergval I, Dheda K, Böttger E, van Pittius NG, Bosman M et al (2012) Mycobacterium tuberculosis population structure determines the outcome of genetics-based second-line drug resistance testing. Antimicrob Agents Chemother 56(5):2420–2427
49. Gygli SM, Keller PM, Ballif M, Blöchliger N, Hömke R, Reinhard M et al (2019) Whole-genome sequencing for drug resistance profile prediction in mycobacterium tuberculosis. Antimicrob Agents Chemother 63(4):e02175–e02118

50. Fonseca J, Knight G, McHugh T (2015) The complex evolution of antibiotic resistance in Mycobacterium tuberculosis. Int J Infect Dis 32:94–100
51. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE (2004) The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism novel insights into drug mechanisms of action. J Biol Chem 279(38):40174–40184
52. O'Sullivan DM, Hinds J, Butcher PD, Gillespie SH, McHugh TD (2008) Mycobacterium tuberculosis DNA repair in response to subinhibitory concentrations of ciprofloxacin. J Antimicrob Chemother 62(6):1199–1202. https://doi.org/10.1093/jac/dkn387
53. Willby M, Sikes RD, Malik S, Metchock B, Posey JE (2015) Correlation between GyrA substitutions and ofloxacin, levofloxacin, and moxifloxacin cross-resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 59(9):5427–5434
54. Beceiro A, Tomás M, Bou G (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26(2):185–230
55. Katayama K, Noguchi K, Sugimoto Y (2014) Regulations of P-glycoprotein/ABCB1/MDR1 in human cancer cells. New J Sci 2014:476974
56. Tovilla-Zárate CA, Vargas I, Hernández S, Fresán A, Aguilar A, Escamilla R et al (2014) Association study between the MDR1 gene and clinical characteristics in schizophrenia. Rev Bras Psiquiatr 36(3):227–232
57. Schinkel A (1998) Pharmacological insights from P-glycoprotein knockout mice. Int J Clin Pharmacol Ther 36(1):9–13
58. Pontual Y, Pacheco VS, Monteiro SP, Quintana MS, Costa MJ, Rolla VC et al (2017) ABCB1 gene polymorphism associated with clinical factors can predict drug-resistant tuberculosis. Clin Sci 131(15):1831–1840
59. Koch A, Cox H, Mizrahi V (2018) Drug-resistant tuberculosis: challenges and opportunities for diagnosis and treatment. Curr Opin Pharmacol 42:7–15
60. Clatworthy AE, Pierson E, Hung DT (2007) Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3(9):541
61. Martínez OF, Cardoso MH, Ribeiro SM, Franco OL (2019) Recent advances in anti-virulence therapeutic strategies with a focus on dismantling bacterial membrane microdomains, toxin neutralization, quorum-sensing interference and biofilm inhibition. Front Cell Infect Microbiol 9:74
62. Bald D, Villellas C, Lu P, Koul A (2017) Targeting energy metabolism in Mycobacterium tuberculosis, a new paradigm in antimycobacterial drug discovery. MBio 8(2):e00272–e00217
63. Singh A, Maurya A, Kant S, Umrao J, Kushwaha R, Nag V et al (2013) Rapid detection of drug resistance and mutational patterns of extensively drug-resistant strains by a novel GenoType® MTBDRsl assay. J Postgrad Med 59(3):179
64. Lino CA, Harper JC, Carney JP, Timlin JA (2018) Delivering CRISPR: a review of the challenges and approaches. Drug Deliv 25(1):1234–1257
65. Singh AK, Carette X, Potluri L-P, Sharp JD, Xu R, Prisic S et al (2016) Investigating essential gene function in Mycobacterium tuberculosis using an efficient CRISPR interference system. Nucleic Acids Res 44(18):e143–e143
66. Sharma D, Bisht D (2017) Role of bacterioferritin & ferritin in M. tuberculosis pathogenesis and drug resistance: a future perspective by interactomic approach. Front Cell Infect Microbiol 7:240
67. Lemmer Y, Kalombo L, Pietersen R-D, Jones AT, Semete-Makokotlela B, Van Wyngaardt S et al (2015) Mycolic acids, a promising mycobacterial ligand for targeting of nanoencapsulated drugs in tuberculosis. J Control Release 211:94–104
68. Moore DA (2007) Future prospects for the MODS assay in multidrug-resistant tuberculosis diagnosis. Future Microbiol 2:97
69. Zumla A, Nahid P, Cole ST (2013) Advances in the development of new tuberculosis drugs and treatment regimens. Nat Rev Drug Discov 12(5):388

Infection-Induced Oxidative Stress in Chronic Respiratory Diseases

8

Shakti D. Shukla, Kanth Swaroop Vanka, Anne Chevalier, Wai Ching Chong, Kavita Pabreja, Madhur D. Shastri, and Ronan F. O'Toole

Abstract

Globally, the burden of chronic respiratory diseases (CRDs) is increasing rapidly. These include asthma, chronic respiratory obstructive diseases (COPD) and cystic fibrosis (CF). Patients with CRDs often exhibit increased levels of oxidant burden in the lungs that is primarily due to chronic exposure to deleterious particles, including cigarette smoke, air pollution, occupational exposure to chemicals and fumes and a variety of allergens. In homeostasis, a delicate balance exists between the pro-oxidant and antioxidant molecules/entities. Both structural and immune cells, when encountering these foreign particles, generally respond by triggering pro-oxidative stress-related pathways in the lungs, thereby disturbing the pulmonary redox homeostasis. Moreover, patients with CRDs are also susceptible to frequent/recurrent microbial infections that lead to worsening of disease which often requires hospitalizations. Several pathogens, such as *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*,

S. D. Shukla (✉) · K. S. Vanka · A. Chevalier
Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute and The University of Newcastle, Newcastle, NSW, Australia
e-mail: shakti.shukla@newcastle.edu.au

W. C. Chong
Department of Molecular and Translational Science and Centre for Cancer Research, Hudson Institute of Medical Research, Monash University, Clayton, VIC, Australia

K. Pabreja
Priority Research Centre for Healthy Lungs, School of Medicine and Public Health, Hunter Medical Research Institute, The University of Newcastle, Newcastle, NSW, Australia

M. D. Shastri
School of Health Sciences, College of Health and Medicine, University of Tasmania, Launceston, TAS, Australia

R. F. O'Toole
School of Molecular Science, La Trobe University, Melbourne, VIC, Australia

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_8

Mycobacterium tuberculosis, *Aspergillus fumigatus*, etc., have the ability to elicit pro-oxidant pathways in the respiratory tract. Also, these pathogens are equipped with enzymatic and non-enzymatic mechanisms to neutralize host-associated oxidative molecules that facilitate the persistence of these pathogens in the lungs. We will discuss the CRD/pathogen-triggered oxidative stress in the lungs. We will also discuss the microbial mechanisms that may further increase oxidative stress in patients with CRDs that potentially results in the heightened inflammatory response in the lungs. Finally, we will discuss the current treatment strategies to limit the oxidative response-associated lung pathologies.

Keywords

Airway infections · Asthma · Chronic obstructive pulmonary diseases · Cystic fibrosis · Oxidative stress

8.1 Introduction

The global burden of chronic lung diseases (CRD) is rapidly increasing which presents a unique challenge to healthcare professionals to devise more effective strategies for managing these conditions. An important aspect of lung pathogenesis in patients with CRDs is the increased levels of oxidative stress [1]. The human body is equipped to maintain a delicate balance between increased oxidative stress factors and antioxidant defence mechanisms. An imbalance between oxidant-antioxidant mechanisms/factors, i.e. increases in reactive oxygen species (ROS) or reactive nitrogen species (RNS) or their intermediates, in conjunction with depletion of innate antioxidant mechanisms (such as superoxide dismutase, glutathione and catalase), leads to a condition which is generally referred to as oxidative stress (OS) [1].

A range of microbial infections has the potential to elicit oxidative burden in the lungs, which usually becomes even more effective in conjunction with downregulating the host antioxidant defence mechanisms. In this review article, we will summarize the key oxidant-antioxidant molecules, the role of oxidative stress in major CRDs (chronic obstructive pulmonary disease, asthma and cystic fibrosis) and the role of key pathogenic microbes in regulating pulmonary oxidative burden. We will then briefly discuss the roles of major co-infections in inducing the oxidative stress markers in the lungs. Finally, we will discuss the currently investigated therapeutic strategies to maintain oxidant-antioxidant homeostasis in the lungs.

8.2 Key Molecules in Oxidative Stress

8.2.1 Pro-oxidant Molecules

A number of factors elicit the oxidative response in the lungs, including but not limited to altered oxygen tension, microbial infections, various environmental factors (cigarette smoke, air pollution, occupational exposure, etc.) and systemic diseases [2]. ROS are referred to as molecules that are highly potent to either accepting or donating a free electron, which also render these oxygen-containing entities somewhat unstable with high potential reactivity with other biological molecules [3, 4]. ROS generated takes place in aerobic cells/organisms by intracellular reactions, which commences with the reduction of molecular oxygen by one electron [3]. This leads to the production of hyperoxide/superoxide O_2^-, which is highly unstable and rapidly reduced to hydrogen peroxide H_2O_2, which is relatively stable and often migratory [3]. In the presence of iron, H_2O_2 may result in highly reactive hydroxyl radical $^{\bullet}OH$ (Fenton's reaction) [3]. Oxidative burden increases when the generation of free radicals is substantially higher than the capacity of innate antioxidant defences. Several potential free radicals contributing to oxidative stress burden include alkoxyl radical ($RO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), hypochlorite anion (OCl^-), singlet oxygen ($^1O_2$), ozone (O_3), nitric oxide ($^{\bullet}NO$), peroxynitrite ($ONOO^-$), nitrogen dioxide ($^{\bullet}NO_2$) and nitrogen oxides (NOx) [4].

8.2.2 Key Antioxidant Molecules

The respiratory airway epithelium effectively counters the exogenous oxidative burden by utilizing innate antioxidant defence system. Major molecules with antioxidative activity include glutathione (GSH); vitamins A, C and E; uric acid; β-carotene; and a variety of antioxidant enzymes (e.g. superoxide dismutase, catalase and peroxidases). Notably, GSH is one of the most important antioxidant mechanisms in lung epithelium [5].

8.3 Microbial Infections in Chronic Respiratory Diseases

CRDs, including asthma, chronic obstructive respiratory disease (COPD) and cystic fibrosis (CF), are highly heterogeneous lung conditions that are further complicated by frequent microbial infections, which are often considered as major comorbidities in these difficult-to-treat diseases [6]. The major pathogens implicated in lung infections include viruses (rhinoviruses, respiratory syncytial virus, influenza virus and metapneumoviruses), bacteria (*Streptococcus pneumonia*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*), fungi (*Aspergillus fumigatus* and *Candida albicans*) and atypical bacteria (*Chlamydia pneumonia*) [7]. The generation of OS is an important and one of the initial mechanisms to limit the damage caused by microbial pathogens in

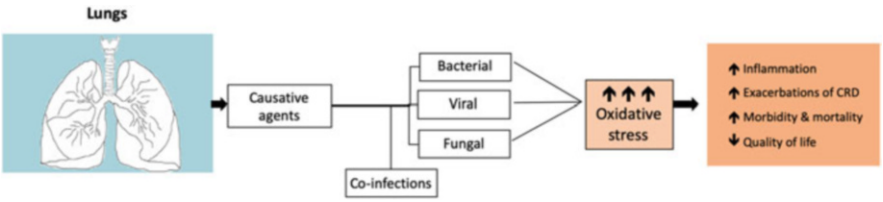


Fig. 8.1 Infection-induced oxidative stress in the lung and associated pathology in chronic respiratory diseases

the lungs. However, the microorganisms have developed strategies, especially enzymatic, to counter the host OS [8]. Moreover, these acute or chronic infections often contribute to a heightened burden of OS in the host that leads to tissue damage resulting in colonization/multiplication of pathogens in the lung [9]. Co-infections with more than one pathogen are extremely deleterious for the host. For instance, viral infection-induced increased production of ROS and RNS leads to the dysfunctional epithelial barrier and lung injury, which then further increases the host susceptibility to secondary infections (Fig. 8.1) [9].

8.4 Mechanism of Induction of Oxidative Stress in the Lung

The lung is highly susceptible to OS as it is continuously challenged by ROS and RNS, which either acquired exogenously or synthesized endogenously by metabolic activity. Under physiological conditions, the lung is exposed to a huge amount of environmental oxygen together with other ecological substances that increase the amount of ROS and RNS in the lung. In this case, exogenous ROS and RNS will be the leading cause of OS in the lung. The sources of exogenous ROS and RNS include cigarette smoke, inhaled oxidants, and airborne pollutants. There are more than 4700 chemical substances and a high number of oxidants found in cigarette smoke [10]. Moreover, it is demonstrated that smokers inhale more than 1000 different types of oxidants, including superoxide anion (O_2^-), ∗NO, sulphur dioxide (SO_2) and hydrogen peroxide (H_2O_2), contained in each puff of smoke [11]. While the actual mechanism of smoking-induced OS in the cell remains obscure, there are pieces of evidence that confirm the correlation between cigarette smoke and redox imbalance in smokers, present in either gas or tobacco residue of cigarette smoke [10, 12]. Beside pre-existing oxidants, ∗NO can react with O_2^- under diffusion-limited reaction and produce $ONOO^-$ that further increases the ROS levels in the lung [13]. On the other hand, there are large amounts of semiquinone radicals in tobacco residue of cigarette smoke, which can react with O_2^- and produce ∗OH and H_2O_2 continuously under aqueous condition [14, 15].

Apart from cigarette smoke, inhaled airborne pollutants also elevate ROS and RNS levels. Based on the US Environmental Protection Agency, the burden of airborne particles in environmental air is steadily increasing worldwide, and the key sources of these airborne particles include the chemical or non-chemical

industrial product (66%), transportation (27%) and fuel combustion (35%) [16]. While there is a heterogeneous mixture of airborne particulates varying in size, shape, structure, biopersistance, surface reactivity and solubility within the polluted air, these particulates are collectively categorized based on their size fractions as particulate matter (PM) [17–19]. Coarse particles with PM more than 2.5 μM (PM $>$ 2.5) mainly consist of dust from the road, agriculture or construction work, whereas fine and ultrafine particles with PM range less than 2.5 μM (PM $<$ 2.5) mostly originate from mining, fuel combustion and transportation [20]. Among these, fine and ultrafine particles exert more significant damage to upper and lower respiratory airways compared to coarse particles as they have a larger surface-to-mass ratio [20]. In Sprague-Dawley rat models, exposure to PM2.5 for 10 weeks increases blood O_2^- level. Interestingly, this OS effect is reversible with *N*-omega-nitro-L-arginine methyl ester, which is an inhibitor for nitride oxide synthase (NOS) [21]. Further studies also link NOS with increased ROS production and reduced antioxidant capacity, suggesting an essential role played by NOS in redox reaction [22, 23]. Moreover, several studies report that mice challenged with PM2.5 showed not only elevated ∗NO but also increased cytochrome P450 and glutathione S-transferase levels, indicating the involvement of these oxidase synthases in exogenous induction of OS [24].

ROS and RNS can also be produced endogenously in our body, either by chemical reaction with certain inducers or as a defence mechanism against invading pathogens. Heavy metals, such as mercury, nickel, or cadmium, can deplete glutathione and reduce the antioxidant capability, whereas arsenic can compete and bound to thiols and enhance H_2O_2 formation. Hence, these metals can either directly or indirectly increase the accumulation of ROS in the body. On the other hand, ROS is also being produced endogenously as a defence mechanism against exogenous pathogens or foreign materials including bacteria, parasite, fungi and allergens [1]. In this case, immune cells, including neutrophils, eosinophils and macrophages, enhance the production of O_2^- and H_2O_2 via mitochondrial respiration, NADPH oxidase, xanthine/xanthine oxidase or cytochrome P450 [25–29]. Likewise, phagocytes, such as neutrophils and macrophages, are also equipped with haem peroxidase and myeloperoxidase that can produce potent oxidants, hypochlorous and hypobromous acid [26].

Interestingly, a few studies have also proposed a link between OS and endoplasmic reticulum stress (ERS) [30, 31]. ERS is a condition where there is an accumulation of misfolded proteins in the cells, which can enhance the production of ROS via oxidative protein refolding process [32]. Also, an increase in ROS can damage ER and further increase the production of misfolded proteins which exacerbates the ERS, and the vicious cycle of ERS and OS continuously worsens the cell cycle [32, 33]. However, the precise mechanism of this ERS-OS cycle in the human respiratory system remains obscure, and further studies are warranted to fully understand the role of ERS-induced OS in the lung.

8.5 Oxidative Stress in Chronic Respiratory Diseases

COPD is a complex non-communicable disease that is primarily characterized by airway inflammation, emphysema and decline in lung function capacity in response to chronic exposure to noxious particles and gases [34]. The major risk factor for COPD includes long-term exposure to cigarette and non-cigarette smoke (i.e. ambient, indoor and occupational) [35]. Asthma is another non-communicable disease that is characterized by chronic lung inflammation which results in airflow limitation, hyperreactivity and airway remodelling [36, 37]. Cystic fibrosis (CF) is primarily a genetic disorder, which is characterized by underlying mutation(s) in a gene coding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. *CFTR* is an ion channel regulator of basic ions, such as chloride, bicarbonate, sodium and fluid fluxes, at epithelial surfaces [38]. These CRDs are difficult to treat and currently available therapies only provide symptomatic relief to patients.

There is now substantial evidence that there is an increase in the levels of oxidants and decrease in protective antioxidant levels in the lung of patients with CRDs [39–41]. For instance, the levels of H_2O_2 in exhaled breath condensate (EBC) is significantly increased in COPD, dependent on the severity of COPD, with exacerbating COPD patients showing the highest levels (mean = 0.600 μM), followed by patients with stable COPD (mean = 0.205 μM), and lowest in healthy controls (mean = 0.029 μM) [42]. Moreover, other key markers of oxidative burden, including 8-isoprostane [43], malondialdehyde [44], nitrotyrosine and inducible nitric oxide synthase (iNOS) [45], have been shown to be elevated in COPD when compared to healthy participants. In addition to increased levels of pro-oxidants in the lung, the antioxidant levels, especially SOD and glucocorticoid receptor, are shown to be significantly depleted in COPD patients [46].

Patients with asthma exhibit increased levels of eosinophil peroxidase (EPO) and myeloperoxidase (MPO) in the peripheral blood, induced sputum and BAL fluid [47]. Moreover, patients with asthma also exhibited increased levels of superoxide production from leukocytes, increased total nitrites and nitrates, elevated protein carbonyls, and increased lipid peroxidation products, and reductions in protein sulfhydryls in plasma, indicative of heightened oxidant burden in asthma [48].

Patients with CF also exhibit increased oxidative stress irrespective of dietary intake of antioxidants. For instance, 8-iso-PGF2alpha concentration in plasma was significantly higher in CF patients (214 pg/mL) compared to age- and gender-matched healthy individuals (135 pg/mL) [49]. Moreover, plasma antioxidant concentrations (vitamins E and C, β-carotene) were reduced in CF patients [49]. Furthermore, patients experiencing CF exacerbations demonstrate increased oxidative stress, as well as sputum concentrations of bioactive lipid mediators (8-iso-PGF2alpha, cysteinyl leukotrienes and prostaglandin (PG)-E2) [50]. Notably, there was a strong negative correlation between FEV1 %predicted and sputum 8-iso-PGF2alpha, cysteinyl leukotrienes and prostaglandin (PG)-E2 [50].

8.6 Bacterial Infections and Oxidative Stress in Lungs

8.6.1 *Streptococcus pneumoniae*

S. pneumoniae, a gram-positive bacterium that commonly causes community-acquired pneumonia, induces oxidative stress, which is most likely due to pneumococcal autolysin, *LytA*, and independent of H_2O_2 produced by both bacterial and host metabolism [51]. Especially, the upper airway epithelial cells are found to be highly sensitive to the oxidative stress induced by *S. pneumonia*. Host's immune defences commonly respond to pneumococcal invasion by increased production of superoxide's by polymorphonuclear leukocytes (neutrophils) as a key defence [52]. In vitro analysis using neutrophils acquired from diseased individuals showed that *S. pneumonia* alone was unable to induce superoxide production during its logarithmic growth phase; however, the autolysis of the cells triggered the respiratory burst of neutrophils. Furthermore, pneumococcal lysis resulted in the release of bacterial components that come in contact with host respiratory epithelia, thereby resulting in a reduction in the levels of the antioxidant enzyme, namely, glutathione (GSH), potentially via nuclear factor erythroid 2-related factor 2 (*Nrf2*). Importantly, pretreatment of respiratory cells with the *Nrf2* inducer (resveratrol) prevented pneumococci-related oxidative stress induction [51]. In addition, another study demonstrated the role of glutathione (GSH) as one of the major antioxidative stress proteins which are protective against ROS/RNS, toxic metal concentrations and osmotic and acidic stress, which is synthesized by bacteria or acquired from extracellular environment (host) through ABC transporter substrate binding protein *GshT*. Mutations in *GshT* and *gor* (glutathione reductase) genes has led to increased pneumococcal sensitivity to host-produced superoxides [53]. Further, studies using superoxide dismutase 3-deficient mice ($SOD3^{-/-}$) found that *SOD3* deficiency leads to increased levels of ROS in phagosomal cells initiating early neutrophilic apoptosis in pneumococcal infections [54].

8.6.2 *Haemophilus influenza*

Haemophilus influenza is a gram-negative, facultative anaerobic coccobacillus that resides in the upper airways of children and adults as a commensal. It can be broadly divided into two subgroups based on the presence or absence of external capsule, i.e. encapsulated or typeable *Haemophilus influenza* strains (HiB) and non-capsulated or non-typeable *Haemophilus influenza* strains (NTHi). The encapsulated stains are further subdivided into seven types (a–g) based upon the chemical nature and antigenic determinants located on the capsule, which provide added protection against host immune defences and cause critical conditions, such as meningitis and septicaemia, in case the host conditions become favourable for bacterial growth, e.g. pre-existing disease and/or certain pharmacotherapies, whereas the non-typeable strains are found to cause or exacerbate various respiratory tract disorders driven by increased oxidative stress levels. NTHi strains are the most

common bacteria isolated from the human subjects suffering from chronic respiratory diseases, including COPD, bronchiectasis, asthma and cystic fibrosis [55]. Importantly, NTHi possesses increased tolerance towards the oxidative stress (H_2O_2, hydroxyl radicals, other radicals produced through Fenton's reaction) produced endogenously by the host and other invading microflora, especially co-pathogen *S. pneumoniae*, through various defence mechanisms to survive in the hostile environment [56]. Studies conducted on NTHI isolate strain 86-028NP have shown that the bacteria have the ability to encode genes for catalase (*Hkte*), peroxiredoxin/glutaredoxin (*Pgdx*) and ferritin-like proteins (*Dps*), which provides a protective shield against host/self-produced free radicals (aerobic respiration or enzyme activity like NADPH oxidases) [57]. King et al. showed that NTHi was capable of inducing increased ROS production in human fibroblasts, epithelial cells, macrophages and neutrophils, with highest levels of ROS recorded in phagocytic cells. The study also revealed that the production of the oxidant 3-nitrotyrosine (*3-NT*) in mice lungs was increased in response to NTHi infections [58]. Furthermore, the above results were backed up by other independent studies conducted in patients suffering from COPD elsewhere [59]. Thus, in addition to highly pathogenic pneumococci, a less virulent bacterium such as NTHicloud also elevates the oxidative stress levels and exacerbates the pre-existing conditions in patients suffering from chronic respiratory illnesses.

8.6.3 *Moraxella catarrhalis*

Moraxella catarrhalis is a gram-negative, aerobic diplococcus well known for its fastidious nature and identified to exclusively cause respiratory infections in the upper respiratory tract (acute otitis media (15–20%)) or exacerbate pre-existing respiratory disorders like COPD (10%), particularly in infants and children. *M. catarrhalis* strains are found to produce β-lactamase making them resistant to a wide spectrum of antibiotics that are having a β-lactam ring in their structure (penicillin, ampicillin, cephalosporins, etc.) [60, 61]. Studies show that *M. catarrhalis* codes for *SodA* and *OxyR*, a *LysR* family transcriptional regulator protein that provides resistance against endogenous (detoxification) and exogenous (host produced) oxygen free radicals and hydrogen peroxide (H_2O_2). In addition, they possess genes coding for *KatA* (predicted catalase) and *AhpCF* (alkyl hydroperoxide) which were upregulated upon exposure to a dose-dependent increase in H_2O_2 levels [61, 62]. The ability of *M. catarrhalis* to induce oxidative burst in human subjects was studied using adenocarcinoma human alveolar basal epithelial cells (A549) and isolated human alveolar epithelial cells. The infection with *M. catarrhalis* was able to induce increased monocyte burst and superoxide generation compared to inflammatory cytokines like *TNFα*, *1L1β* or *IFNγ* [63]. Recent studies found that *M. catarrhalis* infection can elicit a dose-dependent activation of bronchial epithelial cells to induce activation of *IL8* gene transcription resulting in strong inflammatory response resulting in an increased influx of immune cells,

especially neutrophils, which leads to elevated oxidative stress and drives the pathogenesis of COPD [64].

8.6.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa, a gram-negative, rod-shaped opportunistic pathogenic bacterium, could induce lethal diseases in immunocompromised hosts. It is well known to cause or exacerbate pre-existing respiratory illnesses like cystic fibrosis and severe COPD [65]. *P. aeruginosa* APO1 encodes a unique gene *ospR* (oxidative stress response and pigment production regulator), a global regulator gene that can sense oxidative stress and regulate other metabolic pathways (β-lactam resistance, pigment production) and provides defence against host produced H_2O_2. The deletion of *ospR* gene knockouts showed decreased resistance against oxidation stress [66]. In addition, *P. aeruginosa* APO1 encodes for alkyl hydroperoxide reductase (*AhpB* and *AhpC*), SOD and catalases (*KatA* and *KatB*) which protect the bacterial cell from endogenous/exogenous (host) peroxides [67]. Studies showed that the expression of gene enolase *eno* is a key virulence factor, as the mutations in *eno* gene have led to increased susceptibility of the pathogen's neutrophilic killings [68].

8.7 Viral Infections and Oxidative Stress in the Lungs

8.7.1 Rhinoviruses

Rhinovirus (RV) infections are well documented to be associated with the development of respiratory illnesses like common cold, sinusitis and otitis media and also intervene in exacerbations of chronic respiratory disorders like asthma and COPD. Several cross-sectional studies have shown the direct correlation between the severity of symptoms associated with rhinovirus infection and the concentration of interleukin 8 (*IL8*), a cytokine involved in the recruitment of neutrophils. An in vitro study using Beas-2b cells showed that the RV was able to stimulate the *IL-8* production by generating oxidative species and subsequent activation of *NF-KB* during viral replication [69]. In addition, other studies showed that the blocking of viral replication had no effect in regulating the oxidative stress in patients infected with RV virus since the presence of RV at the cell surface is enough to trigger the production of oxidative spp. [70]. Furthermore, the RV-1B infection was found to cause impairment in mitochondrial respiration, increased proton leak across the barrier and increased pro-inflammatory cytokines leading to increased oxidative stress levels [71]. A similar study using primary human nasal and bronchial epithelial cells, ex vivo conditions, showed that the infection with rhinovirus or RIG-1 triggered a robust NRF2-dependent oxidative stress response in later cells compared with nasal epithelial cells, which shows the differed sensitivity of various cells to RV infection within respiratory tract [72].

8.7.2 Respiratory Syncytial Viruses

Respiratory syncytial virus or RSV is a well-known causative agent in inducing upper and lower airway respiratory infections, particularly in children. There are no effective treatments or accurate knowledge of RSV-induced cellular oxidative stress mechanisms. In vitro and in vivo studies using A549, alveolar type II-like epithelial cell lines and small airway epithelial cells showed that RSV infection generates lipid peroxidation products and ROS and significantly decreases the levels of glutathione (GSH), along with the levels of sodium dismutase 1 and 3, catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST), which creates an imbalance in ROS/antioxidant defence mechanisms leading to cellular oxidative stress and oxidative damage in the lungs [73, 74]. Importantly, it was found that RSV infections actually downregulate the expression of various antioxidant enzyme (AOEs) by reducing the levels of nuclear and total cellular Nrf2 transcription factor [75], which compromises the antioxidative stress mechanisms of the host which leads to elevated oxidative stress levels and injury of tissue.

8.7.3 Influenza Virus

Influenza virus is a type of RNA virus known to cause pulmonary pandemics across the globe. Based on the antigenic determinants on the surface area, they are further classified into three subgroups (A, B and C), among which *influenza virus A* is known to be associated with various respiratory illness and flu pandemics across the globe [76]. Studies using pathogenic strains like H5N1 infecting the lung epithelial cells (in vitro) have shown the downregulation of superoxide dismutase SOD1 in both transcriptome and translational levels and increased production of ROS. Forced expression of SOD1 showed a positive effect, i.e. decreased the H5N1-induced production of ROS/viral replication and inflammatory cytokines [77]. In addition, similar studies carried elsewhere have also shown the increased mRNA expression of major antioxidant enzymes (AOEs) like Mn-superoxide dismutase, 2,3 dioxygenase. Further, in vivo studies carried out by Buffington et al. confirm the increased production of superoxide anion radical in cells of bronchoalveolar fluid (BALF), and increased hydrogen peroxide content aids the in vitro results [78].

8.8 Fungal Infections and Oxidative Stress in the Lungs

Fungal pathogens implicated in lung diseases are increasing rapidly, primarily in individuals with a compromised immune system or those exhibiting higher susceptibility due to underlying lung diseases such as COPD [79]. Present trends of prescribing long-term immune-suppressing regimens, such as oral and/or inhaled corticosteroids for the management of COPD and asthma, also contribute substantially to the increasing prevalence of fungal lung infections, which could be invasive if not diagnosed and treated appropriately [79]. Importantly, *Aspergillus* spp. is the

most common fungal pathogen affecting human health, which can cause allergic bronchopulmonary aspergillosis, aspergilloma and invasive pulmonary aspergillosis [80]. Moreover, sensitization to *A. fumigatus* has been shown to be associated with the development of fixed airflow obstruction, more bronchiectasis and increased sputum neutrophils in severe asthmatics [81]. Furthermore, both neutrophils and eosinophils were elevated in sputum obtained from patients with allergic bronchopulmonary aspergillosis [82]. A prospective observational study of 89 cystic fibrosis patients spanned over 11 years reported the airway colonization of *Candida albicans*, which was associated with increased rates of exacerbations, reduction in FEV1 and reduction in BMI [83]. *C. albicans* also co-colonize with *Pseudomonas sp*. [83]. Long-term or intermittent colonization of lungs with fungal pathogens could result in increased oxidative stress or further increase the oxidative stress and associated pathology in the lungs of patients with an underlying chronic disease.

Fungal spores from *Aspergillus flavus*, *Aspergillus penicillioides*, *Penicillium citrinum* and *Penicillium chrysogenum*, when administered intranasally into BALB/c mice, resulted in significantly increased production of reactive oxygen species (malondialdehyde and myeloperoxidase) and reductions in antioxidant markers (SOD and GSH) [84]. Host immune cells, primarily macrophages and neutrophils, when contacted by fungal pathogens, often generate ROS and RNS that is highly toxic to fungi due to damage to DNA, proteins and lipids. Several mechanisms have been proposed that result in fungi-induced oxidative stress in the lungs. In an in vitro model, murine alveolar macrophages showed significantly increased levels of nitric oxide (NO) production when co-incubated with *Cryptococcus neoformans* and *A. fumigatus* [85]. This, in part, was mediated by interferon gamma (IFN-gamma), as IFN-gamma pretreated alveolar macrophages exhibited increased accumulation and attachment of *A. fumigatus* conidia [85]. Also, NO production from alveolar macrophages correlated with the killing of *C. neoformans* that was confirmed by NO inhibitor [85]. In another study, the conidicidal activity of mice alveolar macrophages against *A. fumigatus* was found to be mediated by reactive oxidant intermediates, namely, NADPH oxidase and inducible nitric oxide synthase [86], which was confirmed by administration of NADPH oxidase inhibitor, which had a dual function of reducing the production of reactive oxidant intermediates and subsequent inhibition of *A. fumigatus* killing [86]. For instance, mice exposed to *Aspergillus fumigatus* exhibited increased endoplasmic reticulum (ER) stress markers, unfolded protein response (UPR)-related proteins, phosphorylated Akt, production of mitochondrial reactive oxygen species (mtROS), eosinophilic allergic inflammation and airway hyperresponsiveness (AHR) in the lung [87]. Administration of PI3K-δ inhibitor (IC87114) resulted in reduced *A. fumigatus*-induced mtROS production, as well as amelioration of ER stress upon administration of NecroX-5, a mtROS scavenger [87]. The authors also showed inhibition of ER stress by blocking PI3K-δ in primary tracheal epithelial cells in vitro [87].

Despite the luxury of oxidative stress-dependent defence mechanisms in the host, major fungal pathogens mount an effective response to counter host oxidative stress that then results in the establishment of disease [88]. These mechanisms could be

either enzymatic or non-enzymatic in nature and are extensively reviewed by Missall et al. [88]. Briefly, the major antioxidant enzymes in fungi include superoxide dismutase (SOD), catalases, thiol peroxidases, glutaredoxins, GSH peroxidases and GSH S-transferases and methionine sulfoxide reductase [88]. The non-enzymatic mechanisms include modulation of excessive host oxidative stress via melanin, mannitol and trehalose [88].

The increased oxidative stress has been reported in patients with chronic lung diseases. Both chronic and intermittent fungal infections could lead to a further increase in oxidative stress in the lungs, thereby contributing to overall increases in the inflammation and associated pathophysiology.

8.9 Atypical Bacterial Infections and Oxidative Stress in the Lungs

While often considered as non-significant pathogens in the lungs, atypical bacteria are now recognized as causative of acute and chronic, mild to severe respiratory infections with pulmonary and extra-pulmonary symptoms [89, 90]. The most common atypical bacteria responsible for pneumonia are *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila* and the zoonotic bacteria *Coxiella burnettii* and *Francisella tularensis* [90]. These bacteria are facultative or obligate intracellular pathogens that replicate inside specific host cells such as lung epithelial cells, airway macrophages, monocytes and neutrophils [89, 91–93]. In host cells, atypical bacteria are constitutively exposed to oxidative stress. Indeed, infection by *Mycoplasma pneumoniae* induces reactive oxygen species (ROS) released from the A549 human lung carcinoma cell line [94]. Similarly, infection of a murine macrophage cell line by *Chlamydia pneumoniae* prompted an increase in NADPH oxidase and cytochrome c oxidase activities, thereby favouring ROS production [95]. As mentioned earlier, this oxidative burst is an innate immune mechanism allowing protection of host cells against pathogens, including atypical bacteria. In vitro treatments of murine primary macrophages or peritoneal exudate cells with inducer of oxidative stress such as interferon gamma (IFNγ) and sodium nitroprusside reduce levels of infections by *Coxiella burnetii* and *Francisella tularensis* [92, 93, 96], while treatments with inhibitors of inducible nitric oxide synthase (iNOS), catalase (hydrogen peroxide [H_2O_2] scavenger) or a catalyst of peroxynitrite ($ONOO^-$) can restore infection levels [92, 96]. Similarly, treatments with inhibitors of NO synthase lead to an increase in the frequency of replicative vacuoles of *Coxiella burnetii* in infected primary macrophages [93]. In vivo data confirm the critical role of the oxidative burst in the protection against infection, as mice deficient in $p47^{phox}$ (the main regulator of NADPH oxidase activation) and iNOS were more susceptible to intraperitoneal infection by *Coxiella burnetii* compared to WT mice [96].

Oxidative stress can protect host cells against atypical infection by enhancing the killing capacity of phagocyte cells, as ROS formation was shown to increase the bactericidal ability of neutrophils against *Legionella pneumophila* in a murine model of experimental airway infection [97]. In addition to this direct action, infection-

induced oxidative stress can also activate critical signalling pathways. Indeed, infection with *Mycoplasma hyopneumoniae* and *Mycoplasma ovipneumoniae*, responsible for pneumonia in swine, goat and sheep, can increase ROS production, decrease antioxidant levels and disrupt the integrity of the mitochondrial membrane, resulting in caspase-mediated apoptosis in, respectively, porcine alveolar macrophage 3D4/21 cells or air-liquid interface culture of sheep bronchial epithelial cells [98–100]. Inhibition of NO synthase and treatments with oxidants were able to restore caspase activation and limit apoptosis, suggesting that oxidative stress activates caspase-mediated apoptosis in infected macrophages [98].

Atypical bacteria possess several mechanisms to survive oxidative burst, such as producing proteins able to directly detoxify their intracellular replicative vacuoles from ROS. For instance, *Coxiella burnetii* possess a thiol-specific peroxidase and *Legionella pneumophila* express alkyl hydroperoxide reductases (AhpC1 and AhpC2D) and an outer membrane channel tunnel protein TolC that participates in either efflux or degradation of ROS [101–103]. In addition, atypical bacteria can affect the cellular redox balance and inhibit the host NADPH oxidase. Indeed, *Mycoplasma pneumoniae* possess a glycerol 3-phosphate oxidase named GlpD that can produce H_2O_2 in a glycerol-dependent manner, thereby increasing oxidative burst and cytotoxicity in infected cells [104]. In more, the lipid-associated membrane proteins (LAMPS) of *Mycoplasma pneumoniae* have been shown to disrupt host signalling and induce the translocation of the transcription factor nuclear factor E2-related factor 2 (Nrf2) to the nucleus of the infected THP-1 monocyte cells, reducing oxidative stress and expression of pro-inflammatory mediators [91]. *Francisella tularensis* can overcome high levels of ROS in infected macrophages and epithelial cells through the transcriptional regulator OxyR that favours antioxidants by binding to their promoter regions [105, 106]. These antioxidants can prevent the accumulation of ROS in macrophages and the subsequent activation of the NF-κB pathway and secretion of pro-inflammatory mediators, consequently increasing survival of *Francisella tularensis* [107, 108]. Neutrophils infected by *Coxiella burnetii* were shown to lack translocation of the NADPH oxidase components $p47^{phox}$ and $p67^{phox}$ from the cytosol to the membrane, which resulted in a defect in NADPH oxidase assembly and in production of the reduced amount of ROS compared to neutrophils infected by *Staphylococcus aureus* or *Escherichia coli* [109]. Confirming these results, an acid phosphatase of *Coxiella burnetii* has been shown to prevent the release of reactive oxygen intermediates through inhibition of the formation of the NADPH oxidase complex in polymorphonuclear leukocytes [110].

Infection-induced ROS can affect the pathogenesis of the lung by damaging the host DNA, cytotoxicity and induction of apoptosis [94, 98–100, 104]. Using a murine model of 16 weeks of environmental cigarette smoking and *Mycoplasma pneumoniae* infection, a study suggests that enhanced levels of oxidative stress resulting from infection can exacerbate lung diseases in smokers. In summary, they show that the increase in the antioxidant glutathione in the epithelium lining fluid of cigarette-exposed mice was impaired when mice were infected intranasally by *Mycoplasma pneumoniae*. In addition, smoke-exposed infected mice displayed higher levels of oxidized glutathione compared to smoke-exposed noninfected mice

consequently of a drop in glutathione reductase levels, thus implying that *Mycoplasma pneumoniae* infection can increase oxidative stress in the lungs of mice exposed to smoke [111]. Overall, the oxidative status of infected cells and the capacity of atypical bacteria to survive to those conditions are key determinants of the outcome and pathogenesis of respiratory infection by atypical bacteria.

8.10 Viral-Bacterial Co-infection and Oxidative Stress

In the human respiratory tract, respiratory viruses, such as influenza, can interact with either exogenous bacterial pathogen or host-commensal flora, and these interactions can reduce host-microbe defence system and leave the host susceptible to a secondary infection [112]. This viral-bacterial co-infection is common in patients with chronic respiratory disease; however, it has a significant impact on the outcome of the treatment. One of the great examples to illustrate the devastating effect of viral-bacterial co-infection is the 1918 influenza pandemic, also referred to as "Spanish flu" [113]. The influenza pandemic killed more than 50 million people and was mainly due to the secondary bacterial infection, such as *Staphylococcus aureus* and *Streptococcus pneumonia*, rather than from virus alone [113]. Research in this field has led to advances in understanding that viral infection can favour secondary bacterial infection by different pathways. These include impairing the barrier function [114, 115], creating a microenvironment that promotes bacterial growth and survival [116, 117], reducing responsiveness and effectiveness of immune system [118, 119] or increasing the availability of different receptors for bacterial binding [120, 121].

Growing number of studies suggest a possible role of OS in the viral co-infection. For instance, a study documented that post-influenza *Staphylococcus aureus* causes severe lung inflammation because of NADPH oxidase 2 (NOX2)-dependent and OS-associated inflammatory response [122]. NOX2 is a catalytic subunit for NADPH oxidase, and it is used for the production of ROS and oxidative burst, an essential anti-*Staphylococcus aureus* immune defence process [122]. However importantly, the oxidative burst is a double-edged sword since it is a non-specific process and often causes lethal damage to surrounding tissues and also disrupts the redox balance in the tissues. One of the studies showed that compromised host immune response during influenza initiates NOX2-dependent oxidative burst which when further challenged by *Staphylococcus aureus* caused systemic, lethal damage in the host respiratory tract [123].

Besides influenza and *Staphylococcus aureus* co-infection, *Mycobacterium tuberculosis* and HIV co-infection, which are discussed in later sections, also set an exemplary example of the effect of OS during viral-bacterial co-infections.

8.11 Co-infection of *Mycobacterium tuberculosis* and HIV

Tuberculosis (TB) is a major cause of mortality globally. It is estimated that in 2017 alone, 1.6 million people died from the disease making it the largest cause of death due to a single infectious agent [124]. While most cases are attributable exclusively to infection with *Mycobacterium tuberculosis* (MTB), approximately, 300,000 TB mortalities occurred in individuals who were also living with HIV. Furthermore, of the 10 million people who developed TB during 2017, 920,000 (9%) were HIV-positive [124]. Therefore, MTB/HIV co-infection is a major feature of the biology of TB internationally.

It has been well established that HIV/AIDS weakens the CD4+ T cell-mediated immune response, thereby increasing susceptibility to the development of TB [125–129]. The risk of death in MTB/HIV co-infected TB patients is significantly higher than in TB patients who are HIV-negative [130–132]. TB accounts for approximately 26% of AIDS-related deaths, making it a dominant cause of mortality in HIV/AIDS cases [133].

The structural damage to the lungs of pulmonary TB patients involves extensive fibrosis, cavitation, traction bronchiectasis, bronchostenosis or parenchymal destruction [134, 135]. There is growing evidence that TB disease also changes parameters in the host that favour HIV replication such that TB hastens the progression of HIV/AIDS. For example, MTB infection has been shown to result in upregulated viral gene expression in HIV transgenic mice which can be suppressed through treatment with antitubercular drugs [136]. The induction of HIV gene expression by co-infection with MTB is dependent on the surface presentation of Toll-like receptor 2 (TLR2) [137]. This activity is mediated by an interaction between TLR-2 and a constituent of the MTB membrane, phosphatidylinositol mannoside 6 (PIM6) [138].

Interestingly, a recent study has reported that macrophages infected with virulent MTB release exosomes that induce oxidative stress in neighbouring noninfected immune cells [139]. This was associated with reactivation of HIV in monocytes and lymphocytes which was abrogated through the administration of a known antioxidant, *N*-acetylcysteine [139]. The reactivation of HIV by MTB-specific exosomes was accompanied by altered expression of genes associated with redox metabolism including upregulation of component genes of superoxide-producing enzyme-NADPH oxidase, e.g. cytochrome B-245 beta chain (CYBB) and neutrophil cytosolic factors 1 (NCF1) and 2 (NCF2) [139].

While the initiation of a respiratory burst to produce reactive oxygen species is an important immune response by macrophages for the killing of MTB [140], this defence mechanism may paradoxically participate in the reactivation of HIV replication and in the elevation of titres of the virus in a co-infected host. Therefore, it is apparent that TB control programmes are integral in reducing the incidence of MTB/HIV co-infection and hence slowing the progression of HIV/AIDS disease.

8.12 Co-infection of *Mycobacterium tuberculosis* and Fungi

Patients with pre-existing TB are more susceptible to pulmonary fungal infections. The prevalence of *Candida* co-infection in TB patients was reported to be approximately 15–32% [141]. As stated earlier, *M. tuberculosis* induces ROS production from alveolar macrophages that is a major host-defence mechanism against TB development/progression [139]. There is good evidence that the diagnosis of fungal infections, namely, invasive pulmonary aspergillosis, is often misdiagnosed as the development of TB in patients receiving various organ transplantations and the incidence of misdiagnosis is significantly higher in certain geological locations [142]. Moreover, a recent study confirmed the diagnosis of chronic pulmonary aspergillosis (CPA) in treated TB patients and recommended a combination of CPA diagnosis strategies to increase the sensitivity of diagnosis by combining chest X-ray cavitation, serological assays with *Aspergillus* specific IgG, chronic cough or haemoptysis assays [143]. The killing of *Aspergillus* conidia is primarily mediated by phagocytes, including alveolar macrophages and neutrophils, most importantly by ROS-mediated pathways [144]. However, *Aspergillus* constitutes a range of antioxidant mechanism to evade phagocytic killing, including a range of ROS scavengers [144]. The combination of TB-fungal infections could lead to further increases in OS burden in the lungs, thus enabling both pathogens to persist. Also, increased OS may lead to elevated inflammatory responses leading to heightened pulmonary damage that may aid in the dissemination of both TB and fungal pathogens.

8.13 Treatments

Infection-induced oxidative stress in the lungs is generally deleterious, particularly in patients with pre-existing chronic diseases. Although currently prescribed treatment regimens are beneficial in alleviating the symptoms of CRDs, these treatments do not completely cure the disease. Moreover, an important aspect of CRDs, i.e. oxidative stress, is only beginning to be investigated in the context of management of CRDs. Few examples of antioxidative drugs include flavonoids, glutathione (GSH), *N*-acetylcysteine, ascorbic acid (vitamin C) and tocopherol (vitamin E), 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone), ebselen, etc. [145]. Supplementing current treatment regimens with antioxidant drugs could be beneficial to limit microbial infections in CRDs, especially the microbial pathogens that are equipped with antioxidant mechanisms. However, these antioxidant therapies have been only marginally successful in preventing and/or treating CRDs. One possible explanation may be the inability of these therapies in distinguishing the pathways that are harmful in increased ROS/RNS activities from those that are actually beneficial in nature. Thus, refining the existing knowledge may ensure the reduction in OS-related lung pathology and will potentially reduce pulmonary inflammation that is a hallmark feature of CRDs, which will be essential in improving the overall quality of life.

8.14 Summary and Conclusion

Oxidative stress is an integral mechanism to counter foreign particles, including microbial pathogens, in the lungs. However, the chronic exposure to exogenous factors, such as cigarette smoke, air pollution and recurrent infections, may lead to dysregulation of the oxidant-antioxidant balance in the lung. Furthermore, increased oxidative stress markers are often reported in both pulmonary and systemic samples of patients with CRDs. Major microbial lung pathogens are shown to possess sophisticated mechanisms to counter the host oxidants and survive/persist in the lungs. Notably, several pathogens increase the oxidative burden in the host either by activating ROS/RNS production or by reducing host antioxidant defence mechanisms. This persistent microbial load, as well as an increased oxidative burden, may lead to a heightened risk of further lung pathology and tissue destruction, potentially leading to increased risk of morbidity and mortality in patients with CRDs. Although antioxidant therapies, drugs that may restore the balance between oxidants and antioxidants, are either currently available or under intense investigation, these remain under-/unutilized in patients with CRDs. Focused investigations are needed to fully understand the effects of microbial pathogens in the lungs of CRD patients.

Acknowledgment The authors express their since gratitude to the University of Newcastle in providing the resources.

References

1. Park HS, Kim SR, Lee YC (2009) Impact of oxidative stress on lung diseases. Respirology 14 (1):27–38
2. Rogers LK, Cismowski MJ (2018) Oxidative stress in the lung – the essential paradox. Curr Opin Toxicol 7:37–43
3. Tkaczyk J, Vizek M (2007) Oxidative stress in the lung tissue--sources of reactive oxygen species and antioxidant defence. Prague Med Rep 108(2):105–114
4. Domej W, Oettl K, Renner W (2014) Oxidative stress and free radicals in COPD--implications and relevance for treatment. Int J Chron Obstruct Pulmon Dis 9:1207–1224
5. Galli F et al (2012) Oxidative stress and antioxidant therapy in cystic fibrosis. Biochim Biophys Acta 1822(5):690–713
6. Sethi S (2010) Infection as a comorbidity of COPD. Eur Respir J 35(6):1209–1215
7. Cookson W, Cox MJ, Moffatt MF (2018) New opportunities for managing acute and chronic lung infections. Nat Rev Microbiol 16(2):111–120
8. Ivanov AV, Bartosch B, Isaguliants MG (2017) Oxidative Stress in Infection and Consequent Disease. Oxid Med Cell Longev 2017:3496043
9. Novaes RD, Teixeira AL, de Miranda AS (2019) Oxidative stress in microbial diseases: pathogen, host, and therapeutics. Oxid Med Cell Longev 2019:8159562
10. Karademirci M, Kutlu R, Kilinc I (2018) Relationship between smoking and total antioxidant status, total oxidant status, oxidative stress index, vit C, vit E. Clin Respir J 12(6):2006–2012
11. Church DF, Pryor WA (1985) Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64:111–126
12. Napierala M et al (2019) The effect of maternal tobacco smoking and second-hand tobacco smoke exposure on human milk oxidant-antioxidant status. Environ Res 170:110–121

13. Munakata S et al (2018) Oxidative stress responses in human bronchial epithelial cells exposed to cigarette smoke and vapor from tobacco-and nicotine-containing products. Regul Toxicol Pharmacol 99:122–128
14. Nakayama T, Church DF, Pryor WA (1989) Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. Free Radic Biol Med 7(1):9–15
15. Zang L-Y, Stone K, Pryor WA (1995) Detection of free radicals in aqueous extracts of cigarette tar by electron spin resonance. Free Radic Biol Med 19(2):161–167
16. Kaiser J (2000) Evidence mounts that tiny particles can kill. Science 289(5476):22–23
17. Nel A et al (2006) Toxic potential of materials at the nanolevel. Science 311(5761):622–627
18. Oberdörster G, Ferin J, Lehnert BE (1994) Correlation between particle size, in vivo particle persistence, and lung injury. Environ Health Perspect 102(suppl 5):173–179
19. Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839
20. Rivera Gil P et al (2010) Correlating physico-chemical with toxicological properties of nanoparticles: the present and the future. ACS Nano 4(10):5527–5531
21. Sun Q et al (2008) Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK. Arterioscler Thromb Vasc Biol 28(10):1760–1766
22. Sun L et al (2013) Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol 10 (1):43
23. Xu X et al (2010) Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. Arterioscler Thromb Vasc Biol 30(12):2518–2527
24. Li R et al (2015) Effects of ambient PM 2.5 on pathological injury, inflammation, oxidative stress, metabolic enzyme activity, and expression of c-fos and c-jun in lungs of rats. Environ Sci Pollut Res 22(24):20167–20176
25. Toufektsian M-C et al (2001) Cardiac toxicity of singlet oxygen: implication in reperfusion injury. Antioxid Redox Signal 3(1):63–69
26. Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4 (3):181
27. McNally JS et al (2005) Regulation of xanthine oxidoreductase protein expression by hydrogen peroxide and calcium. Arterioscler Thromb Vasc Biol 25(8):1623–1628
28. Babior B, Lambeth J, Nauseef W (2002) The neutrophil NADPH oxidase. Arch Biochem Biophys 397(2):342–344
29. Butler J, Hoey BM (1993) The one-electron reduction potential of several substrates can be related to their reduction rates by cytochrome P-450 reductase. Biochim Biophys Acta Prot Struct Mol Enzymol 1161(1):73–78
30. Wang Q-L et al (2019) Ginsenoside Rg1 regulates SIRT1 to ameliorate sepsis-induced lung inflammation and injury via inhibiting endoplasmic reticulum stress and inflammation. Mediators Inflamm 2019:6453296
31. Chen X et al (2018) Heme oxygenase-1 reduces sepsis-induced endoplasmic reticulum stress and acute lung injury. Mediators Inflamm 2018:9413876
32. Chong W, Shastri M, Eri R (2017) Endoplasmic reticulum stress and oxidative stress: a vicious nexus implicated in bowel disease pathophysiology. Int J Mol Sci 18(4):771
33. Rao X et al (2018) Effect of particulate matter air pollution on cardiovascular oxidative stress pathways. Antioxid Redox Signal 28(9):797–818
34. Agustí A, Celli B (2017) Natural history of COPD: gaps and opportunities. ERJ Open Res 3 (4):00117-2017
35. Kc R et al (2018) The role of environmental exposure to non-cigarette smoke in lung disease. Clin Transl Med 7(1):39
36. Holgate ST (2013) Mechanisms of asthma and implications for its prevention and treatment: a personal journey. Allergy Asthma Immunol Res 5(6):343–347
37. Boushey HA, Fahy JV (1995) Basic mechanisms of asthma. Environ Health Perspect 103 (Suppl 6):229–233

38. Muhlebach MS et al (2016) Biomarkers for cystic fibrosis drug development. J Cyst Fibros 15 (6):714–723
39. Dua K et al (2019) Increasing complexity and interactions of oxidative stress in chronic respiratory diseases: an emerging need for novel drug delivery systems. Chem Biol Interact 299:168–178
40. Santus P et al (2014) Oxidative stress and respiratory system: pharmacological and clinical reappraisal of N-acetylcysteine. COPD 11(6):705–717
41. Ciencewicki J, Trivedi S, Kleeberger SR (2008) Oxidants and the pathogenesis of lung diseases. J Allergy Clin Immunol 122(3):456–468. quiz 469–70
42. Dekhuijzen PN et al (1996) Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 154(3 Pt 1):813–816
43. Montuschi P et al (2000) Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. Am J Respir Crit Care Med 162(3 Pt 1):1175–1177
44. Bartoli ML et al (2011) Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. Mediators Inflamm 2011:891752
45. Ricciardolo FL et al (2005) Nitrosative stress in the bronchial mucosa of severe chronic obstructive pulmonary disease. J Allergy Clin Immunol 116(5):1028–1035
46. Zeng M et al (2013) Local and systemic oxidative stress status in chronic obstructive pulmonary disease patients. Can Respir J 20(1):35–41
47. Sahiner UM et al (2011) Oxidative stress in asthma. World Allergy Organ J 4(10):151–158
48. Nadeem A et al (2003) Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 111(1):72–78
49. Wood LG et al (2001) Oxidative stress in cystic fibrosis: dietary and metabolic factors. J Am Coll Nutr 20(2 Suppl):157–165
50. Reid DW et al (2007) Oxidative stress and lipid-derived inflammatory mediators during acute exacerbations of cystic fibrosis. Respirology 12(1):63–69
51. Zahlten J et al (2014) Streptococcus pneumoniae–induced oxidative stress in lung epithelial cells depends on pneumococcal autolysis and is reversible by resveratrol. J Infect Dis 211 (11):1822–1830
52. Rigby KM, DeLeo FR (2012) Neutrophils in innate host defense against Staphylococcus aureus infections. Semin Immunopathol 34:237
53. Potter AJ, Trappetti C, Paton JC (2012) Streptococcus pneumoniae uses glutathione to defend against oxidative stress and metal ion toxicity. J Bacteriol 194(22):6248–6254
54. Anthony D et al (2019) Excessive reactive oxygen species inhibit IL-17A^{+} γδ T Cells and innate cellular responses to bacterial lung infection. Antioxid Redox Signal 32(13). https://doi.org/10.1089/ars.2018.7716
55. Baron S (1996) Classification--medical microbiology. University of Texas Medical Branch at Galveston, Galveston, TX
56. Harrison A, Bakaletz LO, Munson RS Jr (2012) Haemophilus influenzae and oxidative stress. Front Cell Infect Microbiol 2:40
57. Harrison A, Baker BD, Munson RS (2015) Overlapping and complementary oxidative stress defense mechanisms in nontypeable Haemophilus influenzae. J Bacteriol 197(2):277–285
58. King PT et al (2015) Nontypeable Haemophilus influenzae induces sustained lung oxidative stress and protease expression. PLoS One 10(3):e0120371
59. Prasad J et al (2013) Nontypeable haemophilus influenzae (NTHi) and lung oxidative stress. Eur Respir J 42:P247
60. Goldstein EJ, Murphy TF, Parameswaran GI (2009) Moraxella catarrhalis, a human respiratory tract pathogen. Clin Infect Dis 49(1):124–131
61. Murphy TF et al (2005) Moraxella catarrhalis in chronic obstructive pulmonary disease: burden of disease and immune response. Am J Respir Crit Care Med 172(2):195–199

62. Luke NR, Karalus RJ, Campagnari AA (2002) Inactivation of the Moraxella catarrhalis superoxide dismutase SodA induces constitutive expression of iron-repressible outer membrane proteins. Infect Immun 70(4):1889–1895
63. Rosseau S et al (2005) Moraxella catarrhalis–infected alveolar epithelium induced monocyte recruitment and oxidative burst. Am J Respir Cell Mol Biol 32(2):157–166
64. Slevogt H et al (2006) Moraxella catarrhalis induces inflammatory response of bronchial epithelial cells via MAPK and NF-κB activation and histone deacetylase activity reduction. Am J Physiol Lung Cell Mol Physiol 290(5):L818–L826
65. Faure E, Kwong K, Nguyen D (2018) Pseudomonas aeruginosa in chronic lung infections: how to adapt within the host? Front Immunol 9:2416
66. Lan L et al (2010) Pseudomonas aeruginosa OspR is an oxidative stress sensing regulator that affects pigment production, antibiotic resistance and dissemination during infection. Mol Microbiol 75(1):76–91
67. Hare NJ et al (2011) Proteomics of the oxidative stress response induced by hydrogen peroxide and paraquat reveals a novel AhpC-like protein in Pseudomonas aeruginosa. Proteomics 11 (15):3056–3069
68. Weng Y et al (2016) Pseudomonas aeruginosa enolase influences bacterial tolerance to oxidative stresses and virulence. Front Microbiol 7:1999
69. Biagioli MC et al (1999) The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. Free Radic Biol Med 26(3-4):454–462
70. Kaul P et al (2000) Rhinovirus-induced oxidative stress and interleukin-8 elaboration involves p47-phox but is independent of attachment to intercellular adhesion molecule-1 and viral replication. J Infect Dis 181(6):1885–1890
71. Wark P et al (2016) Effect of oxidative stress and rhinovirus infection on mitochondrial/endoplasmic reticular function in human primary bronchial epithelial cells. Eur Respir J 48: PA5011
72. Mihaylova VT et al (2018) Regional differences in airway epithelial cells reveal tradeoff between defense against oxidative stress and defense against rhinovirus. Cell Rep 24 (11):3000–3007.e3
73. Hosakote YM et al (2011) Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis. Am J Respir Crit Care Med 183(11):1550–1560
74. Hosakote YM et al (2009) Respiratory syncytial virus induces oxidative stress by modulating antioxidant enzymes. Am J Respir Cell Mol Biol 41(3):348–357
75. Komaravelli N et al (2015) Respiratory syncytial virus infection downregulates antioxidant enzyme expression by triggering Nrf2 degradation. FASEB J 29(1 Suppl):718.25
76. Shi T (2018) The etiological role of common respiratory viruses in acute respiratory infections in older adults: a systematic review and meta-analysis. ERJ 52(62 Suppl). https://doi.org/10.1183/13993003.congress-2018.PA4504
77. Lin X et al (2016) The influenza virus H5N1 infection can induce ROS production for viral replication and host cell death in A549 cells modulated by human Cu/Zn superoxide dismutase (SOD1) overexpression. Viruses 8(1):13
78. Schwarz KB (1996) Oxidative stress during viral infection: a review. Free Radic Biol Med 21 (5):641–649
79. Limper AH (2010) The changing spectrum of fungal infections in pulmonary and critical care practice. Proc Am Thorac Soc 7(3):163–168
80. Bulpa P, Dive A, Sibille Y (2007) Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 30(4):782–800
81. Fairs A et al (2010) IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. Am J Respir Crit Care Med 182(11):1362–1368
82. Wark PA et al (2000) Induced sputum eosinophils and neutrophils and bronchiectasis severity in allergic bronchopulmonary aspergillosis. Eur Respir J 16(6):1095–1101

83. Chotirmall SH et al (2010) Sputum Candida albicans presages FEV(1) decline and hospital-treated exacerbations in cystic fibrosis. Chest 138(5):1186–1195
84. Odebode A, Adekunle A (2019) Biomarkers of oxidative stress as indicators of fungi environmental pollution in Balb/c albino mice monitored from South West, Nigeria. J Pathog 2019:9
85. Gross NT et al (1999) Production of nitric oxide by rat alveolar macrophages stimulated by Cryptococcus neoformans or Aspergillus fumigatus. Med Mycol 37(3):151–157
86. Philippe B et al (2003) Killing of Aspergillus fumigatus by alveolar macrophages is mediated by reactive oxidant intermediates. Infect Immun 71(6):3034–3042
87. Lee KS et al (2016) Phosphoinositide 3-kinase-δ regulates fungus-induced allergic lung inflammation through endoplasmic reticulum stress. Thorax 71(1):52–63
88. Missall TA, Lodge JK, McEwen JE (2004) Mechanisms of resistance to oxidative and nitrosative stress: implications for fungal survival in mammalian hosts. Eukaryot Cell 3 (4):835–846
89. Blasi F (2004) Atypical pathogens and respiratory tract infections. Eur Respir J 24(1):171–181
90. Cunha BA (2006) The atypical pneumonias: clinical diagnosis and importance. Clin Microbiol Infect 12(Suppl 3):12–24
91. Hu J et al (2017) Nrf2 regulates the inflammatory response, including heme oxygenase-1 induction, by mycoplasma pneumoniae lipid-associated membrane proteins in THP-1 cells. Pathog Dis 75(4)
92. Lindgren H et al (2005) The contribution of reactive nitrogen and oxygen species to the killing of Francisella tularensis LVS by murine macrophages. Microbes Infect 7(3):467–475
93. Zamboni DS, Rabinovitch M (2003) Nitric oxide partially controls Coxiella burnetii phase II infection in mouse primary macrophages. Infect Immun 71(3):1225–1233
94. Sun G et al (2008) Mycoplasma pneumoniae infection induces reactive oxygen species and DNA damage in A549 human lung carcinoma cells. Infect Immun 76(10):4405–4413
95. Azenabor AA et al (2005) Elicitation of reactive oxygen species in Chlamydia pneumoniae-stimulated macrophages: a Ca2+-dependent process involving simultaneous activation of NADPH oxidase and cytochrome oxidase genes. Med Microbiol Immunol 194(1-2):91–103
96. Brennan RE et al (2004) Both inducible nitric oxide synthase and NADPH oxidase contribute to the control of virulent phase I Coxiella burnetii infections. Infect Immun 72(11):6666–6675
97. Ziltener P, Reinheckel T, Oxenius A (2016) Neutrophil and alveolar macrophage-mediated innate immune control of legionella pneumophila lung infection via TNF and ROS. PLoS Pathog 12(4):e1005591
98. Bai F et al (2013) Mycoplasma hyopneumoniae-derived lipid-associated membrane proteins induce apoptosis in porcine alveolar macrophage via increasing nitric oxide production, oxidative stress, and caspase-3 activation. Vet Immunol Immunopathol 155(3):155–161
99. Jiang Z et al (2017) Capsular polysaccharide of mycoplasma ovipneumoniae induces sheep airway epithelial cell apoptosis via ROS-dependent JNK/P38 MAPK pathways. Oxid Med Cell Longev 2017:6175841
100. Li Y et al (2016) Mycoplasma ovipneumoniae induces sheep airway epithelial cell apoptosis through an ERK signalling-mediated mitochondria pathway. BMC Microbiol 16(1):222
101. Ferhat M et al (2009) The TolC protein of Legionella pneumophila plays a major role in multi-drug resistance and the early steps of host invasion. PLoS One 4(11):e7732
102. LeBlanc JJ, Davidson RJ, Hoffman PS (2006) Compensatory functions of two alkyl hydroperoxide reductases in the oxidative defense system of Legionella pneumophila. J Bacteriol 188(17):6235–6244
103. Hicks LD et al (2010) A DNA-binding peroxiredoxin of Coxiella burnetii is involved in countering oxidative stress during exponential-phase growth. J Bacteriol 192(8):2077–2084
104. Hames C et al (2009) Glycerol metabolism is important for cytotoxicity of Mycoplasma pneumoniae. J Bacteriol 191(3):747–753
105. Ma Z et al (2016) Elucidation of a mechanism of oxidative stress regulation in Francisella tularensis live vaccine strain. Mol Microbiol 101(5):856–878

106. Honn M et al (2017) Lack of OxyR and KatG results in extreme susceptibility of Francisella tularensis LVS to oxidative stress and marked attenuation in vivo. Front Cell Infect Microbiol 7:14
107. Rabadi SM et al (2016) Antioxidant defenses of Francisella tularensis modulate macrophage function and production of proinflammatory cytokines. J Biol Chem 291(10):5009–5021
108. Melillo AA, Bakshi CS, Melendez JA (2010) Francisella tularensis antioxidants harness reactive oxygen species to restrict macrophage signaling and cytokine production. J Biol Chem 285(36):27553–27560
109. Siemsen DW et al (2009) Inhibition of the human neutrophil NADPH oxidase by Coxiella burnetii. Microbes Infect 11(6-7):671–679
110. Hill J, Samuel JE (2011) Coxiella burnetii acid phosphatase inhibits the release of reactive oxygen intermediates in polymorphonuclear leukocytes. Infect Immun 79(1):414–420
111. Kariya C et al (2008) Mycoplasma pneumoniae infection and environmental tobacco smoke inhibit lung glutathione adaptive responses and increase oxidative stress. Infect Immun 76 (10):4455–4462
112. Bellinghausen C et al (2016) Viral–bacterial interactions in the respiratory tract. J Gen Virol 97 (12):3089–3102
113. Morens DM, Taubenberger JK, Fauci AS (2008) Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 198(7):962–970
114. Zeng H et al (2013) Tropism and infectivity of influenza virus, including highly pathogenic avian H5N1 virus, in ferret tracheal differentiated primary epithelial cell cultures. J Virol 87 (5):2597–2607
115. Faris AN et al (2016) Rhinovirus delays cell repolarization in a model of injured/regenerating human airway epithelium. Am J Respir Cell Mol Biol 55(4):487–499
116. Pettigrew MM et al (2014) Dynamic changes in the Streptococcus pneumoniae transcriptome during transition from biofilm formation to invasive disease upon influenza A virus infection. Infect Immun 82(11):4607–4619
117. Siegel SJ, Roche AM, Weiser JN (2014) Influenza promotes pneumococcal growth during coinfection by providing host sialylated substrates as a nutrient source. Cell Host Microbe 16 (1):55–67
118. Neagos J et al (2015) Epigenetic regulation of tolerance to Toll-like receptor ligands in alveolar epithelial cells. Am J Respir Cell Mol Biol 53(6):872–881
119. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34(5):637–650
120. Avadhanula V et al (2007) Nontypeable Haemophilus influenzae and Streptococcus pneumoniae bind respiratory syncytial virus glycoprotein. J Med Microbiol 56(9):1133–1137
121. Hafez MM, Abdel-Wahab KS, El-Fouhil DF (2010) Augmented adherence and internalization of group A Streptococcus pyogenes to influenza A virus infected MDCK cells. J Basic Microbiol 50(S1):S46–S57
122. Sun K, Metzger DW (2014) Influenza infection suppresses NADPH oxidase–dependent phagocytic bacterial clearance and enhances susceptibility to secondary methicillin-resistant Staphylococcus aureus infection. J Immunol 192(7):3301–3307
123. Sun K et al (2016) Nox2-derived oxidative stress results in inefficacy of antibiotics against post-influenza S. aureus pneumonia. J Exp Med 213(9):1851–1864
124. World Health Organization (2018) Global tuberculosis report. WHO, Geneva
125. Chaisson RE, Martinson NA (2008) Tuberculosis in Africa--combating an HIV-driven crisis. N Engl J Med 358(11):1089–1092
126. Geldmacher C et al (2010) Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection. J Exp Med 207(13):2869–2881
127. Geldmacher C et al (2008) Early depletion of Mycobacterium tuberculosis-specific T helper 1 cell responses after HIV-1 infection. J Infect Dis 198(11):1590–1598

128. Hwang JH et al (2013) Incidence and risk factors of tuberculosis in patients with human immunodeficiency virus infection. J Korean Med Sci 28(3):374–377
129. Swaminathan S et al (2000) Risk of development of tuberculosis in HIV-infected patients. Int J Tuberc Lung Dis 4(9):839–844
130. Mabunda TE, Ramalivhana NJ, Dambisya YM (2014) Mortality associated with tuberculosis/HIV co-infection among patients on TB treatment in the Limpopo province, South Africa. Afr Health Sci 14(4):849–854
131. Podlekareva DN et al (2014) Short- and long-term mortality and causes of death in HIV/tuberculosis patients in Europe. Eur Respir J 43(1):166–177
132. Zenner D et al (2015) Impact of TB on the survival of people living with HIV infection in England, Wales and Northern Ireland. Thorax 70(6):566–573
133. Pawlowski A et al (2012) Tuberculosis and HIV co-infection. PLoS Pathog 8(2):e1002464
134. Jordan TS, Spencer EM, Davies P (2010) Tuberculosis, bronchiectasis and chronic airflow obstruction. Respirology 15(4):623–628
135. Dheda K et al (2005) Lung remodeling in pulmonary tuberculosis. J Infect Dis 192 (7):1201–1209
136. Scanga CA, Bafica A, Sher A (2007) Viral gene expression in HIV transgenic mice is activated by Mycobacterium tuberculosis and suppressed after antimycobacterial chemotherapy. J Infect Dis 195(2):246–254
137. Bafica A et al (2003) Cutting edge: in vivo induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2. J Immunol 171(3):1123–1127
138. Larson EC et al (2017) Mycobacterium tuberculosis reactivates latent HIV-1 in T cells in vitro. PLoS One 12(9):e0185162
139. Tyagi P et al (2020) Mycobacterium tuberculosis reactivates HIV via exosomes mediated resetting of cellular redox potential and bioenergetics. bioRxiv 11:e03293-19. https://doi.org/10.1101/629048
140. Shastri MD et al (2018) Role of oxidative stress in the pathology and management of human tuberculosis. Oxid Med Cell Longev 2018:7695364
141. Fontalvo DM et al (2016) Tuberculosis and pulmonary candidiasis co-infection present in a previously healthy patient. Colomb Med 47(2):105–108
142. Kim SH et al (2015) Invasive pulmonary aspergillosis-mimicking tuberculosis. Clin Infect Dis 61(1):9–17
143. Page ID et al (2019) Chronic pulmonary aspergillosis commonly complicates treated pulmonary tuberculosis with residual cavitation. Eur Respir J 53(3):1801184
144. Dagenais TRT, Keller NP (2009) Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. Clin Microbiol Rev 22(3):447–465
145. Suzuki K (2009) Anti-oxidants for therapeutic use: why are only a few drugs in clinical use? Adv Drug Deliv Rev 61(4):287–289

Oxidative Stress in Depression and Other Comorbid Disorders

9

Shvetank Bhatt, Dilip Kumar Pandey, Chandragouda R. Patil, and A. N. Nagappa

Abstract

Reactive oxygen species (ROS) play an important role in the pathophysiology of central nervous system (CNS) disorders such as depression, Alzheimer's disease, and Parkinson's disease. A decreased antioxidant capacity may not be able to protect against ROS, resulting in damage to endogenous vital body molecules such as fat, DNA, and protein. The increased oxidative stress linked with increased expression of nuclear factor (NF)-κβ, which is responsible for modulation of various proinflammatory cytokines and neuronal inflammation, results in neuronal death. Moreover, increased oxidative stress also stimulates the caspase-3-mediated apoptotic pathway and leads to neuronal death.

Coexistence of magnified oxidative stress and the appearance of depressive disorders is evidenced by enhanced lipid peroxidation. Earlier studies reported that malondialdehyde (MDA), a metabolized output of lipid peroxidation, was found in greater amounts in the biomatrix of patients with CNS disorders as compared with control subjects. Reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) enzyme levels/activity play significant roles in antioxidant defense mechanisms. High GSH levels lead to scavenging of free radicals. Some previous reports have shown that lower levels of the antioxidant GSH play an important role in the pathophysiology of CNS disorders. The CAT enzyme catalyzes the conversion of hydrogen peroxide into water and oxygen. Previous studies have reported decreases in CAT and SOD levels in the prefrontal

S. Bhatt (✉) · A. N. Nagappa
Amity Institute of Pharmacy, Amity University Madhya Pradesh (AUMP), Gwalior, Madhya Pradesh, India

D. K. Pandey
Department of Pharmacy, Birla Institute of Technology & Science, Pilani, Pilani, Rajasthan, India

C. R. Patil
Department of Pharmacology, RC Patel Institute of Pharmaceutical Education and research, Shirpur, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_9

cortex, the hippocampus, and the striatum of stressed mice, indicating depression-like behavior. CAT mediates signaling in cell growth abnormality, the death of neurons, sugar metabolism, and platelet aggregation.

SOD is another important antioxidant enzyme, which promotes dismutation of superoxide into oxygen and hydrogen peroxide. SOD is an important enzyme involved in CNS disorders, cofactored with copper and zinc. Mice deficient in SOD_2 have been shown to have major oxidative stress and died soon after birth. Overall increased levels or imbalances of oxidative stress enzymes are responsible for various CNS disorders. The roles of these enzymes or oxidative stress markers in various disorders are discussed in various sections of this book chapter.

Keywords

Neurological disorders · Oxidative stress · SOD · GSH · CAT

9.1 Outline

- Oxidative stress
- Oxidant and antioxidant systems
- Role of oxidative stress in various neurological disorders
- Expert opinion

9.2 Introduction to Oxidative Stress

Oxidative stress is a disturbance or imbalance between the production of reactive oxygen species (ROS; i.e., free radicals) and antioxidant defense mechanisms [1]. In other words, oxidative stress is the result of production of oxygen radicals in cells, which can overpower the normal antioxidant mechanism(s) [2]. This imbalance may be responsible for the pathogenesis of various central nervous system (CNS) disorders such as depression, anxiety, Alzheimer's disease (AD), and Parkinson's disease (PD). Individual internal defense mechanisms such as antioxidants (tocopherols, ascorbic acid, and glutathione) or enzymes involved in oxygen radical scavenging (catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD)), which are required for fighting oxidative damage to proteins, lipids, and DNA, can lead to cytotoxicity/genotoxicity and sometimes lead to carcinogenesis, when affected mutated cells can proliferate [2]. ROS serve as an important mediators in various physiological roles (i.e., cell signaling), and they are generated in the body as by-products of biotransformation of oxygen. In addition, different environmental stressors (e.g., ultraviolet (UV) light, x-rays, pollutants, and metallic ions), xenobiotics (e.g., antiblastic drugs), and external substances (e.g., cigarette smoke, pesticides, and ozone) can cause the formation of free radicals in the body and contribute to greater ROS production, causing an imbalance that leads to cell and

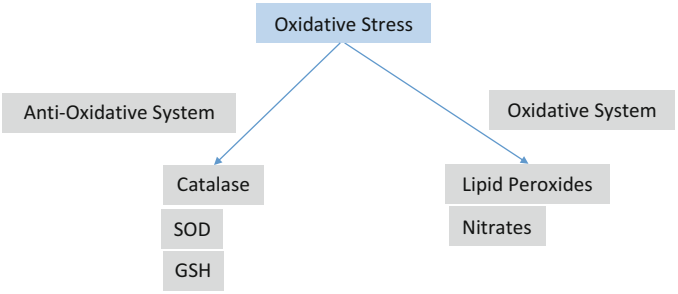


Fig. 9.1 Oxidative and antioxidative systems. *GSH* glutathione, *SOD* superoxide dismutase

tissue damage due to excessive oxidative stress [3]. The basic components of the oxidative and antioxidative systems are shown in Fig. 9.1.

Hydroxyl ion radicals (•OH), superoxide free radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are frequently defined as ROS and are responsible for generation of oxidative stress; these free radicals are generated as by-products of various biological activities in vivo [4].

Production of ROS takes place in mitochondria in a normal physiological process; that is, $O_2^{\bullet -}$ can be formed by the cellular respiration process, by lipoxygenases (LOX) and cyclooxygenases (COX) during the biotransformation of arachidonic acid, and by vascular endothelial and inflammatory cells [5]. Oxidative stress has been associated with various CNS disorders, such as PD, AD, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), depression, and memory loss [6–8]. In different sections of this book chapter, we discuss the role of oxidative stress in some of these disorders.

9.3 The Antioxidant System

Oxidative stress initiates structure and function alterations of key biomolecules. To overcome uncontrolled generation of free radicals, cells have evolved defense mechanisms for protection against ROS-mediated oxidative damage. These include antioxidant defense mechanism(s) to monitor the generation of ROS. Antioxidants are present in lesser concentration in the cells and significantly delay or prevent oxidation of various cellular oxidizable substrates. Antioxidants work by donating their electrons to ROS and thereby reduce their adverse effects. Three prominent mechanisms of antioxidants are (1) minimization of ROS production, (2) scavenging of ROS, and (3) repair of damaged target molecules [9].

9.4 Oxidative Stress in Depression

The World Health Organization (WHO) previously stated that by the year 2020, depressive disorder would be the illness with the maximum burden of disease. In particular, unipolar major depressive disorder is the psychiatric disorder with the highest prevalence and incidence rates, is cost intensive, and has relatively high morbidity [10]. Oxidative stress plays a crucial role in the pathogenesis of depression. Data from various studies have suggested that the severity of depression increases with increases in oxidative stress. Increased oxidative stress in unipolar depressive disorder is mediated by elevated concentrations of free radicals. According to studies, important causes of major depression are inflammation, autoimmune disease-induced tissue damage, and prolonged exposure to stress, leading to oxidative stress. In several studies, malondialdehyde (MDA) levels have been found to be increased in depression [11]. Moreover, long-term stress is closely related to various neurological disorders, including depression. Excessive stress leads to alterations in neuronal signaling, immune responses, the cardiovascular system, the neuroendocrine system, and the sympathetic nervous system via activation of the hypothalamus–pituitary–adrenal (HPA) axis [12, 13]. In normal physiological conditions, oxygen-derived species are converted into less toxic compounds with participation of the most significant antioxidant enzymes such as SOD, CAT, and GSH [14]. Increases in stress cause deregulation of the antioxidant activity of enzymes such as GSH, SOD, and CAT, and increases lipid peroxide and nitrite levels in the brain structure of rodents [15, 16], leading to depression.

Increased activity of ROS causes damage to endogenous biological molecules such as protein, fat, and DNA [17–19]. Increased oxidative stress leads to increased expression of nuclear factor (NF)-κβ, which leads to increased levels of various proinflammatory cytokines (such as TNF-α and interleukin (IL)-1β) and neuronal inflammation, followed by neuronal death. In addition to neuronal inflammation, oxidative stress activates caspase-3-mediated apoptotic neuronal death, as shown in Fig. 9.2.

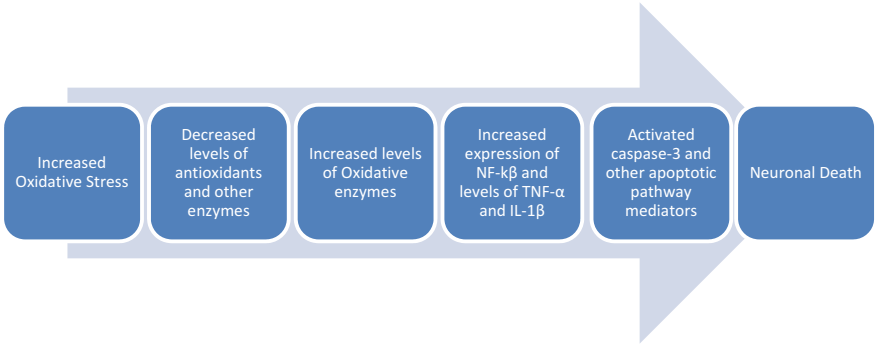


Fig. 9.2 Increased oxidative stress leads to neuronal death in depression. *IL* interleukin, *NF* nuclear factor, *TNF* tumor necrosis factor

9.5 Oxidative Stress in Alzheimer's Disease

Alzheimer's disease is the most common cause of disability in individuals over the age of 60 years. Oxidative stress plays an important role in the pathophysiology of AD, a destructive disorder in the elderly. The brain is more prone than other organs to be affected by oxidative stress, and most of the components of neurons (lipids, proteins, and nucleic acids) can be oxidized in AD as a result of mitochondrial dysfunction, increased metal levels, inflammation, and β-amyloid (Aβ) peptide accumulation. Many blood/serum markers of oxidative stress have been identified in AD patients or related animal models, including protein carbonyls and 3-nitrotyrosine [20, 21], 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), malondialdehyde (MDA) [12], 4-hydroxynonenal (4-HNE), and F2-isoprostanes (F2-IsoPs). Some studies have suggested that mitochondrial dysfunction, metal accumulation, Tau hyperphosphorylation, and Aβ peptide accumulation are basic mechanisms involved in increased oxidative stress. Aβ accumulation is the most important factor in the progression of AD; it disrupts the electron transport chain (ETC) by reducing the activities of some important enzymes and affects mitochondrial function [22, 23]. In addition, soluble Aβ is related to rises in H_2O_2 levels and reductions in the activity of cytochrome c oxidase in mice [24]. Moreover, treatment with Aβ in isolated mitochondria causes oxidative injury to the membrane of mitochondria, impairs lipid polarity, and inhibits the most necessary enzymes of the respiratory chain [25, 26].

Abnormally high accumulation of Zn, Cu, and Fe has been seen in patients with AD. Interaction of these metal ions leads to increases in oxidative stress. By binding to these metal ions, Aβ produces ROS through redox activity. However, metal chelators reduce the activity of Aβ and prevent aggregation of metal overload. For example, binding of Aβ to cupric ion forms a cuproenzyme-like complex and during this process, formation of Aβ free radicals and hydrogen peroxide take place.

In addition, oxidative stress has a genetic link with AD, particularly with respect to apolipoprotein E4 (ApoE4) [27]. Moreover, higher levels of F2-isoprostanes have been observed in patients with AD. A high incidence of cell death or apoptosis has been observed in patients with AD, which is further associated with oxidative stress [28] (Fig. 9.3).

9.6 Oxidative Stress in Anxiety

Consumption of O_2 with less antioxidant defenses and more fat content make the brain highly vulnerable to redox imbalances. Recently, many studies have suggested that oxidative stress is associated with anxiety and depression, as well as comorbid depression and anxiety. In the process of high oxidative stress, the lipid-rich constitution of the brain enhances peroxidation of lipids, leading to a decrease in the strength of cellular membranes, as well as damage to membrane proteins, and also inactivating receptors, enzymes, and ion channels. This overall process

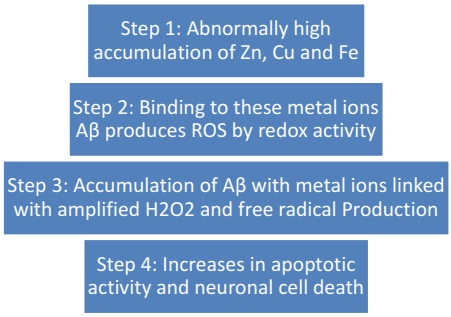


Fig. 9.3 Role of oxidative stress in the progression of Alzheimer's disease. *Aβ* β-amyloid, *ROS* reactive oxygen species

imbalance in oxidative stress can alter neuronal signaling, neuronal function, and overall brain activity.

In 2005, Hovatta et al. [29] identified a close relationship between antioxidative defense mechanisms and anxiety-related phenotypes in six inbred mouse strains. They observed that in the brain, expression of the glutathione reductase 1 and glyoxalase 1 genes (which are related to antioxidant metabolism) is highly correlated with phenotypes of anxiety. Overexpression of the aforementioned enzymes in the cingulated cortex of the mouse brain results in an increase in anxiety-like behavior, while inhibition of glyoxalase 1 expression produces low-anxiety mice.

Some studies have also demonstrated the role of the HPA axis in anxiety. The literature shows a link between anxiety and oxidative stress. Berry et al. [30] showed that in wild mice, pain sensitivity and emotional behavior increase with age, likely because of the increase in oxidative stress and the reduction in antioxidant mechanisms. They demonstrated that deletion of the p66Shc gene (which is responsible for the regulation of reactive species metabolism) leads to a reduction in anxiety-like behavior and oxidative stress in mice. Desrumaux et al. [31] correlated a reduction in vitamin E levels with an increase in anxiety-like behavior. Moreover, decreased levels of antioxidant enzymes such as SOD and glutathione peroxidase (GPx) and higher lipid peroxide levels have been observed in patients with anxiety, particularly those suffering with obsessive–compulsive disorder and panic disorder. Similarly, Yasunari et al. [32] found a marked link between ROS and anxiety in patients with hypertension. Some recent studies have also demonstrated a positive correlation between oxidative stress markers and human aging [33] (Fig. 9.4).

9.7 Oxidative Stress in Schizophrenia

It is well known that both genetic and environmental factors are involved in the pathogenesis of schizophrenia. Oxidative stress has an important place in the pathophysiology of schizophrenia, which may lead to novel treatment strategies.

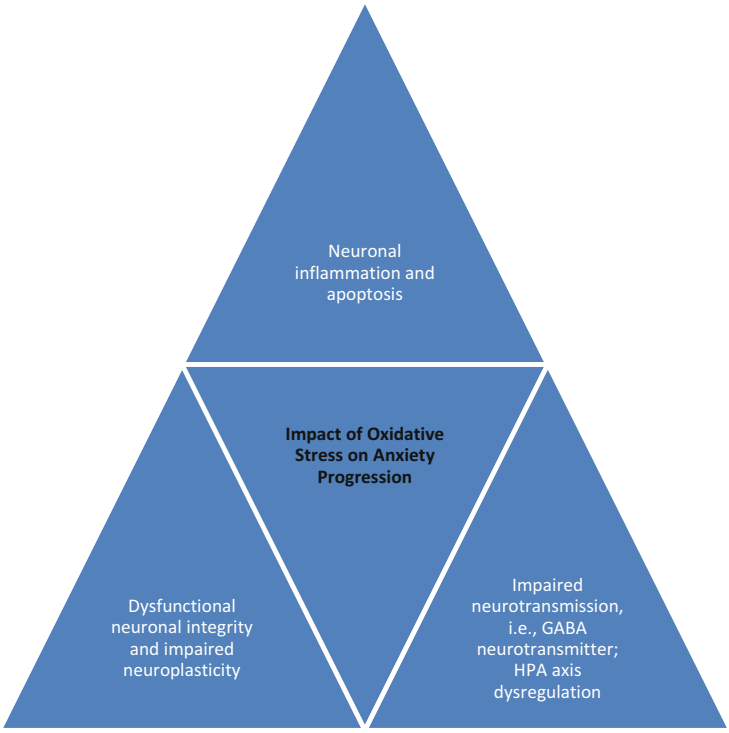


Fig. 9.4 Role of oxidative stress in the progression of anxiety. *GABA* γ-aminobutyric acid, *HPA* hypothalamus–pituitary–adrenal

Recent human studies support the concept that oxidative stress plays a putative role in the progression of schizophrenia. Elevated levels of oxidative enzymes and decreased levels of antioxidant enzymes are observed in early schizophrenia. High levels of oxidative enzymes result in damage to lipids, proteins, nucleic acids, and tissue. Incapability of the antioxidant defense system has been observed both in patients with neuroleptic-naive first-episode schizophrenia and in patients with chronic schizophrenia being treated with medication, and indicates the presence of oxidative stress in schizophrenia. Bitanihirwe and Woo [34] and Huang and Liu [35] have noted that levels of erythrocyte thiobarbituric acid reactive substances (TBARS) in patients with chronic schizophrenia treated with stable daily doses of neuroleptics and serum levels of MDA in patients newly affected with schizophrenia are significantly increased. Moreover, increased lipid peroxidation levels have been found in the hippocampus and thalamus of patients with schizophrenia. A recent meta-analysis has shown that there is an increase in the levels of lipid peroxidation products and NO (discussed in further detail below) in schizophrenia, while SOD activity has been found to be significantly decreased in this disorder [36]. This analysis also reported that the activities of GPx and CAT are not affected in patients

Fig. 9.5 Roles of genetic and environmental factors and inflammasomes in the progression of oxidative stress and the pathophysiology of schizophrenia. *AP* activator protein, *ERK* extracellular signal-related kinase, *IFNγ* interferon-γ, *JNK* c-Jun NH_2-terminal protein kinase, *NF* nuclear factor, *TNF* tumor necrosis factor

with schizophrenia [36]. However various studies have reported lower, normal, and increased levels of CAT in the brains of patients with schizophrenia. The same pattern has been found for SOD. Postmortem studies of human brains have reported a 40% depletion of GSH levels in the caudate nucleus of patients with schizophrenia [37] (Fig. 9.5).

The levels of plasma antioxidants (uric acid, albumin, and bilirubin) have been reported to be significantly decreased in schizophrenia [38–40]. These findings were found to be independent of smoking status [38]. Plasma levels of α-tocopherol [41] and ascorbic acid have also been reported to be decreased in patients with schizophrenia. In contrast, thioredoxin levels have been shown to be increased during the acute phase of schizophrenia [42] but become normalized in patients with chronic schizophrenic on long-term antipsychotic pharmacotherapy [42].

9.8 Impact of Oxidative Stress on Parkinson's Disease

The mechanisms responsible for neuronal degeneration in PD are highly complex and remain to be completely elucidated. The majority (90–95%) of PD cases are idiopathic. In both idiopathic and genetic cases of PD, oxidative stress is thought to be the generic underlying mechanism causing cellular dysfunction and demise [43]. Although the exact mechanism pertaining to ROS generation related to PD is still unknown, many theories have been proposed in support of the role of oxidative stress in PD. In this chapter, we summarize the major sources of oxidative stress and oxidative cellular dysfunction, and their impacts on the progress and maintenance of PD. Extensive production of ROS in the brain may provide an explanation for the magnitude of the role that these reactive molecules play in PD. ROS are generally produced in the cell in the mitochondrial ETC or redox reactions, and they are in fact essential constituents of cellular homeostasis [44]. The oxidative process alters mitochondrial respiration and induces a change in the permeability of transition pores in brain mitochondria [45]. Examples of ROS are the superoxide anion radical (O_2^{2-}), the hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). The superoxide anion, which is mainly generated by mitochondrial Complexes I and III of the ETC, is highly reactive and can easily cross the inner mitochondrial membrane, forming

H_2O_2 [46]. Extensive production of ROS in the brain possibly provides an explanation for the significance of the role that these reactive molecules play in the pathophysiology of PD. The brain ingests about 20% of the oxygen source in the body, and a substantial portion of that oxygen is converted to ROS. ROS can be generated in the brain from several sources, in both neurons and glia, with the ETC being the major contributor at the mitochondrial level [23]. Considerable experimental evidence suggests that a significant contributor to dopaminergic (DAergic) neuronal loss in the brain of patients with PD is ROS, resulting from dopamine metabolism, low GSH levels, and high levels of iron and calcium in the substantia nigra pars compacta (SNpc) [47].

9.9 Mitochondrial Dysfunction in Parkinson's Disease

Mitochondria are dynamic organelles with many purposes. Besides their participation in energy generation, they are closely engaged in calcium homeostasis, stress response, and cell death pathways [48]. Mitochondrial dysfunction was first linked to PD upon the recognition of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD among some drug abusers, and the finding of substantial DAergic neuron loss in their substantia nigra on postmortem investigation [49]. MPTP crosses the blood–brain barrier and is taken up by astrocytes, where it is metabolized into 1-methyl-4-phenylpyridinuim (MPP+) by monoamine oxidase B (MAO-B) and is released into the extracellular space. MPP+ is a substrate for the dopamine transporter and is taken up selectively into DAergic neurons, where it inhibits Complex I of the mitochondrial ETC [50]. Damage to mitochondrial Complex I in the ETC causes leakage of electrons, which in turn causes ROS generation. As such, the Complex I inhibitors rotenone and MPTP, when injected intraperitoneally, exert preferential cytotoxicity to the DAergic neurons [51]. Additional evidence for mitochondrial dysfunction linked to oxidative stress and dopamine (DA) cell damage comes from findings that mutations in genes that encode proteins such as α-syn, parkin, DJ-1, or PINK are linked to familial forms of PD. Mutations in these genes affect mitochondrial function and integrity, and are associated with increases in oxidative stress. ROS influence proteasomal, lysosomal, and mitochondrial function, which in turn regulate the cellular response to oxidative damage [52]. Protein misfolding along with mutation in genes may play significant roles in the occurrence of deleterious events involved in the neurodegenerative process of PD [53].

9.10 Physiological and External Determinants of Oxidative Stress in Parkinson's Disease

9.10.1 Aging

Age is the major risk factor for PD, with an exponential increase of the disease incidence above the age of 65 years [54]. Age-linked mitochondrial dysfunction and subsequent increased ROS production seem to be essential aspects of neurodegenerative disorders that develop later in life. In the brains of elderly patients with PD, high intensities of mitochondrial DNA (mtDNA) deletions are observed in pigmented neurons of the substantia nigra [55]. Furthermore, with aging, mitochondrial function weakens concomitantly with alterations in their morphology and decreased numbers [56], and age-related decreased autophagy results in the accumulation of defective mitochondria [57].

9.10.2 Toxins

The risk of PD may be greater after exposure to multiple pesticides than after exposure to any one pesticide alone [58]. In addition, consumption of pesticide-contaminated well water may increase the risk of PD [59]. Although these toxins act through different mechanisms, they have common characteristics of enhanced oxidative stress due to increased ROS production. An example is the herbicide paraquat, which has been linked to an increased risk of PD [60]. Another pesticide, rotenone, can freely access cellular membranes and stores in mitochondria, where it hinders Complex I by weakening oxidative phosphorylation [61]. With these observations it can be summarized that toxins play significant roles in ROS and PD. The effects of environmental factors on the progression of PD are shown in Fig. 9.6.

9.11 Expert Opinion

Redox degenerative chain reactions in living systems inevitably produce ROS and their derivatives. Oxidative stress is the consequence of an imbalance in the equilibrium of pro-oxidant/antioxidant homeostasis, leading to the generation of toxic ROS, such as hydrogen peroxide, organic hydroperoxides, nitric oxide, superoxide, and hydroxyl radicals. Information is accumulating steadily that supports the general importance of oxidative damage to tissue and cellular components as a primary or secondary causative factor in aging processes and many different human diseases such as PD, AD, and depression.

On the basis of various literature reports and research work, we can conclude that oxidative stress plays a major role in the pathophysiology of various CNS disorders. The main culprits causing disease in all of these cases are imbalances between oxidative and antioxidant metabolism processes. These imbalances lead to increases

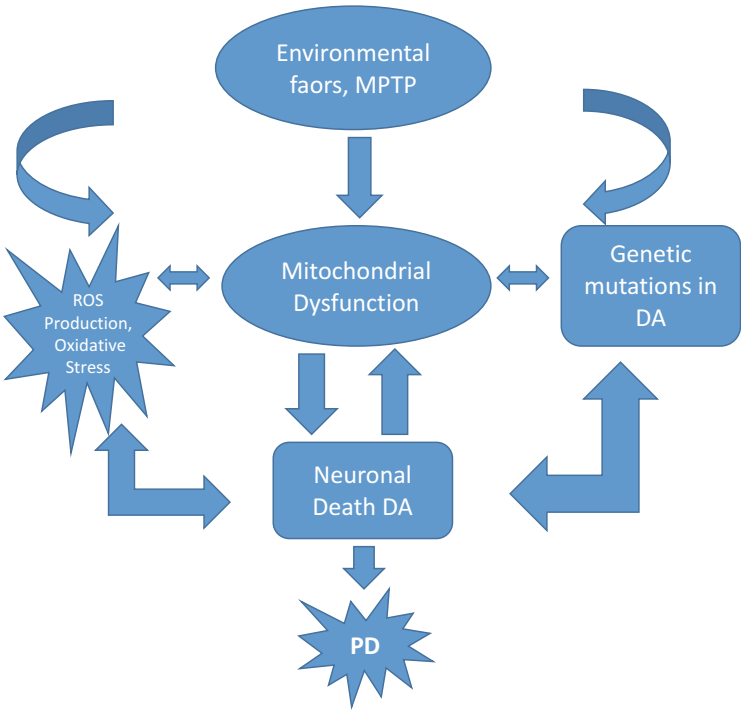


Fig. 9.6 Factors and events associated with Parkinson's disease (PD). *DA* dopamine, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *ROS* reactive oxygen species

in the activity of inflammasomes and other apoptotic mediators such as caspase-3, and finally lead to neuronal death.

References

1. Betteridge DJ (2000) What is oxidative stress? Metabolism 49:3–8
2. Gagné F (2014) Biochemical ecotoxicology: principles and methods. Elsevier, Amsterdam
3. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A (2017) Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev 2017:8416763
4. Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N, Li M, Pappa A, Martinez-Fong D, Del Razo LM, Quintanilla-Vega B, Franco R (2014) Antioxidant gene therapy against neuronal cell death. Pharmacol Ther 142:206–230
5. Al-Gubory KH, Garrel C, Faure P, Sugino N (2012) Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. Reprod Biomed Online 25:551–560
6. Christen Y (2000) Oxidative stress and Alzheimer disease. Am J Clin Nutr 71:621S–629S
7. Halliwell B (2012) Free radicals and antioxidants: updating a personal view. Nutr Rev 70:257–265

8. Singh RP, Sharad S, Kapur S (2004) Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. J Indian Acad Clin Med 5(3):218–225
9. Birben E, Sahiner UM, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5(1):9–19
10. Michel TM, Pülschen D, Thome J (2012) The role of oxidative stress in depressive disorders. Curr Pharm Des 18:5890–5899
11. Bajpai A, Verma AK, Srivastava M, Srivastava R (2014) Oxidative stress and major depression. J Clin Diagn Res 8:CC04–CC07
12. Dayas CV, Buller KM, Crane JW, Xu Y, Day TA (2001) Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. Eur J Neurosci 14:1143–1152
13. Reyes TM, Walker JR, DeCino C, Hogenesch JB, Sawchenko PE (2003) Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus. J Neurosci 23:5607–5616
14. Maes M, Galecki P, Chang YS, Berk M (2011) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. Prog Neuropsychopharmacol Biol Psychiatry 35:676–692
15. Kumari B, Kumar A, Dhir A (2007) Protective effect of non-selective and selective COX-2-inhibitors in acute immobilization stress induced behavioural and biochemical alterations. Pharmacol Rep 59:699–707
16. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN (1996) Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J 10:1532–1538
17. Bilici M, Efe H, Koroglu MA, Uydu HA, Bekaroglu M, Deger O (2001) Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by anti-depressant treatments. J Affect Disord 64:43–51
18. Eren I, Naziroglu M, Demirdas A, Celik O, Uguz AC, Altunbasak A, Uz E (2007) Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat. Neurochem Res 32:497–505
19. Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R (2003) Oxidative damage and major depression: the potential antioxidant action of selective serotonin reuptake inhibitors. Redox Rep 8:365–370
20. Beal M (2002) Oxidatively modified proteins in aging and disease. Free Radic Biol Med 32:797–803
21. Butterfield D, Kanski J (2001) Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mech Ageing Dev 122:945–962
22. Wang X, Su B, Siedlak SL, Moreira PI, Fujioka H, Wang Y et al (2008) Amyloid-beta overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. Proc Natl Acad Sci U S A 105:19318–19323
23. Yan MH, Wang X, Zhu X (2013) Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. Free Radic Biol Med 62:90–101
24. Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH (2006) Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. Hum Mol Genet 15:1437–1449
25. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA (2002) Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem 80:91–100
26. Rodrigues CM, Sola S, Brito MA, Brondino CD, Brites D, Moura JJ (2001) Amyloid beta-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. Biochem Biophys Res Commun 281:468–474
27. Ramassamy C, Averill D, Beffert U et al (2000) Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. Neurobiol Dis 7:23–37
28. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ II (1998) Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. Ann Neurol 44:410–413

29. Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM, Ellison JA, Schadt EE, Verma IM, Lockhart DJ, Barlow C (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature 438:662–666
30. Berry A, Capone F, Giorgio M, Pelicci PG, de Kloet ER, Alleva E et al (2007) Deletion of the life span determinant p66Shc prevents age-dependent increases in emotionality and pain sensitivity in mice. Exp Gerontol 42:37–45
31. Desrumaux C, Risold PY, Schroeder H, Deckert V, Masson D, Athias A et al (2005) Phospholipid transfer protein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. FASEB J 19:296–297
32. Yasunari K, Matsui T, Maeda K, Nakamura M, Watanabe T, Kiriike N (2006) Anxiety-induced plasma norepinephrine augmentation increases reactive oxygen species formation by monocytes in essential hypertension. Am J Hypertens 19:573–578
33. Pandey KB, Rizvi SI (2010) Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxid Med Cell Longev 3:2–12
34. Bitanihirwe BK, Woo TU (2011) Oxidative stress in schizophrenia: an integrated approach. Neurosci Biobehav Rev 35:878–893
35. Huang D, Liu S (2017) Oxidative stress and schizophrenia. J Psychiatry Brain Sci 2(2):4
36. Zhang M, Zhao Z, He L, Wan C (2010) A meta-analysis of oxidative stress markers in schizophrenia. Sci China Life Sci 53:112–124
37. Yao JK, Leonard S, Reddy R (2006) Altered glutathione redox state in schizophrenia. Dis Markers 22:83–93
38. Reddy R, Keshavan M, Yao JK (2003) Reduced plasma antioxidants in first-episode patients with schizophrenia. Schizophr Res 62:205–212
39. Yao JK, Reddy R, van Kammen DP (1998) Reduced level of plasma antioxidant uric acid in schizophrenia. Psychiatry Res 80:29–39
40. Yao JK, Reddy RD, van Kammen DP (2001) Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. CNS Drugs 15:287–310
41. McCreadie RG, MacDonald E, Wiles D, Campbell G, Paterson JR (1995) The Nithsdale schizophrenia surveys. XIV: Plasma lipid peroxide and serum vitamin E levels in patients with and without tardive dyskinesia, and in normal subjects. Br J Psychiatry 167:610–617
42. Zhang XY, Chen DC, Xiu MH, Wang F, Qi LY, Sun HQ, Chen S, He SC, Wu GY, Haile CN, Kosten TA, Lu L, Kosten TR (2009) The novel oxidative stress marker thioredoxin is increased in first-episode schizophrenic patients. Schizophr Res 113:151–157
43. Nakabeppu Y, Tsuchimoto D, Yamaguchi H, Sakumi K (2007) Oxidative damage in nucleic acids and Parkinson's disease. J Neurosci Res 85:919–934
44. Johnson WM, Wilson-Delfosse AL, Mieyal JJ (2012) Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients 4:1399–1440
45. Berman SB, Hastings TG (1999) Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J Neurochem 73:1127–1137
46. Muller FL, Liu Y, Van Remmen H (2004) Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 279:49064–49073
47. Jenner P, Olanow W (2006) The pathogenesis of cell death in Parkinson's disease. Neurology 66:S24–S36
48. Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552:335–344
49. Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979–980
50. Vila M, Przedborski S (2003) Targeting programmed cell death in neurodegenerative diseases. Nat Rev Neurosci 4:365–375
51. Betarbet R, Sherer TB, Greenamyre JT (2002) Animal models of Parkinson's disease. Bioessays 24:308–318
52. Cook C, Stetler C, Petrucelli L (2012) Disruption of protein quality control in Parkinson's disease. Cold Spring Harb Perspect Med 2:a009423

53. Schapira AHV, Olanow CW, Greenamyre JT, Bezard E (2014) Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. Lancet 384:545–555
54. Gibb WR, Lees A (1991) Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 54:388–396
55. Reeve AK, Krishnan KJ, Elson JL, Morris CM, Bender A, Lightowlers RN, Turnbull DM (2008) Nature of mitochondrial DNA deletions in substantia nigra neurons. Am J Hum Genet 82:228–235
56. Shigenaga MKHT, Ames BN (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 91:10771–10778
57. Cuervo AM (2008) Autophagy and aging: keeping that old broom working. Trends Genet 24:604–612
58. Kamel F (2013) Epidemiology: paths from pesticides to Parkinson's. Science 341:722–723
59. Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B (2009) Well-water consumption and Parkinson's disease in rural California. Environ Health Perspect 117:1912–1918
60. Lee PC, Bordelon Y, Bronstein J, Ritz B (2012) Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. Neurol. 79:2061–2066
61. Perier C, Bové J, Vila M, Przedborski S (2003) The rotenone model of Parkinson's disease. Trends Neurosci 26:345–346

Oxidative Stress Monitoring in In Vitro and In Vivo Models

10

Thania Rios Rossi Lima, Bianca Camargo Penteado Sales, and Lilian Cristina Pereira

Abstract

In aerobic organisms, cellular respiration is an essential process that is divided into several steps. The third and last step is called oxidative phosphorylation, which occurs in the mitochondria, specifically in the inner mitochondrial membrane. The mitochondria are considered the cell "powerhouse" because they generate energy through oxidative phosphorylation, which is the main energy source in aerobic organisms. The generated energy is stored in the ATP molecule and is used to maintain various biological processes. Conversely, the mitochondria are also the primary site for reactive oxygen species (ROS) production. If ROS are produced beyond the capacity of antioxidant systems to neutralize them, they induce oxidative stress. This condition is harmful for macromolecules and can lead to cell death. Oxidative stress and its consequences can be monitored by many assays that employ in vitro and in vivo models, which will be discussed throughout this chapter.

T. R. R. Lima · B. C. P. Sales
Department of Pathology, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

Center for Evaluation of Environmental Impact on Human Health (TOXICAM), Botucatu, São Paulo, Brazil
e-mail: thania.rios@unesp.br

L. C. Pereira (✉)
Center for Evaluation of Environmental Impact on Human Health (TOXICAM), Botucatu, São Paulo, Brazil

Faculty of Agronomic Sciences of Botucatu, Department of Bioprocesses and Biotechnology, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
e-mail: lilian.pereira@unesp.br

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_10

Keywords

ROS · Oxidative stress monitoring · Cellular culture · Zebrafish

10.1 Overview of Oxidative Stress

Owing to their bioenergetic functionality, the mitochondria are considered the most important organelle in eukaryotic organisms. They are the center of oxidative phosphorylation (OXPHOS), thereby playing a crucial role in energy metabolism, i.e., adenosine triphosphate (ATP) production, in aerobic cells [1]. OXPHOS is a complex and systematic process that occurs in the inner mitochondrial membrane and involves two interrelated steps: the electron transport chain (ETC) and chemiosmosis. The ETC comprises four distinct protein complexes (I, II, III, and IV) that are inserted in the inner mitochondrial membrane, namely, NADH dehydrogenase, succinate dehydrogenase, ubiquinol-cytochrome c reductase, and cytochrome c oxidase, respectively. Each of these complexes acts as sequential electron carriers via reduction and oxidation (redox) reactions up to a final acceptor, molecular oxygen (O_2), which is reduced to water. The electrons that participate in the ETC come from early cellular respiration stages and are channeled through universal acceptors, the coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) [2, 3]. In association with the four multienzymatic complexes I–IV, other proteins such as coenzyme Q (or ubiquinone), cytochromes, and iron-sulfur proteins are involved in electron transport along the ETC [4]. Simultaneously with the electron transport through the respiratory chain, H^+ ions are pumped into the intermembrane space, generating a proton-motive force. Next, ATP synthase couples the backflow of these protons to ATP synthesis from ADP and phosphate [3–5]. At the end of OXPHOS, a total of 32–34 ATP molecules are obtained. The entire oxidative phosphorylation process has been discussed in previous chapters (see Chap. 1).

When this sequence of events is overstimulated or blocked, electrons leak and reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radical (OH^-), singlet oxygen (O_2), and alpha-oxygen (α-O), are produced. Normally, ROS regulate many cellular processes like proliferation, quiescence, and phenotypic determination, but when such reactive species are generated beyond the neutralizing capacity of antioxidant systems, they can induce an oxidative stress condition that impairs proteins and DNA and which even culminates in cell death [6, 7]. Clearly, the mitochondria are major ROS sources, so they are also primary injury sites. However, other sources have been identified, such as p66shc and Nox4 proteins [6].

Under normal conditions, the antioxidant activity can balance the ROS amount that causes oxidative damage and prevent a target molecule from being damaged by ROS (see Fig. 10.1) [8]. Antioxidant enzymes are necessary to maintain life: they intercept and repair damages in the organism, thanks to their association with the detoxification of xenobiotics. The members of this enzymatic system are catalase

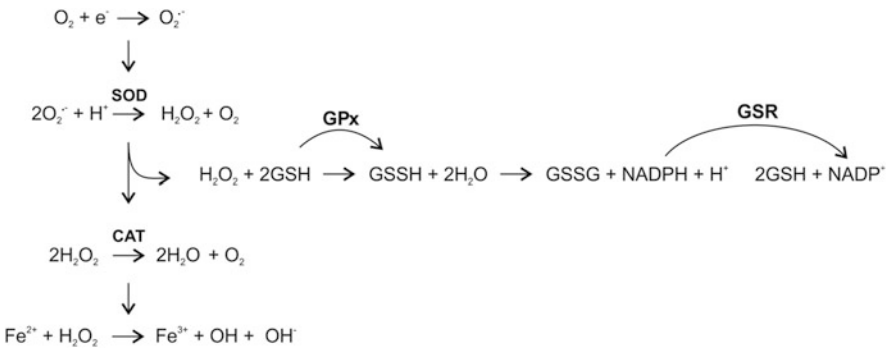


Fig. 10.1 Reactions involved in ROS formation and detoxification. The O_2^- originating from molecular oxygen monoelectronic reduction is converted to H_2O_2 by the enzyme SOD; the enzyme CAT decomposes H_2O_2 into water and oxygen and/or is oxidized by action of the GPx enzyme. Oxidized glutathione is recycled to maintain the reduced glutathione levels. In the presence of some transition metals (e.g., iron and copper), H_2O_2 generates OH^-

(CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), while vitamin E, vitamin C, reduced glutathione (GSH), and other compounds [9, 10] represent nonenzymatic antioxidants.

According to the World Health Organization (WHO), around 22% of all the diseases are associated with environmental factors. Pesticides and metals are among the main environmental toxicants, and they can severely impact animal and human health on the tissue, organ, and systemic levels. Numerous pesticides are known to induce ROS. A classic example is the herbicide paraquat, which inhibits the ETC and preferentially affects the nigrostriatal region, cerebral cortex, and hippocampus. Methylmercury (MeHg), a highly poisonous heavy metal that is released into the environment and which has bioaccumulative properties, is another common example. Currently, exposure to MeHg occurs mainly through contaminated fish and shellfish intake [11]. MeHg increases ROS production and decreases glutathione levels [1, 7]. Because the neuronal tissue has high energy demands and may therefore be a critical ROS accumulation site, exposure to toxic substances and the consequent oxidative stress generation have been suggested to trigger different diseases, especially neurodegenerative diseases (e.g., Parkinson's and Alzheimer's disease).

10.2 Relevance of Experimental Studies and Use of Alternative Methods in Biomedical Research

The intensive use of hazardous compounds in human activities adversely affects the human health and contaminates various environments, including soil, groundwater, and surface water, thereby impairing different ecological levels. Unintentional poisonings are estimated to lead to 193,000 deaths/year globally even though most exposures could be controlled. According to projections of the Organization for Economic Cooperation and Development (OECD), the production of chemicals,

such as household and occupational substances, will grow fast up to 2050, increasing chemical exposure and potential poisoning [12]. Therefore, assessing the hazard and the risk of chemical substances is extremely important in biomedical research. Considering the scientific historical context in the last decades, most of these studies have been carried out with animal models that allow the adverse effects to be characterized and the mode and mechanism of action of the investigated compounds to be understood. Animal models have also played a pivotal role in understanding the pathogenesis of the well-known Zika virus (ZIKV), which emerged suddenly in 2015 and was associated with a widespread outbreak of microcephaly and other severe congenital abnormalities in newborns of infected mothers [13]. Recently, brain organoids have been extensively used to study how ZIKV is associated with neurological alterations [14, 15]. In summary, this technology represents one of the innovations in in vitro methods that mimic an organ through self-organized three-dimensional tissue cultures that are derived from stem cells [16].

In the world scenario, there has been a progressive increase in the use of alternative methods to animal experimentation in different academic research and industrial sector. This has happened due to efforts made by the global scientific community to establish cost-efficient, inhumanity-free, and increasingly predictive experimental models associated with the ethical-social pressure to follow the 3R principles—*Replacement*, *Reduction*, and *Refinement*—postulated by Russell and Burch in the late 1950s [17, 18]. The 3R program aims to develop and to validate new means of evaluation, to harmonize the use of experimental models, to demonstrate the use of new procedures aimed at reducing the number of animals, to replace the use of animals whenever possible, to improve on methods that have already been described to minimize animal discomfort, and to recommend that alternative tests be implemented in vivo and in vitro [17–19]. Although the concern with animal welfare has taken on greater proportions over the past decades, it is nothing new (see Fig. 10.2). The first worldwide act (Martin's Act, 3 Geo IV, c 71) to prevent animal

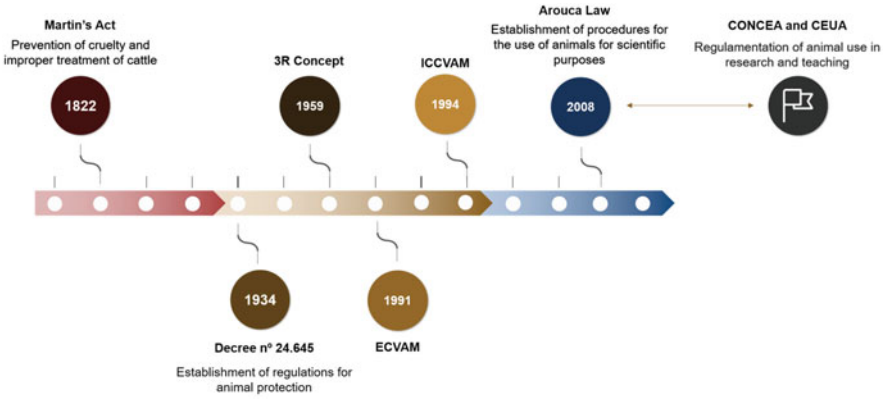


Fig. 10.2 Progress of regulations on animal welfare. Beginning in 1822, with the approval of Martin's Act, and culminating in the emergence of Ethics Committees on Animal Use

cruelty, which discussed the improper treatment of cattle, was implemented in 1822 in the United Kingdom [20]. In Brazil, the first decree on animal welfare requiring adequate animal care and punishment for noncompliance was only endorsed in 1934 (Decree no. 24.645, July 10, 1934) [21]. The Arouca Law no. 11.794, which was sanctioned in 2008, regulates and establishes procedures for the use of animals for scientific purposes. The Arouca Law also created the National Council for Animal Control and Experimentation (CONCEA), which makes the Ethics Committees on the Use of Animals (CEUA) compulsory in institutions that conduct research or teaching activities in order to guarantee adequate animal care and ethical animal management [22]. Such events in Brazil followed the same pathway as other events on animal welfare around the world, including the creation of the European Centre for Validation of Alternative Methods (ECVAM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) [23].

10.2.1 In Vitro Experimental Models with Cell Culture

Despite confusion between the terms "alternative method" and "in vitro method," they are different. Alternative approaches include distinct nonanimal methods like computerized modeling, decision-aiding tools, data sources to enhance sharing, and assessment of existing knowledge or in vitro models [24, 25]. An in vitro method (Latin meaning *in the glass*) entails maintaining a given biological model in a controlled environment outside a living organism. In vitro methods can employ microorganisms (e.g., bacteria or fungi), cells derived from multicellular organisms (e.g., the HepG2 or MCF-7 cell lines), or intracellular components (e.g., mitochondria), among others. In vitro models for cell culture can be prepared by two main techniques: two-dimensional (2D) and three-dimensional (3D) cell culture [26–28].

The 2D cell culture method is widely used because it is easy to handle, its maintenance is inexpensive, and it can be performed with adherent cells or cells in suspension. Although 2D systems are well accepted and have advanced our understanding of cell behavior significantly, they do not represent the biological characteristics and the functional bioactivities that are observed in vivo. The increasing use of in vitro models has stimulated the research and the development of new methods that can reflect the body response to aggression better. In this context, 3D systems can mimic the highly complex physiological environment through cell-cell and cell-extracellular matrix (ECM) interactions. These interactions influence cell differentiation, proliferation, viability, and expression of genes and proteins, and they are tied to the functions of whole organs [26–28]. With respect to the methods that are applied to prepare 3D models, they can be divided into the scaffold-based and the scaffold-free method (see Fig. 10.3). In the scaffold-based method, the cells are encapsulated by a physical structure manufactured from a natural or synthetic material that acts as an ECM, enabling the cells to interact with their surroundings and creating an artificial microenvironment. In scaffold-free models, cells aggregate due to an external force, which stimulates the formation of their own ECM

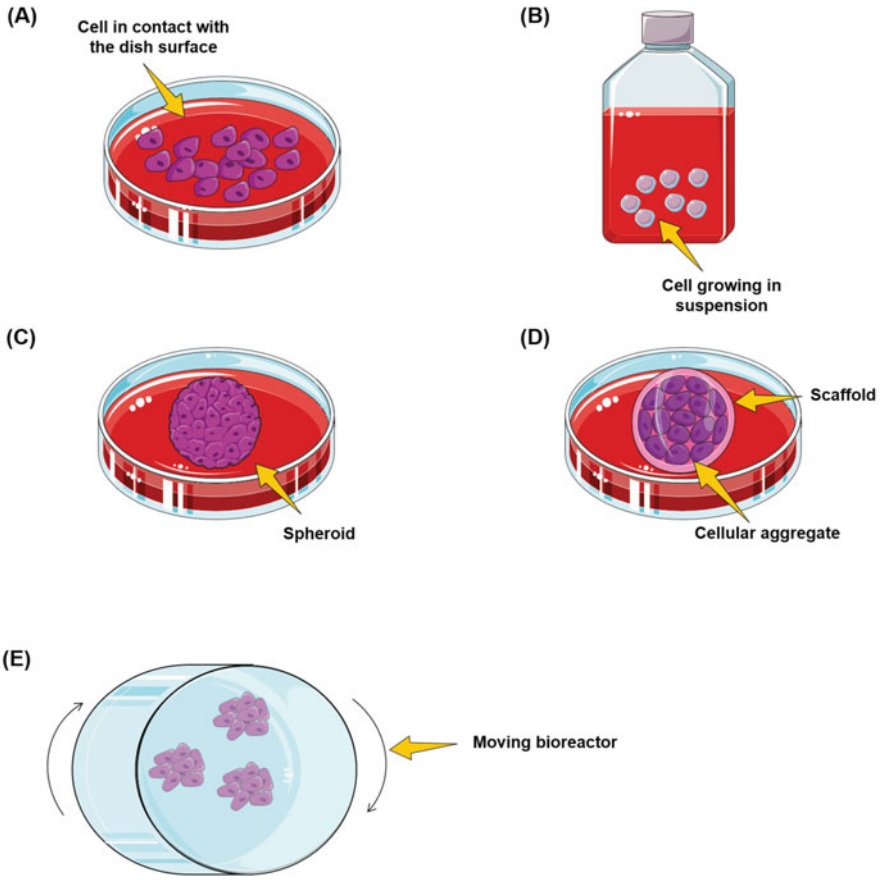


Fig. 10.3 Cell culture methods. (**a**) Cells growing in a monolayer, (**b**) cell culture in suspension, (**c**) scaffold-free 3D culture, (**d**) scaffold-based 3D cell culture, and (**e**) 3D cell culture using bioreactors

components [29, 30]. Nowadays, a 3D culture can be obtained from numerous techniques, such as magnetic levitation, hanging drop, use of Matrigel or Alginate hydrogel, or use of bioreactors. Both the 2D and the 3D systems can be achieved by using cocultures in which different cell types grow together in the same environment. This type of culture enables cells to communicate in almost the same way they interact in organisms. In cocultures, it is possible to distinguish between target cells and assistance cells that support target cell growth and development [26].

The famous statement of the statistician George E. P. Box [31], "essentially, all models are wrong, but some are useful," is not restricted to mathematics, but it certainly extends to biomedical science. Therefore, the more predictive and reproducible the applied model, the greater its reliability. How much an in vitro assay is reliable is directly related to how similar the chosen model is to the cell phenotype within the target tissue in physiological conditions [32]. For example, the size of

spheroids can interfere in the observed outcome: larger spheroids may transport oxygen and nutrients inefficiently, producing areas of necrotic cells at their center. However, under healthy conditions, these models are not recommended for cytotoxicity evaluation, such as those related to ROS generation and oxidative stress induction, because cytotoxicity depends on oxygen availability, and these models may more closely reflect the structure of some tumor types in vivo [30].

10.2.2 Zebrafish as Experimental Model In Vivo

The zebrafish, of South Asian origin, is a small teleost of the *Danio rerio* species measuring about 4 cm [33]. It was described by Francis Hamilton in 1822 and is part of the vertebrate family Cyprinidae, which has the largest number of species. According to European Union regulations, using the zebrafish during embryonic stages (until 72 h) does not upset the animal welfare [34]: the embryos have not reached exogenous feeding, and they do not have the immunological system completely developed, so they are not considered to be animals [35–37]. Therefore, the zebrafish embryos constitute a greatly relevant in vitro model [34, 36, 37]. As an adult, the zebrafish can also be considered an alternative method to the use of conventional animals in research (e.g., rat, mice, and guinea pig) especially if one considers the reduction principle described in the 3R concept. The black and white stripes throughout the body are the most relevant characteristics of the zebrafish in the adult phase [36, 38].

The zebrafish is an important model for research—a large part of its genome has been sequenced, and it shares similarities with other vertebrates, including humans, with whom it has about 70% of gene equivalence (35). In the biomedical area, the zebrafish is an appropriate specimen in studies that focus on ecotoxicology, which encompasses assessing the risk of chemical compounds [36, 39–41]. The zebrafish develops rapidly, is easy to manipulate, and requires a small space for management, breeding, and experiments [36]. Compared to other models, it represents a robust and low-cost model [42]. The zebrafish has a life cycle that involves four different stages (embryonic, larval, juvenile, and adult) (see Fig. 10.4). Most of the time, the life cycle can be monitored under a microscope because the zebrafish is transparent in the early stages, which allows changes in development and particularly in organogenesis to be observed.

As a model organism, the zebrafish in the embryonic phase aids understanding of various biological processes that take place during the development of the analyzed tissue. Indeed, the zebrafish allows noninvasive visualization of the embryo (which has external development) in a transparent egg [36, 41]. Given that the zebrafish develops in translucid structures, it has been used to understand the processes of cell death (apoptosis), differentiation, and proliferation, as well as the cellular redox state, which are essential stages during fish embryogenesis. The model has become highly targeted because such pathways are functionally similar to the corresponding pathways in mammals [43].

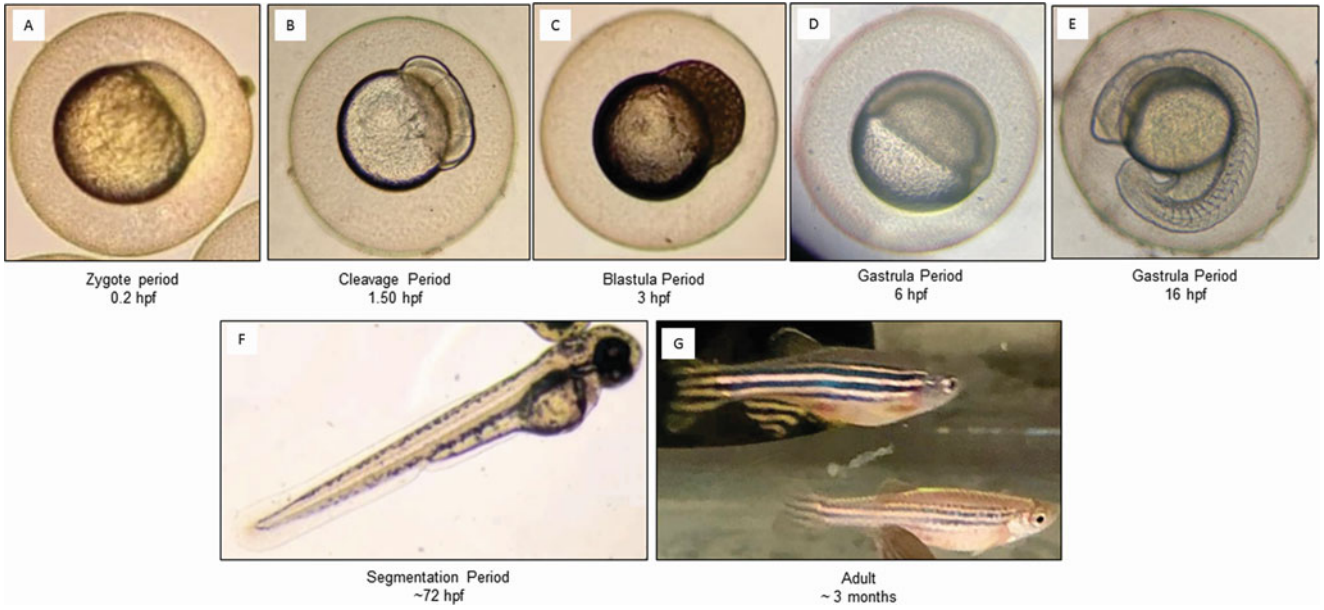


Fig. 10.4 Development of zebrafish, characterized by different embryonic stages: zygote, cleavage, blastula, gastrula, and segmentation (**a**–**f**), until adulthood (**g**). *hpf* hours postfertilization

Contamination of the aquatic environment with a range of toxic agents represented by heavy metals, agrochemicals, and organic compounds [44] has raised the interest in evaluating toxicity in aquatic organisms to assess environmental conditions and human exposure. In this scenario, experimentation with zebrafish is valuable in biomedical research—many studies have helped to understand how environmental contaminants interfere in the development of organisms and to study the toxic effects that these compounds elicit [36, 39, 45].

10.3 Assays for Oxidative Stress Monitoring in Zebrafish and Cell Culture

Countless chemical substances are being continuously released into the environment, including pesticides, polycyclic aromatic hydrocarbons (PAHs), dioxins, and furans produced by urban communities [46]. These chemicals can affect the aquatic ecosystem. In addition, they have bioaccumulative properties, which means that several compounds can reach humans. The biotransformation and detoxification of these chemical substances are an important source of reactive oxygen species (ROS), among which O_2^-, H_2O_2, and OH^- stand out [47]. An imbalance in the ROS amount may result in cellular, molecular, and biochemical damage and culminate in cell death [48–51]. In this sense, oxidative stress can be monitored by various assays that employ ROS as biomarkers and which measure the levels of defense against cellular damage or quantify oxidative damage (see Fig. 10.5) [52, 53]. Some of the chief evaluated endpoints are listed below.

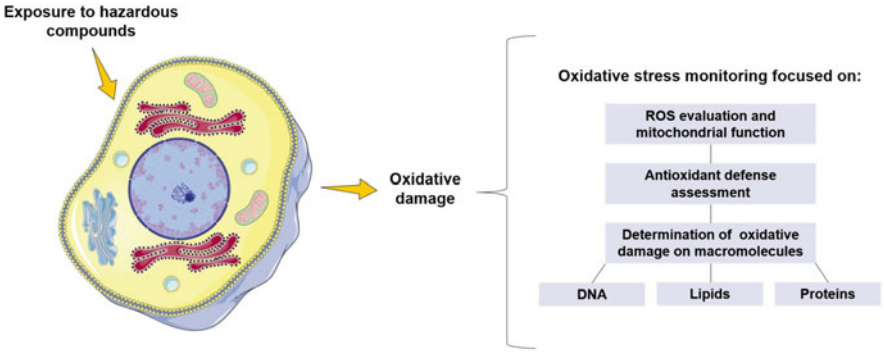


Fig. 10.5 Exposure to hazardous compounds induces oxidative stress, a condition that impairs the biological system at different levels and which can be monitored by focusing on different endpoints, including ROS determination, antioxidant system assessment, and macromolecule oxidative damage evaluation

10.3.1 Reactive Oxygen Species (ROS) Determination

10.3.1.1 Fluorescent Probes

ROS are easily measured in living cells by using fluorescent dye-based assays such as the CM-H_2DCFDA assay. The chloromethyl group facilitates passive CM-H_2DCFDA diffusion into the cell, where the H_2DCFDAacetate groups react with intracellular thiols and are cleaved by intracellular esterases, to give a fluorescent adduct that gets trapped inside the cell. The fluorescence status is directly related to the presence of ROS [54]. Numerous studies with the CM-H_2DCFDA probe have been carried out to evaluate the free radical content in zebrafish and cell culture [55–59]. The advantages of CM-H_2DCFDA include its easy application, its sensitivity to changes in the cell redox state, and its ability to monitor alterations in ROS over time [54]. Amplex® Red (10-acetyl-3,7-dihydroxyphenoxazine) is another example of a probe that can be used to identify and to quantify H_2O_2 or peroxidase activity. In the presence of horseradish peroxidase (HRP), the Amplex® Red reagent reacts stoichiometrically with H_2O_2, to yield the highly fluorescent resorufin [60, 61].

10.3.2 Antioxidant Defense Evaluation

10.3.2.1 Superoxide Dismutase Activity (SOD)

The enzyme superoxide dismutase (SOD) converts O_2^- to H_2O_2 [62]. SOD contains two subunits: the CuZn-SOD subunit (bearing copper and zinc in its active site) is present in the cell cytoplasm, mainly in lysosomes and peroxisomes, whereas the Mn-SOD subunit (bearing manganese in its active site) participates in O_2^- regulation in the intramitochondrial space and therefore acts against oxidative stress [63]. Several methods can be used to assess SOD activity, including spectrophotometric measurements, calculated according to the standard curve and expressed as U/mg of protein [62, 64–66]. Nitroblue tetrazolium (NBT) reduction is an additional method to determine SOD activity that indicates O_2^- production. SOD competes with NBT for O_2^-, and the percent inhibition of NBT reduction is related to the amount of SOD that is present in the medium [67].

10.3.2.2 Catalase Activity (CAT)

Catalases are enzymes that can metabolize H_2O_2 to water and molecular oxygen [63]. They are abundant in peroxisomes, which are vesicles enveloped by a membrane [47]. To determine CAT activity in aquatic organisms (like the zebrafish) and cell cultures, the H_2O_2 consumption rate is most commonly measured by spectrophotometry [68–70].

10.3.2.3 Glutathione System (GR, GPx, and GST)

Glutathione (GSH) is a broad nonlinear tripeptide that is present in all aerobic organisms. GSH plays an essential role in the balance of ROS formation, in the cell redox status maintenance, in the defense against electrophilic agents, and in the

detoxification of xenobiotics [71–73]. Glutathione reductase (GR) reduces oxidized glutathione (GSSG) to GSH while simultaneously oxidizing β-nicotinamide adenine dinucleotide phosphate (β-$NADPH_2$). The GSH/GSSG ratio is crucial to intracellular homeostasis maintenance. However, when cells are subject to an oxidative stress condition, GSSG can accumulate, and the GSH/GSSG ratio can consequently decrease. Therefore, the GSH/GSSG ratio is a useful indicator of oxidative stress in cells and tissues and can be assessed by reacting GSH with Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid (DTNB)), which can be spectrophotometrically quantified at 412 nm [71]. Glutathione-S-transferase (GST) conjugates the electrophilic xenobiotics to GSH, allowing the transport system to eliminate these conjugates to the extracellular medium. GPx and GST are important biomarkers [74, 75]. The GST, GPx, and GR activities in zebrafish are usually measured in tissues by using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The formation of the adduct CDNB, S-2, 4-dinitrophenyl glutathione, is monitored by measuring the increase in the absorbance rate at 340 nm for 2 min in a spectrophotometer. The specific GR and GPx activities are spectrophotometrically obtained from NADPH oxidation [76–78].

10.3.2.4 Analysis of Other Thiol Groups

Apart from the aforementioned assays, thiol levels can be evaluated in zebrafish larvae by incubation with the fluorescent dye monobromobimane (mBrB). Decreased thiol levels can be associated with thiol consumption during detoxification [79, 80].

Another method to evaluate thiol levels in cell cultures is to measure the fluorescence intensity produced by the interaction between biological thiols and the dye Granada Green dinitrobenzene sulfonate (GGDNBS). This assay helps to determine cellular oxidative stress on the basis of changes in biothiol concentrations [81].

10.3.3 DNA Damage, Lipid Peroxidation, and Protein Oxidation

Oxidative stress damages macromolecules. Hence, the extent of DNA damage and the levels of lipid peroxidation and protein oxidation are an indirect way of assessing a stress condition.

10.3.3.1 DNA Damage

8-Hydroxydesoxyguanosine (8-OHdG) is one of the most common markers of oxidative DNA damage [82]. The increase in 8-OHdG activity in tissues corresponds to the oxidative stress level. 8-OHdG has been used to evaluate zebrafish and cellular DNA injury by ELISA or immunofluorescence [83–86].

10.3.3.2 Lipid Peroxidation

Numerous biochemical events occur during oxidative stress, which can lead to deleterious effects on the lipid membranes, also called lipid peroxidation (LP) [8]. Malondialdehyde (MDA) is a product of lipid peroxidation that is applied

as an indicator of oxidative stress. MDA levels can be estimated by reaction with 2-thiobarbituric acid (TBA) in an assay known as TBARs. Each MDA molecule reacts with two TBA molecules, to form products that can be quantified by spectrophotometry [87–90]. In cell cultures, LP can also be estimated by detecting hydroperoxides, such as 4-hydroxynonenal (4-HNE), by GC-mass spectrometry analysis [91].

10.3.3.3 Protein Determination

Protein oxidation occurs by action of ROS, to generate stable products that serve as biomarkers in in vitro and in vivo models. Oxidative stress can modify the protein structure and consequently alter the cellular functions and the normal intracellular equilibrium, affecting cell viability [6, 7]. To determine protein in cells and tissues, the Bradford technique is frequently employed. The Bradford protein assay is based on the interaction between the protein molecules and the Coomassie dye under acidic conditions, which results in a color change from brown to blue [59, 77, 92]. The carbonyl assay is also a common biomarker of protein damage: carbonyl groups can arise as a result of protein binding to aldehydes (including many of the aldehydes emerging during lipid peroxidation) or direct protein oxidation by ROS, and they can be measured spectrophotometrically or by ELISA [93].

10.4 Conclusion

This chapter has described that ROS formation and accumulation triggers oxidative stress, consequently inducing severe damage to cellular components, including DNA, proteins, and lipids. Most of the existing protocols for oxidative stress assessment can be applied in in vivo and in vitro studies. Although there are numerous assays for this purpose, it is noteworthy that oxidation-reduction reactions can also occur during sample manipulation. To avoid this, minimal sample manipulation and sample protection from heat and light are recommended. Apart from careful performance of all the procedures, critical analysis of the results is crucial for the generation of reliable data.

References

1. Roubicek DA, De Souza-pinto NC (2017) Mitochondria and mitochondrial DNA as relevant targets for environmental contaminants. Toxicology 391:100–108
2. Friedman JR, Nunnari J (2014) Mitochondrial form and function. Nature 505:535
3. Nelson DL, Cox MM (2014) Princípios de bioquímica de Lehninger, 6th edn. Porto Alegre, Artmed
4. Hüttemann M, Lee I, Samavati L, Yu H, Doan JW (2007) Regulation of mitochondrial oxidative phosphorylation through cell signaling. Biochim Biophys Acta Mol Cell Res 1773 (12):1701–1720
5. Augusto O (2006) Radicais livres: bons, maus e naturais, 1st edn. Oficina de Textos, São Paulo. 120 p

6. Blajszczak C, Bonini MG (2017) Mitochondria targeting by environmental stressors: implications for redox cellular signaling. Toxicology 391(April):84–89
7. Zolkipli-Cunningham Z, Falk MJ (2017) Clinical effects of chemical exposures on mitochondrial function. Toxicology 391(April):90–99
8. Halliwell B (2006) Reactive species and antioxidants: redox biology is a fundamental theme of aerobic life. Plant Physiol 141:312–322
9. Hilscherova K, Blankenship AL, Nie M, Coady KK, Upham BL, Trosko JE et al (2003) Oxidative stress in liver and brain of the hatchling chicken (Gallus domesticus) following in ovo injection with TCDD. Comp Biochem Physiol Part C 136:29–45
10. Modesto KA, Martinez CBR (2010) Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 81:781–787
11. Hong Y, Kim Y, Lee K (2012) Methylmercury exposure and health effects. J Prev Med Public Health 45:353–363
12. WHO (2016) International Programme on Chemical Safety. The public health impact of chemicals: knowns and unknowns. WHO, Geneva
13. Caine EA, Jagger BW, Diamond MS (2018) Animal models of Zika virus infection during pregnancy. Viruses 10(598):1–21
14. Higa LM, Trindade P, Delvecchio R, Tanuri A, Rehen SK (2016) Zika virus impairs growth in human neurospheres and brain organoids. Science 352(6287):33–36
15. Janssens S, Schotsaert M, Karnik R, Balasubramaniam V, Dejosez M, Meissner A (2018) Zika virus alters DNA methylation of neural genes in an organoid model of the developing human brain. mSystems 3(1):e00219–e00217
16. de Souza N (2018) Organoids. Nat Methods 15(1):2018
17. Tannenbaum J, Bennett BT (2015) Russell and Burch' s 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci 54(2):120–132
18. Doke SK, Dhawale SC (2015) Alternatives to animal testing: a review. Saudi Pharm J 23 (3):223–229
19. Schechtman LM (2002) Implementation of the 3Rs (Refinement, Reduction, and Replacement): validation and regulatory acceptance considerations for alternative toxicological test methods. ILAR J 43:S85
20. Blosh M (2012) The history of animal welfare law and the future of animal rights. The University of Western Ontario, London, ON. Available at: https://ir.lib.uwo.ca/etd/803
21. Trent N, Edwards S, Felt J, Meara KO (2005) International animal law, with a concentration on Latin America, Asia, and Africa. In: Salem DJ, Rowan AN (eds) The state of the animals III. Humane Society Press, Washington, DC, pp 65–77. Available at: https://animalstudiesrepository.org/sota_2005/5/
22. de Vasconselos RM (2008) Conhecendo a Lei Arouca, Lei no 11.794, de 8 de outubro de 2008, que regula a pesquisa com animais. pp 113–50
23. United States of America' Congress (2000) Public Law 106–545. 106th Public Law 106–545—December 19, 2000. 2721–5
24. Knight A (2008) Non-animal methodologies within biomedical research and toxicity testing. ALTEX 25(3/08):213–231
25. Griesinger C, Desprez B, Coecke S, Casey W, Zuang V (2016) Validation of alternative in vitro methods to animal testing: concepts, challenges, processes and tools. In: Eskes C, Whelan M (eds) Advances in experimental medicine and biology. Springer International Publishing, Cham
26. Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V et al (2018) State of the art paper 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. Arch Med Sci 14(4):910–919
27. Duval K, Grover H, Han L, Mou Y, Pegoraro AF, Fredberg J et al (2017) Modeling physiological events in 2D vs 3D cell culture. Physiology (Bethesda) 32:266–277
28. Joseph JS, Malindisa ST, Ntwasa M (2018) Two-dimensional (2D) and three-dimensional (3D) cell culturing in drug discovery. In: Cell culture. IntechOpen, Rijeka. Available at:

https://www.intechopen.com/books/cell-culture/two-dimensional-2d-and-three-dimensional-3d-cell-culturing-in-drug-discovery
29. Antoni D, Burckel H, Josset E, Noel G (2015) Three-dimensional cell culture: a breakthrough in vivo. Int J Mol Sci 16:5517–5527
30. Knight E, Przyborski S (2015) Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. J Anat 227:746–756
31. Box GEP (1976) Science and statistics. J Am Stat Assoc 71(356):791–799
32. Anadón A, Martínez MA, Castellano V, Martínez-Larrañaga MR (2014) The role of in vitro methods as alternatives to animals in toxicity testing. Expert Opin Drug Metab Toxicol 10 (1):67–79
33. Society A (1974) Spawning cycle and egg production of Zebrafish, Brachydanio rerio, in the laboratory. Am Soc Ichthyol Herpetol (ASIH) 1974(1):195–2014
34. Strahle U, Scholz S, Geisler R, Greiner P, Hollert H, Rastegar S et al (2012) Zebrafish embryos as an alternative to animal experiments - a commentary on the definition of the onset of protect life stages in animal welfare regulations. Reprod Toxicol 33:128–132
35. European Commision (2010) Directive 2010/63/EU of the European parliament and of the Council of 22 September 2010
36. Scholz S, Fischer S, Gündel U (2008) The zebrafish embryo model in environmental risk assessment — applications beyond acute toxicity testing. Environ Sci Pollut Res 15:394–404
37. Teixidó E, Piqué E, Llobet JM (2013) Assessment of developmental delay in the zebrafish embryo teratogenicity assay. Toxicol In Vitro 27:469–478
38. Lawrence C (2007) The husbandry of zebrafish (Danio rerio): a review. Aquaculture 269:1–20
39. Carlsson G, Patring J, Kreuger J, Norrgren L, Oskarsson A (2013) Toxicity of 15 veterinary pharmaceuticals in zebrafish (Danio rerio) embryos. Aquat Toxicol 126:30–41
40. Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E et al (2000) The syntenic relationship of the zebrafish and human genomes. Genome Res 10:1351–1358
41. Hill AJ, Teraoka H, Heideman W, Peterson RE (2005) Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci 86(1):6–19
42. Mcgrath P, Li C (2008) Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discov Today 13(9–10):394
43. Hahn ME, Timme-laragy AR, Karchner SI, Stegeman JJ (2015) Free radical biology and medicine Nrf2 and Nrf2-related proteins in development and developmental toxicity: insights from studies in zebrafish (Danio rerio). Free Radic Biol Med 88:275–289
44. Rashed MN (2019) Cadmium and Lead levels in fish (Tilapia Nilotica) tissues as biological indicator for lake water pollution. Environ Monit Assess 68:75–89
45. Moura MAM, Oliveira R, Jonsson CM, Domingues I, Soares AMVM, Nogueira AJA (2018) The sugarcane herbicide ametryn induces oxidative stress and developmental abnormalities in zebrafish embryos. Environ Sci Pollut Res 25(14):13416–13425
46. Oost D, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149
47. Atli G, Canli M (2007) Enzymatic responses to metal exposures in a freshwater fish Oreochromis niloticus. Comp Biochem Physiol Part C 145:282–287
48. Gravato C, Almeida JR, Silva C, Oliveira C, Soares AMVM (2014) Using a multibiomarker approach and behavioural responses to assess the effects of anthracene in Palaemon serratus. Aquat Toxicol 149:94–102
49. Newman TAC, Carleton CR, Leeke B, Hampton MB, Julia A (2015) Embryonic oxidative stress results in reproductive impairment for adult zebrafish. Redox Biol 6(Dec):648–655
50. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 64:178–189
51. Winston GW, Di Giulio RT (1991) Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat Toxicol 19:137–161

52. Ahmad I, Pacheco M, Santos MA (2006) Anguilla anguilla L. oxidative stress biomarkers: an in situ study of freshwater wetland ecosystem (Pateira de Fermentelos, Portugal). Chemosphere 65:952–962
53. Funesa V, Alhamaa J, Navasb JI, López-Bareaa J, Peinadoa J (2006) Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral. Environ Pollut 139:214–223
54. Eruslanov E, Kusmartsev S (2010) Identification of ROS using oxidized DCFDA and flow-cytometry. In: Methods in molecular biology. Springer, New York, NY
55. Paget V, Moche H, Kortulewski T, Grall R, Irbah L, Nesslany F et al (2015) Human cell line-dependent WC-Co nanoparticle cytotoxicity and genotoxicity: a key role of ROS production. Toxicol Sci 143(2):385–397
56. Lebel CP, Ali SF, McKee M, Bondy C (1990) Organometal-induced increases in oxygen reactive species: the potential diacetate as an index of neurotoxic damage. Toxicol Appl Pharmacol 104:17–24
57. Xu H, Dong X, Zhang Z, Yang M, Wu X, Liu H (2015) Assessment of immunotoxicity of dibutyl phthalate using live zebrafish embryos. Fish Shellfish Immunol 45:286–292
58. Usenko CY, Hopkins DC, Trumble SJ, Bruce ED (2012) Hydroxylated PBDEs induce developmental arrest in zebrafish. Toxicol Appl Pharmacol 262(1):43–51. https://doi.org/10.1016/j.taap.2012.04.017
59. Guilherme D, Paganotto L, De Brum P, Rodrigues N, Carvalho D, Rubim A et al (2018) N-acetylcysteine inhibits Mancozeb-induced impairments to the normal development of zebrafish embryos. Neurotoxicol Teratol 68(April):1–12
60. Dębski D, Smulik R, Zielonka J, Michałowski B (2016) Mechanism of oxidative conversion of Amplex® Red to resorufin: pulse radiolysis and enzymatic studies. Free Radic Biol Med 95:323–332
61. Zhao B, Summers FA, Mason RP (2012) Photooxidation of Amplex Red to resorufin: implications of exposing the Amplex Red assay to light. Free Radic 53(5):1080–1087
62. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247(10):3170–3175
63. Medica E (1979) Oxygen free radicals and tissue damage. Ciba Found Symp (65):1
64. Zhang Y, Zheng Q, Pan J, Zheng RL (2004) Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin Pharmacol Toxicol 95:53–58
65. Paravani EV, Simoniello MF, Poletta GL, Casco VH (2019) Cypermethrin induction of DNA damage and oxidative stress in zebrafish gill cells. Ecotoxicol Environ Saf 173(August 2018):1–7
66. Marcon M, Mocelin R, Benvenutti R, Costa T, Herrmann AP, De Oliveira DL et al (2018) Environmental enrichment modulates the response to chronic stress in zebrafish. J Exp Biol 221:jeb176735
67. Weydert CJ, Cullen J (2010) Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5(1):51–66
68. Ove P, Lieberman I (1958) Enzyme activity levels in mammalian cell cultures. J Biol Chem 233 (3):634–636
69. Shao B, Zhu L, Dong M (2012) DNA damage and oxidative stress induced by endosulfan exposure in zebrafish (Danio rerio). Ecotoxicology 21:1533–1540
70. Bartoskova M, Dobsikova R, Stancova V, Pana O, Zivna D, Plhalova L et al (2014) Norfloxacin — toxicity for zebrafish (Danio rerio) focused on oxidative stress parameters. Biomed Res Int 2014:6
71. Halliwell B (2001) Role of free radicals in the neurodegenerative diseases therapeutic implications for antioxidant treatment. Drugs Aging 18(9):685–716
72. Reischl E, Luiz A, Luis J, Wilhelm D (2007) Distribution, adaptation and physiological meaning of thiols from vertebrate hemoglobins. Comp Biochem Physiol 146(Part C):22–53
73. Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem 97:55–74

74. Stegeman JJ, Renton KW, Woodin BR, Addison RF (1990) Experimental and environmental induction of cytochrome P450E in fish from Bermuda waters. J Exp Mar Biol Ecol 138:49–61
75. Taylor P, Bucheli TD, Fent K, Bucheli TD, Fent K (2012) Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. Crit Rev Environ Sci Technol 25(3):201–267
76. Habig WH, Pasbst MJ, Jakoby WB (1974) Glutathione S-transferases. J Biol Chem 249 (22):7130–7140
77. Nunes MEM, Müller TE, Murussi C, Aline MB, Gomes JLC, Marins AT et al (2018) Oxidative effects of the acute exposure to a pesticide mixture of cypermethrin and chlorpyrifos on carp and zebrafish – a comparative study. Comp Biochem Physiol Part C 206–207(October 2017):48–53
78. Divišová L, Dobšíková R, Blahová J, Plhalová L, Hostovsky M (2013) Oxidative stress responses in zebrafish Danio rerio after subchronic exposure to atrazine. Food Chem Toxicol 61:82–85
79. Meyer JN, Leung MCK, Rooney JP, Sendoel A, Hengartner MO, Kisby GE et al (2013) Mitochondria as a target of environmental toxicants. Toxicol Sci 134(1):1–17
80. Pitt JA, Trevisan R, Massarsky A, Kozal JS, Levin ED, Di RT (2018) Maternal transfer of nanoplastics to offspring in zebrafish (Danio rerio): a case study with nanopolystyrene. Sci Total Environ 643:324–334
81. Puente-Muñoz V, Paredes JM, Resa S, Vílchez JD, Zitnan M, Miguel D et al (2019) New thiol-sensitive dye application for measuring oxidative stress in cell cultures. Sci Rep 9:1659
82. Thompson HJ, Heimendinger J, Haegele A, Sedlacek SM, Gillette C, Neill CO et al (1999) Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. Carcinogenesis 20(12):2261–2266
83. Topal A, Alak G, Altun S, Serkan H (2017) Evaluation of 8-hydroxy-2-deoxyguanosine and NFkB activation, oxidative stress response, acetylcholinesterase activity, and histopathological changes in rainbow trout brain exposed to linuron. Environ Toxicol Pharmacol 49:14–20
84. Zhu B, Wang Q, Shi X, Guo Y, Xu T, Zhou B (2016) Effect of combined exposure to lead and decabromodiphenyl ether on neurodevelopment of zebrafish larvae. Chemosphere 144:1646–1654
85. Park C, Cha H, Hong SH, Kim G, Kim S, Kim H et al (2019) Protective effect of Phloroglucinol on oxidative stress-induced DNA damage and apoptosis through activation of the Nrf2/HO-1 signaling pathway in HaCaT human keratinocytes. Mar Drugs 17(255):1–16
86. Aydin UR, Akpinar KE, Hepokur C, Erd D (2018) Assessment of toxicity and oxidative DNA damage of sodium hypochlorite, chitosan and propolis on fibroblast cells. Braz Oral Res 32:1–8
87. Ferreira ALA, Matsubara LS (1997) Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. Rev Assoc Med Bras 43(1):61–68
88. Pilz J, Meineke I, Gleiter CH (2000) Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. J Chromatogr B 742:315–325
89. Özgüner MF, Delibaş N, Tahan V, Koyu A, Köylü H (1999) Effects of industrial noise on the blood levels of superoxide dismutase, glutathione peroxidase and malondialdehyde. Eastern J Med 4(1):13–15
90. Yagi K et al (1976) A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 15:212–216
91. Araseki M, Kobayashi H, Hosokawa M, Miyashita K (2005) Lipid peroxidation of a human hepatoma cell line (HepG2) after incorporation of linoleic acid, arachidonic acid, and docosahexaenoic acid. Biosci Biotechnol Biochem 69(3):483–490
92. Kumar N, Awoyemu O, Willis A, Schmitt C, Ramalingam L, Moustaid-Moussa N et al (2019) Comparative lipid peroxidation and apoptosis in embryo-larval zebrafish exposed to three azole fungicides, Tebuconazole, Propiconazole, and Myclobutanil, at environmentally relevant concentrations. Environ Toxicol Chem 38(7):1455–1466
93. Halliwell B, Whiteman M (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 142:231–255

Effects of Yoga on Oxidative Stress During Aging

11

Riya Gupta and Pawan K. Maurya

Abstract

As research in medicine flourishes, so does the life expectancy. The average life expectancy is consistently growing and has come to be 70 years for most adults in the developed and developing countries. However, even as the life expectancy is growing, the lifespan, i.e. the age for healthy living, isn't growing as much. In diseases such as dementia, brain functioning is easily depleted in the later years. A good and natural way to combat this is yoga. Yoga is a process to open up the mind to work at its full potential. Through this review, we wanted to see if this is also true for the elderly, whose brain functioning is already depleted. Can yoga rejuvenate the minds of the elderly?

Keywords

Yoga · Oxidative stress · Aging

11.1 Introduction

11.1.1 Process of Aging

The twenty-first century is seeing an aging population like never before, and it has now become a challenge for the developed countries to combat aging. An international agency has been created to research on this issue. The "International Agency for Research on Aging" is affiliated to an intergovernmental institution like the

R. Gupta
Amity Education Group, Suffolk, NY, USA

P. K. Maurya (✉)
Department of Biochemistry, Central University of Haryana, Mahendragarh, Haryana, India
e-mail: pkmaurya@cuh.ac.in

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_11

World Health Organization. It is a path for promoting gerontology (study on human aging) and promotes research on geroprotectors for humans [1]. The main goal of gerontology and preventive medicine is to extend the healthy lifespan. Medications like antioxidants, calorie restriction mimetics, autophagy inducers, etc. that target aging all help in promoting and extending healthy lifestyle in various animal species. The geroprotectors.org database currently lists more than 200 substances that help in increasing lifespan [1].

Human aging is associated with many pathways, and potential interventions may help to prevent it. Many pathways that have been affected have been identified, and the behavioural, dietary and pharmacological approaches to prevent these disorders are being identified. New anti-aging drugs are regularly being developed and are going to be of great help in the upcoming years [1]. Successful aging is used to describe aging that occurs throughout the lifespan in gerontology. Successful aging looks at aspects like longevity, life satisfaction, no disability, growth, active engagement in life and independence. This term can also be referred to as "active aging" or "vital aging" or "productive aging" as aging should be accompanied by sustained health and vitality, where elderly are also contributors to the society instead of being dependent on one another, that is, the ability of maintaining functionality as long as possible [2]. One approach that has been available since hundreds of years but is yet to gain popularity for anti-aging is the practice of Yoga. Yoga has been commonly practised in Hinduism and has scientifically been proven to reduce oxidative stress from the body—one of the key factors in combating aging.

The Hindu culture believes in a four-stage life model of aging. In this stage, youth is the preparation age (Brahmacharya), where one studies and prepares for the later stages in life. The second stage (Grihastha) is the productive stage which involves family and work and ways of contributing to the society. The third stage is the transition to a more self-oriented person (Vanaprastha, the "retired person") and leads a more introspective life. In this stage, individuals renounce from the home and work practices and spend more of their time in meditation and prayer. This life is filled with a general sense of happiness and a feeling of readiness to let the son of the family take hold of the house. This gives them enough time to contemplate over their death and rebirth. At this stage one can choose to become a hermit or get involved in active worship of the Hindu gods and goddesses. The last stage is known as Sannyasa, the "ascetic". This is the when one is free from all the attachment to desires, hopes, fears, duties and responsibilities of the previous stages of life. At this stage one becomes holy and can attain enlightenment and achieve the true wisdom of the cosmos [2]. One thing that is common throughout the lifecycle in the Hindu culture is the practice of yoga, ensuring the connection between body and mind at all stages.

11.2 Mechanisms of Aging

It has been a dream since the dawn of civilization to slow down aging. All old civilizations and cultures have stories like the fountain of youth or other writings which clearly indicate the fondness with youth and anti-aging during those times. In

fact, the fascination with aging has not ended. Scientists all over the world have been anticipating for the day when the process of aging could be slowed down. The breakthrough research on aging is however new. Since a long time people have thought of aging as a natural process which occurs due to the wear and tear of the body due to natural and inevitable processes [3].

To slow down the process of aging, however, it is necessary to know the reasons behind aging. But till date the mechanisms behind aging are still not understood to a great extent [4]. It is known however that aging is a degenerative process and occurs due to cellular-, tissue- and organ-level damage accumulated over the years. Several theories on aging have been proposed to explain this phenomenon. The most applauded theory that has taken the centre stage has been the mitochondrial free radical theory of aging (MFRTA) [4].

According to the MFRTA theory, aerobic metabolism produces toxic byproducts, i.e. the ROS which cause oxidative damage due to their high chemical reactivity and various cellular macromolecules which are injured [4]. The main production site of superoxide is the respiratory chain (RC) located in the inner mitochondrial membrane. During electron transport, abundant amount of ROS complexes I and III are formed in the mitochondria. SOD converts the superoxide anion into hydrogen peroxide. In the presence of transition metals, hydrogen peroxide turns into a highly reactive hydroxyl radical through the Fenton reaction. Hydroxyl radical is considered the most damaging form of ROS and causes oxidative damage in almost all types of molecules in the cell including proteins, nucleic acids and lipids [4].

Another process of aging understood by scientists is that it is controlled by signalling pathways and transcription factors through complicated biological processes [3]. For example in the case of yeast as explained by Mark A. McCormick et al., two methods can be used to assay aging in yeast: chronological lifespan (CLS) and replicative lifespan (RLS). RLS is the number of daughter cells a mother can produce till it stops dividing; it is proposed as an assay which can help identify conserved pathways that affect the viability of dividing cells in humans. In fact, it was identified that the genes that were responsible for RLS in yeasts were the same as the genes that cause aging in worms. Mark A. McCormick et al. [5] furthermore studied many pathways that a yeast undergoes in the process of aging.

Their study has identified the role of Los1-mediated tRNA in the dietary restriction in downstream transport. In many aging pathways, the nuclear tRNAs providing longevity are being identified which were not considered earlier. The data showed Rad53 and mTOR are regulated by DR pathway and have mechanisms that influence the localisation of Los1. This is in accordance with prior study that Rad53 regulates Los1 in response to damage of DNA. Ntg1 is translocated during stress on mtDNA, and hexokinase Hxk2 which is responsible for nutrient responsiveness and cAMP-dependent protein kinase A (PKA) signalling pathway are all necessary for the regulation of Rad53 gene checkpoint which responses to DNA damage. With these results, it can be noted that both Hxk2 and PKA act in the DR pathway in yeast. This leads to the conclusion that neither DR nor overexpression of Rad53 is enough to induce the damage checkpoint of DNA when there is no DNA damage.

Thus, the connections between DNA maintenance, nutrient response pathways, tRNA transport and aging can be contrived [5].

For a cell to survive basal level of oxidative stress, reactive oxygen species and reactive nitrogen species are essential [6]. If the oxidative stress is severe, then it may also lead to oxidative damage and cell death. The level of oxidative stress at a moderate level can actually be beneficial and adapt to cellular responses better. The pathological challenges in aging and aging-associated diseases such as ischemia can be better handled in case of moderate-level oxidative stress. Moderate level of oxidative stress can even be termed as positive stress as done by Liang Jun-Yang in his review. Oxidative stress causes formation of lipid peroxidation and protein oxidation which at a moderate level can actually benefit the aging process. Since this is true, positive oxidation can even be used as a way to overcome the aging process. It can be used to target aging and aging-related diseases. However, even though this basal-level oxidative stress seems to be good, when the oxidation level increases to a severe level, it causes damage to cells and impairs their self-repair activity, thus causing cell death [6].

Through the last three decades, studies have shown changed patterns in the DNA methylation process due to chronological aging during the entire lifespan of a person [7]. This phenomenon is known as the epigenetic drift. This phenomenon accounts that epigenetic similarities among youngsters are lost over time and turned into divergent methylomes found in the elderly population. DNA methylation is an irreversible change involving hypermethylation and hypomethylation of the methylome and also causes progressive accumulation of epigenetic damage due to environmental factors or due to errors in the transmission of epigenetic information. This leads to different unpredictable methylomes in the aging population. This indicates that these changes are not programmed changes but occur randomly. Also several studies show aging-associated differentially methylated regions which create clusters of consecutive CpG sites which create changes over time in the same direction. The presence of DMRs indicates that biological mechanisms are involved in aging process or longevity. The initial analyses on aging are focused on the loci of candidate with a potential relevance of aging-related diseases. Altogether, the conclusion from this study shows that aging is somewhat similar to cancer as it is also associated with changes in DNA methylation which are gradual but profound where epigenome is marked by genome-wide hypomethylation and site-specific hypermethylation preferentially occurring at CGI promoters [7].

11.2.1 Methods to Overcome Aging

Aging and the diseases associated with it are the current big challenges in developing as well as developed countries [8]. Even though we see the life expectancy has increased manyfold in the recent years to an average of 100 in the developed countries, it is still not considered as an increase in the healthy life expectancy also termed as healthspan. Due to this, studies on longevity extension have gained a

lot of scepticism as it is determined to leave a lot of elderly population and thus the prevalence of aging-related diseases [8].

Experiments on invertebrates and rodents have shown that a major extension of the lifespan can take place when induced to chronic dietary restrictions (DR) and mutation in growth and nutrient pathways. This can extend their longevity from 30% to 50%. Dietary restriction has also been found to reduce the occurrence of aging-related function and many diseases like tumour, cardiovascular disease and neurodegeneration. It also protects against diabetes, cardiovascular disease, sarcopenia, cancer and neurodegeneration [8] in certain brain regions in an experiment conducted on rhesus monkeys and also extended their lifespan. In the case of humans, DR continued for a long term causes several changes both metabolic and molecular in nature and protects against aging-related pathologies, and this includes changes in type 2 diabetes markers, cardiovascular diseases, hypertension and dementia. Chronic DR also reduces age expected alterations like autonomic function, myocardial stiffness and changes in skeletal muscle gene expression. Even though all these positive changes come with chronic dietary restrictions, it also comes with undesirable side effects. Thus, instead of recommending to make drastic nutritional changes, scientists are trying to come up with drugs that target the nutrient response pathways which are safer and can be used more effectively [8]. However, for now, just calorie restriction is not the ultimate answer to achieving a healthy lifespan.

Another instance where diet was seen as an important factor in aging can be seen by analysing the traditional Okinawa diet and see how it affects in impairing symptoms of aging [9]. The basic ingredient in the Okinawa traditional diet is the root vegetables. These include sweet potato, soybean-based foods, green and yellow vegetables and medicinal plants. Also food items like lean meats, marine foods, fruit, medicinal garnishes, tea, spices and alcohol are also moderately consumed. This diet resembles to other diets like the Mediterranean diet, DASH diet and portfolio diet which are also considered as healthy diets. These can help reduce the risk of cardiovascular diseases and other age-associated diseases. These diet include unrefined carbohydrates, moderate protein like vegetables/legumes, fish and lean meats as sources. It also includes the intake of healthy fats like higher mono-/polyunsaturated fats, lower levels of saturated fat and foods rich in omega − 3. By taking healthy fats, it reduces inflammation and optimizes cholesterol and other risk factors. Plant-rich diets have lower caloric density and high amount of phytonutrients and antioxidants which is beneficial for one's well-being. And also as it has been mentioned before with the presence of antioxidants, superoxides can be converted back into non-harming form and thus decrease the level of cellular destruction. Other features of this diet are low glycaemic load, less inflammation and oxidative stress and modulation of aging-related biological pathways. Thus a thoughtful diet can help reduce the risk of chronic aging diseases and promote healthy aging and longevity [9].

As calorie restriction has become the most discussed method of anti-aging, many drugs have also been generated to create this effect [10]. Pathways involved in energy sensing, i.e. insulin/IGF1 signalling, sirtuins and mammalian target of

rapamycin (mTOR), are considered to be the pathways modified due to anti-aging actions of DR. Compounds involved in these pathways are DR mimetics (also metformin), resveratrol (sirtuins) and rapamycin (mTOR). Rapamycin was found to have increased both the healthspan and lifespan in mice. However, rapamycin does have negative effects like developing an induction of insulin resistance, potentially limiting its translation into humans. In an experiment by Yu Z et al., it was found that both DR and rapamycin inhibit lipogenesis, activate lipolysis and increase serum levels of nonesterified fatty acids and that only DR is able to activate β-oxidation of the fatty acids leading to the production of ketone bodies [10].

Vitamin C has also shown some anti-aging effects. It is essential for the synthesis of collagen biosynthesis and serves as a cofactor for prolyl and lysyl hydroxylase which are the key enzymes needed to cross-link and stabilize collagen fibres. Vitamin C is also involved in the activation of transcription factors which help in the synthesis of collagen and stabilizes procollagen messenger RNA (mRNA) which regulates synthesis of type I and III collagen. Vitamin C also decreases the degradation of collagen and increases its gene expression and tissue inhibitor MMP-1 synthesis. Three percent topical Vitamin C applied daily over a 4-month period in a study led to a significant increase in the density of dermal papillae [11].

Recently, in a study, it was observed that metformin, i.e. the drug used to cure type 2 diabetes, has shown some anti-aging effects. It was found by Cabreiro et al. that 25, 50 and 100 mM of metformin managed to increase lifespan by, respectively, 18%, 36% and 3% in a study on *C. elegans*. And another study by Martin-Montalvo et al. done on mouse breeds showed an increase in lifespan by 4–6% due to metformin. However, when a dose higher than 1% was given, it was found to be toxic. Another study claimed that when metformin is started at an early stage, the mean lifespan increases by 14% which is approximately 1 month, whereas when started at a later stage, the effect is less. Study on metformin done in the UK Prospective Diabetes Study (UKPDS) showed decrease in cardiovascular disease, overall mortality and incidence of cancer, as compared to the other antidiabetic drugs [12].

11.2.2 Yoga and Aging

Yoga which encompasses meditation and pranayam (breathing practices) has also found to delay the process of aging. Yogic practices are clearly shown to evolve strength, muscles, body flexibility and also improve cardiovascular and respiratory functions. It can even help in the recovery from different kinds of addictions, reduce the level of stress and anxiety, depression and chronic pain, improve sleep patterns and enhance overall well-being and quality of life. These practices facilitate oxygenated blood to various organs and body tissues; improve proper coordination of the body, emotions and mind; and eliminate waste. A longer healthspan can be sustained when physical, mental and emotional body is fit and leads to a longer life span [13].

11.2.3 What Is Yoga?

Yoga is an ancient science, initially originated in India. Over the years, its physical practice and teachings about the way of life have grown popular in the Western countries. Physical yoga includes disciplines like asanas (postures), dhyana (meditation) and pranayama (breathing techniques). Yoga teaches us tools that are used to withdraw one's senses (pratyahara), develop unwavering awareness (dhyana) and concentrate the mind (dharana). These tools can be manifested in oneself by dedicated practice of yoga. More than stretching, yoga comprises movements with efficient breathing. Many health benefits are related to yoga like physical stamina, balance, flexibility and relaxation. In addition to this, yoga appears to offer psychological benefits, through its mindfulness training which involves meditation and its combination of perceptive and interceptive awareness. With regular practice of these skills, one can increase its awareness and be able to focus profoundly in the present moment without any judgment [14].

Yoga and mindfulness lead to physical and cognitive benefits which may be due to mechanisms including pranayama and the activation of the parasympathetic nervous system, activate an increased volume of grey matter; enlarge the amygdala; increase the perception of body; and increase the functional connectivity within the basal ganglia [14].

Initially yoga originated as physical and spiritual practices which lead to a greater union with true self or the divine. Later, these teachings were compiled, and till the fifteenth century, all these aspects of physical and spiritual health were written down. Modern yoga practices in Western countries are mostly associated with hatha yoga. Hatha yoga compromises of numerous styles all emphasizing different physical and lifestyle practices. Examples of hatha yoga practices are Kripalu Iyengar, Sivananda, Asthanga and Vinyasa and others with more focus on esoteric aspects of yoga philosophy like Sahaj, Kundalini and Siddha. Other yoga regimens, focusing on the physical aspects of yoga, are recently being introduced in fitness training such as Yogafit, Bikram Yoga (a kind of hot yoga) and Power Yoga, mainly in the United States. On the other hand, interventions of yogic practices in healthcare usually refer to various breathing techniques including Kirtan Kriya (meditative chanting from Kundalini yoga), Sudarshan Kriya yoga (rhythmic breathing practices) and yoga Nidra (deep relaxation through meditation).

11.2.4 Memory Implications

Memory has long been known to decline over the years of aging; long-term memories are shortened and easily forgotten with the implications of aging. These mind impairments can even become severe and increase the susceptibility to develop dementia later in life. Out of all the reasons hypothesized for it, it has been concluded by studies that aging lies as the foremost factor for dementia. Another reason for this worry about aging is the fact that by 2030 approximately 23% of the people in the United States will be above 65 years, and this percentage may even be higher in other

countries. This indicates a demographic phenomenon also given the term "silver tsunami" to establish its magnitude.

It is our memories that make us the individuals we are and gives meaning to our lives, and with this impact on memory destruction for a big percentage of population, this can lead to devastating consequences. The US government has projected the cost of $20 trillion to care for dementia over the next 35 years. Therefore, scientific advances for aging-related memory remedies are greatly needed to lower this tremendous economic strain upcoming in the aging developed countries [15]. Many studies have been looked at including the Swedish Betula study on aging, memory and dementias suggesting that hippocampus/MTL activity is altered due to aging. A cross-sectional study of face name-encoding and retrieval showed a decline in hippocampal function across the adult lifespan. The reduction became significant after 65 years. Additional studies showed hippocampal activity is well preserved for older adults and maintained through 15–20 years. A longitudinal study showed that the hippocampal activity during episodic encoding declined significantly over 6 years in older adults with memory decline [16].

These and other studies collectively show that older adults face an episodic memory decline and show a less stronger activity in their hippocampal/MTL region compared to the younger adults. However, this aging-related effect varies in magnitude of the memory impairment. The resting state activity of the cortical seems to be reduced in older age. This was concluded after analysing the Betula study results [16]. The prefrontal cortex to MTL connectivity has been shown to be altered in aging. It was found that the left hippocampus and the right hippocampus interact with each other and several cortical regions, including the left PFC, when episodic memory is encoded in the brain. The connectivity within the network decreases as a function of age and was related positively to performance as well. Thus, it can be concluded from these studies that the efficacy of interactions between the hippocampus and the other regions of brain including the PFC decreases significantly with age [16].

A network of regions called DMN shows activity patterns when the brain is at rest and a decreased mind is engaged in the external environment. This compromises areas of the PCC, medical prefrontal cortex, precuneus and medical temporal lobe structures like hippocampus. DMN is found to be involved in episodic memory retrieval, social cognition, prospective memory encoding, self-referential processing including self-prospecting and internal monitoring, future planning, autobiographical memory retrieval and theory of mind. Research also suggests that during aging, DMN is the main rs-fMRI network that is affected, due to reduced connectivity between anterior and posterior nodes [17].

Research by Harris et al. suggests that yoga may be helpful in enhancing memory, mainly visual memory encoding. Additionally, more yogic practices may cause even further improvements. It was suggested that this betterment was due to increased DMN connectivity in anterior, posterior and frontal medial areas. There are various other studies which support these findings. Wells et al. in their pilot study explored the effect of MBSR vs. usual care on 14 MCI subjects. Later, when structural MRI and rs-fMRI data around the hippocampus and DMN, respectively, were inspected,

enhanced activity of DMN (due to an increase in connectivity between medial prefrontal cortices and PCC and the hippocampus) was seen, and additionally bilateral atrophy in the hippocampus was decreased. It is believed that yoga works by lowering inflammation, lowering stress, increasing antioxidant levels, increasing telomerase activity and enhancing neuroplasticity processes (like producing brain-derived neurotrophic factor). Many other studies have also made the same conclusion regarding meditation effects on yoga. Another example is when Taylor et al. compared DMN functionality between experienced and beginner mediators, and the conclusion was increased functional connectivity between DMN in experienced mediators with an increase in emotional appraisal and self-referential processing. However, their processing of present moment awareness had decreased. It is understood that present moment awareness is involved in verbal memory, processing and recall at the present moment. Then another study with Brewer et al. analysed in a similar way between different levels of meditators. Their criteria for analysis were loving kindness, concentration and choice-less awareness. Experienced mediators had deactivated main DMN nodes (MFC and PCC) and also stronger coupling between PC, dorsal ACC and dorsolateral prefrontal cortices at both baseline and during meditation. This indicates enhanced efficiency of cognitive and self-monitoring control. Kirtan Kriya is a complex form of meditation involving chanting, visualization and hand movements, which is believed to enhance verbal and visual memory as seen in this study. Other mechanism proposed suggests it could be due to increased global attention/awareness and stress reduction. Higher connectivity in the parietal network gives improved visual-spatial memory performance, which is thought to be associated with attention, translation of visual motor information and working memory. These changes on the network connectivity lead to enhancement in efficiency in relevant brain regions including the precuneus cortex, bilateral posterior cingulated cortex, precentral and parietal operculum cortex and postcentral gyrus. It was concluded in the study that yoga improves the connectivity of the white matter and thus helps in reducing neuronal activity resulting in improved visuospatial memory performance [17].

11.2.5 Anxiety

Late-life anxiety hasn't been much investigated; however, the research done concludes it to be true. The elderly often face subsyndromal symptoms and have been reported to a 12-month prevalence rate of 26.2% for subthreshold anxiety versus 5.6% for DSM-IV anxiety disorders. Additionally, these subsyndromal symptoms of anxiety cause severe outcomes like cognitive decline, reduced health-related quality of life and increased healthcare cost. Anxiety has recently been categorized as dimensional rather than categorical construct in the late life by experts. Therefore, one should look for symptoms of anxiety rather than anxiety disorders.

In one study by Pink et al., associations between lower global thickness, lower thickness in frontal and temporal cortical regions and anxiety symptoms were

investigated. This study also took age, sex, antidepressant medication, education, medical comorbidity and global cognition into account. After adjusting depressive symptoms, the results found anxiety symptoms are related to the thickness of insula. This could be due to insula's interconnections between other cortical and subcortical regions. In addition to that, insula is also responsible in emotional responses, and its size and reactivity has been linked to bodily responses and anxiety. Another region that is investigated in anxiety disorders is the amygdala. The amygdalar volume reduces in anxiety disorders [18].

Mostly adults with early stages of dementia or depression have higher rates of anxiety symptoms. They start to anticipate their cognitive decline, better understand their diagnosis and know there is no cure which leads to fear that there is no cure. Other fears include losing their intellectual abilities, embarrassing themselves by forgetting who people are and not being able to follow conversations. All in all, they fear not being able to contribute to the society, and this builds on the anxiety [19].

Stress and its prolonged exposure are shown by various studies in reducing the sense of well-being of the individual. In a work life, one goes through continuous link between self-esteem, self-efficacy and societal status. Other than that, there is also the burden of long working hours, inadequate pay, job dissatisfaction, ambiguous roles and other conflicts associated at the workplace. All of these are examples of workplace stress, and this stress can be caused in other situations including family, relationship and social issues as well. And the modern, fast-paced lifestyle of the society also adds to the increase of stress level [20].

Prolonged exposure to all these stresses can lead to the person experiencing emotional distress, risk of autonomic arousal (anxiety disorder), increased insecurity and anxiety and stress-associated reactions. Overall stress as a result impacts mental health and well-being negatively. The relationship between stress and anxiety is very close. Stress is due to a threat, i.e. a stimulus caused due to a threat in any situation, whereas anxiety is the reaction to stress. Both stress and anxiety are physiologically stimulated by the parasympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Earlier studies have also shown how anxiety is detrimental to mental health. Anxiety negatively impacts one's well-being and is associated with depression, quality of life and decreased self-esteem [20].

A study to test association of mindfulness to combating stress was done on school teachers. Using mindfulness-based stress reduction (MBSR), teachers were measured before and after a 2.5-h weekly session for 8 weeks and 5 h of silence in week 5- and 6-week mindfulness programmes. This showed a reduction in depression and stress in nine school teachers and two teaching assistants ranging from ages late of 20s to late 50s. Depression Anxiety Stress Scales (DASS21) were used to make these measures [20]. In another study by Shirley Telles et al., 118 primary school teachers, naïve to yoga, were given a residential yoga programme to test their levels of anxiety. After the programme, significant level of reduction in the state of anxiety and an improvement in their total well-being were seen, whereas the control group which did not perform any yoga showed no improvement at all [20]. Another study tested on chronically ill patients to measure their anxiety levels also showed an improvement in anxiety levels after a prolonged practice of yoga. It was also

reported from this study that the time per day given on yoga did not make that much an impact on yogic practices than the duration of ongoing yoga practice [21]. As it is known that the DMN is one of the parts of the brain involved in anxiety, it is already seen from the studies on memory that DMN is significantly enhanced after yoga practices and thus can also help in reducing anxiety especially in the elderly. However, some studies on anxiety have also shown no effect of yoga at all. Like in the case of one study, anxiety was tested after acute sessions of YogaFit. The STAAI-Y1 scores for anxiety in this study decreased between the baseline and post-condition, but they were seen to return to baseline post-exposure. Even though there was a decrease in anxiety right after the exercise, this condition did not persist after 30–40 min [22]. There could be many causes of such a result: (1) the duration of yoga practice wasn't enough to make a permanent change in the anxiety levels. (2) Since these were acute sessions of YogaFit, these weren't the right yogic exercises for anxiety, as anxiety would be better combated by meditation and mindfulness exercises.

11.2.6 Distress Tolerance

One of the most common diseases due to aging is CVD (cardiovascular disease). Heart attacks due to cardiovascular dysfunction are the leading cause of deaths in the United States. And this number is growing with the increasing elderly population. Aging causes diminished peripheral vascular function, the main cause of cardiovascular dysfunction and also stress. Peripheral vascular dysfunction also leads to redox imbalance and creates free radicals and oxidative stress leading to impact on the vascular endothelium [23].

A study done by Elissa S et al. revealed that better stress adapted bodies tend to live longer. Resistance to stress is suggested to change the effects of many aging mechanisms. During aging, a large variety of cellular proteins tend to accumulate in insoluble forms causing homeostasis. Mutations that cause stress resistance and longevity slow down the accumulation of these insoluble proteins. In *C. elegans*, enhancing chaperone activity leads to increase in both stress tolerance and lifespan [24]. Even though homeostatis is the main connection seen between stress tolerance and longevity, there are other mechanisms connecting the two that are equally important. Some of these include oxidative stress and environmental toxins, telomere dysfunction or shortness and inflammation [24].

Aging and chronic stress are both associated with altered plasticity of the brain and thus increase in the risk of developing brain disorders. LPA1 is the main receptor for emotion processing in the brain and thus is the ideal receptor to control the impact of stress on behaviour and neurological variables. This receptor also regulates the effective state in aging. Latest research suggests that changes in LPA/LPA1 receptor signalling pathway lead to late-life depression syndromes, thus indicating a clear connection between the two [25].

Low DT, i.e. low distress tolerance, leads to negative emotion and even becomes threatening. Their sufferers are highly inclined to reduce or avoid such experiences.

Due to the disease, these individuals have difficulty in regulating their emotions leading to maladaptive strategies, like overeating to avoid their distress. Hatha Yoga increases one's present awareness and thus acts as a potential to reduce physical and psychological discomfort in patients suffering from DT. In an experiment by Johnna Medina et al., they found that yoga directly enhanced DTS subscales like tolerance (i.e. metacognitions about capacity to handle distress) and absorption (i.e. attention interference during distress). Cognitive components in the brain involved in distressed processing were particularly impacted after 8 weeks of Bikram Yoga practice. Another two factors, i.e. appraisal and regulation, related to emotional and behavioural distress weren't impacted as much. Other studies have also proven in the past that regular mind-body practice leads to enhanced capacity to stay attentive while completing a task. In fact, it is found that experienced yoga practitioners are better at performing cognitive attention tasks than non-meditators. Another experiment showed that some mindfulness decreased the dependence on alcohol for people addicted to it [26].

Reasons of increased DT by yoga interventions were examined, and significant changes in the mental health were observed. Meditation showed increased DTS-absorption scores and mediated a positive effect on emotional eating. Due to improved absorption, emotional eating was reported to improve by 15%. It is inferred that emotional eating is controlled by yoga due to its ability to improve concentration and the ability to think clearly (enhanced cognitive capacity) even when influenced by distress. It is interesting to note that although yoga decreased DT in general, only reductions in the level of absorption helped tackle the criterion for emotional eating [26].

11.2.7 Depression

Population of depressive elderly in the year 2006 according to NHANES data in the United States consisted of 4.1% with major depression, 5.1% other depression and 9.1% any depression, whereas in 2010 between 1.2 and 1.8 million elderly were reported to have depressive disorders which is 3–4.5% of their population. People who are admitted in the nursing homes in this age group have even higher rates of depressive symptoms, i.e. 49.6% [27].

Main symptoms of depression were reported to be "trouble sleeping or sleeping too much"; "feeling tired or having little energy"; "feeling down, depressed or hopeless"; and "little interest in doing things" [27]. The cycle of depression which causes individuals to show depression-related behaviours, feeling and thoughts, if unrecognized and untreated, causes distress and dysfunction. A higher level of care, including hospitalization among the elderly patients, is usually common. Depression reduces the pace of recovery after treatments in individuals [28].

Even though the risk and frequency of depression is clearly known among medically ill older individuals, depression is often unrecognized as a disorder. Depression can be rectified and have treatments; however, GP are usually not able to recognize depression. Thus, efforts need to be made to understand the problems

the elderly are facing to be able to recognize symptoms of depression better. Best way to combat depression is by practising yoga and mindfulness, which will avoid one from even having depression in the first place [28].

The estimate is that there are 350 million people worldwide suffering from depression. It is currently the most common mental illness in the world. In the United States, 16 million people have had depressive episodes in the past year. One's life can be substantially affected by depression, altering their mood and actions. The WHO reports depression as the leading cause of disability worldwide and is believed as the major contributor of the overall global burden of diseases [29]. Depression has been consistently ranked among the most common healthcare which can be self-treated with yoga. Self-treatment methods used by individuals include breathing exercises, relaxation techniques and meditation which are all elements of yoga. Yoga also appeals to depression patients as the perfect remedy mostly because of its ease of access, low cost, high social acceptance and the fact that yoga focuses on the whole person including the mind, body and spirit [30].

In a meta-analysis using 12 randomized controlled trials (RCT), depression was concluded to be better cured by yoga than the other self-care control exercises, such as relaxation or aerobics. Other physical health outcomes due to depression can also be cured by yoga. However, all these studies done on yoga to decrease depression also have many limitations like small sample sizes, lack of outcome assessments, lack of assessment of trainer, lack of intent to treat analysis and also insufficient documentation of randomization procedures [31].

A study by Uebelacker L et al. showed that participants who performed yoga showed lower levels of depression and also an overall improvements in general health, as well as improvement in their social work and role functioning. However none of these changes were acute and could have been better after a 3 months' or 6 months' treatment. When compared to health education however, yoga has a more enduring effect on the patients. In this study participants were also encouraged to practise yoga at home. Thus, this could have made participants better at coping with depressed mood and cognitions, and practising skills regularly and even outside class helped accumulate the beneficial effects pretty well over time. Additionally data also suggests cognitive behavioural therapy is better at preventing relapse than medications, maybe because therapy teaches patients to relate to the negative cognitive content and deal with it better even after therapy [31].

11.2.8 Cognitive Enhancement

After the age of 20, volume of grey matter begins to decrease. The prefrontal cortex constitutes the most atrophy. Changes with age are more moderate in the temporal lobes, and the volume of the hippocampus also decreases. The entorhinal cortex however doesn't show decrease in normal aging; however, it decreases in volume in Alzheimer's. The possible cause of grey matter volume decline is implicated to be the death of neurons, which causes the detrimental effect. Reduction of synaptic density due to decrease in the size and number of connections because of fewer

neurons is well documented in older adults. According to the model created by Terry and Katzman, the cognitive ability of a 130 year old will be the same as of someone with Alzheimer's dementia [32].

The volume of white matter decreases even faster than grey matter with the increasing age. When tested using morphometric methods from autopsy data in a study, a 16–20% decrease in white matter in subjects older than 70 years compared to younger subjects was observed. Shrinkage in white matter was noted in the gyrus rectus, corpus callosum and precentral gyrus. These findings have also been supported by other studies like Rogalski et al., who also described a decrease in parahippocampal white matter. Also studies with diffusion tensor imaging (DTI) show a decline of white matter with age [32].

One study examined the effects of six 60 min of yoga sessions, including mindfulness and improved WM (DS and LNS) functions. DS stands for digit span and LNS for letter number sequencing. The result showed an overall improvement in WM measures [14]. Another study by Nagendra H et al. used engineering student volunteers from IIT, Roorkee, to test the implications of yoga on their cognitive skills. EEG band scores were analysed of these students before and after 5 months of yoga. The results showed an increase of α, β and δ EEG band powers and a decrease in the θ, γ band powers. β band increase shows the enhanced cognitive functions like alertness. In addition, the increased α and decreased δ indicate high vigilance level and thus increased alertness. Thus, the results of increased higher-frequency α and β band powers and decreased lower-frequency θ and δ band powers lead to enhancement of cognitive skills like visual information processing and memory [33].

Other behaviour parameters that can be evaluated on the basis of these results include θ/α, β/α, β/θ, $(\delta + \theta)/\alpha$, $\beta/(\alpha + \theta)$ and $(\delta + \theta)/(\alpha + \beta)$. θ/β ratio indicates arousal in the central nervous system (CNS). This ratio decreased in the yoga group indicating the increase in arousal and thus reflecting a shift in α and θ activity towards β activity. Increased β implicates an increase in the brain cognitive performance like memory, attention and concentration. Also the α/δ ratio was seen to have increased in the yoga practised group. This indicates the brain perfusion index, which indicated the amount of blood flow to different parts of the brain. This was 4.6% higher in yoga practitioners. Due to this, the overall brain functioning is enhanced. LF/HF ratio is expressed in the terms of $(\theta + \delta)/(\alpha + \beta)$ which indicated the balance of sympathovagal in the autonomic nervous system (ANS). This ratio decreased in the yoga group, indicating a better ANS balance. When brain oscillations are slower (θ and δ), more neurons are harmonized across larger brain areas, and faster oscillation (α and β) is involved in harmonizing more focused, smaller neuronal assemblies. The ratio of α/β which analyses decreased desynchronization which is a good thing. On the other hand, the ratio of δ/θ representing synchronization increased. Also α/δ ratio increased in the parietal and temporal lobes and decreased in frontal, central and occipital lobes [33].

11.3 Discussion and Conclusion

All these findings show that yoga is overall beneficial for our well-being. Yoga when implemented to our daily lives can benefit us mentally and physically. Mindfulness helps us get in touch with ourselves internally and introspectively. When the practice of yoga was studied in the elderly, it turned out that they were able to function better which enhanced their cognitive ability. Memory stays as the most important factor in our lives, since it makes us who we are and helps us remember our experiences. It was something that needed to be tested on the elderly, as late-life memory disorders are common.

After having volunteers take yoga classes for an established period of time, the results for the DMN connectivity before and after were recorded. DMN is the area of the brain which connects its right and left ventricles and is responsible for memory recollections. With these results, it was concluded that DMN function is enhanced after yoga practices, thus implementing a better memory due to yoga. On top of this, yoga is also been identified in improving one's distress tolerance. Distress is very common in the elderly as when they stop being able to take control of their lives, they tend to get distressed. Doing yoga on the other hand calms the mind down and takes care of distress. One of the symptoms of distress is overeating, and this was seen to be improved in yoga practitioners in a study.

The most easily combated disease from yoga would be depression. In fact, the ease with which one can access yoga makes it the best remedy for the disease. With meditation and mindfulness exercises, the mind can be more efficient and thus less depressed. Yoga enlivens not only one's body and mind but also one's spirit which should be another way to combat depression. Cognitive functions in the brain also decline with aging. However, with yoga even that is seen to get better due to better blood flow in the brain.

All of these prove the positive effects of yoga in the aging population and thus should be promoted among the young and old alike.

References

1. Anisimov VN, Sidorenko AV (2018) It is necessary to establish an International Agency for Research on Aging. Aging (Albany, NY) 10(5):863–867
2. Martin P et al (2015) Defining successful aging: a tangible or elusive concept? Gerontologist 55 (1):14–25
3. Wiggins J, Bitzer M (2013) Slowing the aging process. Clin Geriatr Med 29(3):721–730
4. Bratic A, Larsson N-G (2013) The role of mitochondria in aging. J Clin Invest 123(3):951–957
5. McCormick MA et al (2015) A comprehensive analysis of replicative lifespan in 4,698 single-gene deletion strains uncovers conserved mechanisms of aging. Cell Metab 22(5):895–906
6. Yan L-J (2014) Positive oxidative stress in aging and aging-related disease tolerance. Redox Biol 2:165–169
7. Zampieri M, Ciccarone F, Calabrese R, Franceschi C, Caiafa P (2015) Reconfiguration of DNA methylation in aging. Mech Ageing Dev 151:60–70
8. Longo VD et al (2015) Interventions to slow aging in humans: are we ready? Aging Cell 14 (4):497–510
9. Willcox DC, Scapagnini G, Willcox BJ (2014) Healthy aging diets other than the Mediterranean: a focus on the Okinawan diet. Mech Ageing Dev 136–137:148–162

10. Yu Z, Wang R, Fok WC, Coles A, Salmon AB, Pérez VI (2015) Rapamycin and dietary restriction induce metabolically distinctive changes in mouse liver. J Gerontol Ser A 70 (4):410–420
11. Al-Niaimi F, Chiang NYZ (2017) Topical vitamin C and the skin: mechanisms of action and clinical applications. J Clin Aesthet Dermatol 10(7):14–17
12. Wang Y-W et al (2017) Metformin: a review of its potential indications. Drug Des Devel Ther 11:2421–2429
13. Rao RV (2018) Ayurveda and the science of aging. J Ayurveda Integr Med 9(3):225–232
14. Brunner D, Abramovitch A, Etherton J (2017) A yoga program for cognitive enhancement. PLoS One 12(8):e0182366
15. Barrientos RM, Kitt MM, Watkins LR, Maier SF (2015) Neuroinflammation in the normal aging hippocampus. Neuroscience 309:84–99
16. Nyberg L (2017) Functional brain imaging of episodic memory decline in ageing. J Intern Med 281(1):65–74
17. Eyre HA et al (2016) Changes in neural connectivity and memory following a yoga intervention for older adults: a pilot study. J Alzheimers Dis 52(2):673–684
18. Pink A et al (2017) Cortical thickness and anxiety symptoms among cognitively normal elderly persons: the Mayo Clinic study of aging. J Neuropsychiatry Clin Neurosci 29(1):60–66
19. Riley RJ, Burgener S, Buckwalter KC (2014) Anxiety and stigma in dementia: a threat to aging in place. Nurs Clin North Am 49(2):213–231
20. Telles S et al (2018) Increased mental well-being and reduced state anxiety in teachers after participation in a residential yoga program. Med Sci Monit Basic Res 24:105–112
21. Telles S, Pathak S, Kumar A, Mishra P, Balkrishna A (2015) Influence of intensity and duration of yoga on anxiety and depression scores associated with chronic illness. Ann Med Health Sci Res 5(4):260–265
22. Albracht-Schulte K, Robert-McComb J (2018) The effects of yoga and quiet rest on subjective levels of anxiety and physiological correlates: a 2-way crossover randomized trial. BMC Complement Altern Med 18(1):280
23. Wray DW, Amann M, Richardson RS (2017) Peripheral vascular function, oxygen delivery and utilization: the impact of oxidative stress in aging and heart failure with reduced ejection fraction. Heart Fail Rev 22(2):149–166
24. Epel ES, Lithgow GJ (2014) Stress biology and aging mechanisms: toward understanding the deep connection between adaptation to stress and longevity. J Gerontol A Biol Sci Med Sci 69 (Suppl 1):S10–S16
25. Moreno-Fernandez RD et al (2018) Stress, depression, resilience and ageing: a role for the LPA-LPA1 pathway. Curr Neuropharmacol 16(3):271–283
26. Medina J, Hopkins L, Powers M, Baird SO, Smits J (2015) The effects of a hatha yoga intervention on facets of distress tolerance. Cogn Behav Ther 44(4):288–300
27. McCall WV, Kintziger KW (2013) Late life depression: a global problem with few resources. Psychiatr Clin North Am 36(4):475–481
28. Smith M, Haedtke C, Shibley D (2015) Late-life depression detection. J Gerontol Nurs 41 (2):18–25
29. Bridges L, Sharma M (2017) The efficacy of yoga as a form of treatment for depression. J Evid Based Complement Altern Med 22(4):1017–1028
30. Prathikanti S, Rivera R, Cochran A, Tungol JG, Fayazmanesh N, Weinmann E (2017) Treating major depression with yoga: a prospective, randomized, controlled pilot trial. PLoS One 12(3): e0173869
31. Uebelacker LA et al (2017) Adjunctive yoga v. health education for persistent major depression: a randomized controlled trial. Psychol Med 47(12):2130–2142
32. Harada CN, Natelson Love MC, Triebel KL (2013) Normal cognitive aging. Clin Geriatr Med 29(4):737–752
33. Nagendra H, Kumar V, Mukherjee S (2015) Cognitive behavior evaluation based on physiological parameters among young healthy subjects with yoga as intervention. Comput Math Methods Med 2015:821061

12 Molecular Level Insight into the Involvement of Heat Shock Proteins in Oxidative-Stress-Mediated Human Diseases

Sangeeta Mitra, Angshuman Bagchi, and Rakhi Dasgupta

Abstract

Heat shock proteins (HSPs) are molecular chaperons that are duly responsible for catalyzing the bonafide folding of incipient proteins as well as refolding of denatured proteins. Under certain pathological conditions, these stress proteins play a few cytoprotective ventures through the commencement of protein folding, repairing, misfolded peptide refolding, as well as feasible degradation of irremediable ones. Elevated reactive oxygen species (ROS) in cellular levels often result in imprudent apoptosis. This subsequently leads to the amplification of inflammatory reactions. This is familiar in the pathogenesis as well as in the succession of various human inflammatory diseases (HIDs), respiratory diseases, cancer, and other deadly diseases. For example, chronic obstructive pulmonary disease (COPD) is designated by an imbalance in oxidants and antioxidants, as well as vivid inflammatory response. Evidence proposes that HSPs have a crucial role in retaining a proper balance between oxidants/antioxidants in COPD patients. HSP 70 usually plays a key role in neuroprotection by delaying the prognosis of neurodegenerative diseases as well as preventing senescence. This chapter discusses how some of the important oxidative-stress-mediated human diseases can be barcoded by several heat shock proteins to redeem their disease-specific aggregation.

Keywords

Heat shock proteins · ROS · Antioxidant effect · Oxidative stress · Protein aggregated diseases

S. Mitra · A. Bagchi (✉) · R. Dasgupta (✉)
Department of Biochemistry and Biophysics, University of Kalyani, Nadia, West Bengal, India
e-mail: angshu@klyuniv.ac.in

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_12

12.1 Introduction

Various research revealed that oxidative stress is a substantial result of the imbalanced production of ROS, as well as due to biological incompetence; proper detoxification of those species results in further damage. The effects of oxidative stress correlate with its impact on cellular arrangement. The elevation of oxidative stress often leads to necrosis and depletion of ATP and also helps to prevent controlled apoptotic death. When normal condition prevails, a large number of cells are kept in a compressing environment preserved by enzymes. In the normal redox state, any kind of imbalance results in toxicity mediated by the production of peroxides and free radicals (enacting nitrogen species/reactive oxygen), which severely damage cell DNA, proteins, and sometimes also lipids. There are mainly two types of ROS; one is less reactive, and the other is aggressive. By virtue of normal aerobic metabolism, less reactive species are evolved at low concentration. ROS species often cause cellular damage, which remains under persistent repairing. In some instances, it is very much evident that due to the oxidoreduction reactions along with transition metals, the less reactive superoxide's are readily transformed into much more aggressive radicals which eventually promotes DNA damage. ROS have a crucial role in cell signaling. In order to cultivate a convenient cellular homeostasis, a suitable harmony must be maintained between the production of reactive oxygen as well as its utilization. Enormous free radicals or ROS either must be converted into metabolically noncalamitous molecules or should have to be neutralized right after their formation. This vigilant mechanism is termed as the antioxidant defense system, which is responsible for inhibiting free-radical-arbitrated damage of cells, resulting in various diseases and aging [1].

12.2 Role of Heat Shock Protein

Depending on their molecular shape and size, Hsps have been restricted to six prime families (small heat shock proteins, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100). The individual family comprises members who are able to be integrally expressed, to be analytically regulated, and to be targeted to various chambers. Grp94 shows a corresponding function in the endoplasmic reticulum, while Hsp90 incorporates its role in both the nuclear and cytosolic compartments. It is found that Hsp70 family members incorporate a highly complex pattern of stress-induced as well as growth-regulated gene expressions. For these reasons, Hsp70 is being chosen often for contrasting subcellular chambers. For example, Hsp70 and Hsc70 (heat shock constitutive 70) proteins are nuclear as well as cytosolic in nature; conversely, Grp74 (glucose-regulated protein 74) is localized in mitochondria, and Grp78 (glucose-regulated protein 78) is an endoplasmic reticulum constrained protein [2] (Table 12.1).

The transcription of genes in Hsp is often synchronized by transcription factors, which belong to the heat shock factor family. This usually verifies the rapid transcriptional activation during stress. The family of Hsf gene includes heat shock

Table 12.1 Heat shock protein families and their detailed features correlated with oxidative stress

Heat stress proteins	Cellular localization	Cellular functions	Important remarks/ features
Hsp10	Mainly mitochondria	Acts as a biomarker in endometrial cancer	Helps in protein folding
Hsp20	Cytoplasm	Vasorelaxation	Apoptosis, cardiac dysfunction
Hsp27	Nucleus, cytosol, endoplasmic reticulum (ER)	Acts as chaperone and accelerates refolding of denatured proteins Serves as biomarker in several diseases, especially in cancer	Cofactor for HSP70
Hsp40	Cytosol	Assists in protein folding	Cochaperoning with Hsp70
Hsp60	Mitochondria/ cytoplasm	Forms hetero-oligomer complexes and thus assembles unfolded proteins	Prevents protein aggregation
Hsp70	Nucleus/cytosol	Aids in degradation and assembling of folded peptides	Integrally expressed molecular chaperones
Hsp90	Nucleus/cytoplasm	Promotes myosin folding complexes to refold the misfolded proteins	Acts as chaperone for protein kinase
Hsp100	Cytoplasm	Facilitates signal transduction	Helps in refolding
Hsp110	Nucleus/cytosol	Facilitates immune response as well as helps in the formation of complexes with Hsp70	Assists to survive under severe stress factors

transcription factor 1 (hsf1), heat shock transcription factor 2 (hsf2), and heat shock transcription factor 4 (hsf4). It is evident that the fundamental Hsps can be traced in a diverse array of multiprotein complexes carrying both the Hsps and their cofactors. Hsp90 is found to be very much dynamic and thus forms complexes comprising a huge number of cellular kinases and transcription factors [3].

Genes that are responsible for encrypting HSPs are also transcriptionally regulated by a variety of physiologic processes. It is not always necessary that they will cogitate on cell stress, which includes the cell cycle, proliferation, and differentiation [4, 5]. Based on these observations, researchers have suggested that Hsp90 and Hsp70 may also have crucial activities during cell growth and can be eventually incorporated with the cell cycle and cell proliferation [6, 7]. The observation also gives evidence that Hsp expression can be harmonized with many of the conditions that subsequently leads to apoptosis. This event very much correlates with some pathologic states such as fever, ischemia, inflammation, and infections [8].

12.3 Relation Between Oxidative Stress and Various Diseases

12.3.1 Oxidative Stress and Respiratory Disease

The distinct imbalance between the reactive oxygen/nitrogen species formed and the cell/tissue damage becomes one of the main reasons for the occurrence of many severe diseases. In humans, oxidative stress results in various diseases in so many ways. Oxidative stress is interrelated with several pathological conditions, along with the accumulation of ROS and mutations in the mitochondrial DNA (mtDNA). It leads to the development of chronic obstructive pulmonary diseases (COPDs). COPD is outlined by an imbalance in oxidants and antioxidants, as well as vivid inflammatory response. Cigarette smoking is a prime factor that facilitates the gradual advancement and progress of COPD. It triggers different free radicals and toxic substances, which adversely affect protease/antiprotease as well as oxidants/antioxidants [9, 10].

COPD is also described as a slowly accelerating state that is categorized by airflow limitation and is inevitable to some extent. One of the major reasons behind the development of this condition is cigarette smoking. Studies have shown that more than 90% of patients with COPD are smokers but not all smokers develop COPD. Evidence shows that 14–20% of cigarette smokers are definitely inclined to its effects and thus experience a rapid decrease in forced expiratory volume in 1 s (FEV1). It subsequently helps to develop the disease further. The elevated oxidant burden in chain smokers can be attributed to the fact that cigarette smoke contains >1015 oxidant/free radical mol/puff. Among these, there are comparatively long-lived tar semiquinone, which is able to produce Fenton-reaction-mediated hydrogen peroxide (H_2O_2) and OH. Several different factors such as infections, air pollutants, and occupational dusts eventually induce COPD as well as promote oxidative stress [11] (Fig. 12.1).

It is observed that the oxidant burden in the lungs of smokers is apparently elevated due to the release of ROS macrophages as well as from neutrophils, and both of them are known for their migration into the lungs of chain and passive cigarette smokers. These two produce ROS by means of the NADPH oxidase system. It is also indicated that with higher levels of COPD, the circulation of neutrophils from the smokers releases more and more oxygen [11].

12.3.2 Oxidative Stress and Inflammatory Disease

Inflammation is defined as a therapeutic condition that is characterized by immune cell infiltration into the vascular wall as well as extra dilation of the immune cells in the tissue. The release of ROS by these cells often leads to severe injury to the tissue. ROS produced by inflammatory cells also induce various avenues that amplify the inflammation. ROS-induced activation of kinases (or enzymes that facilitate phosphorylation), such as protein kinase C (PKC), c-Jun-N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK), subsequently activates the

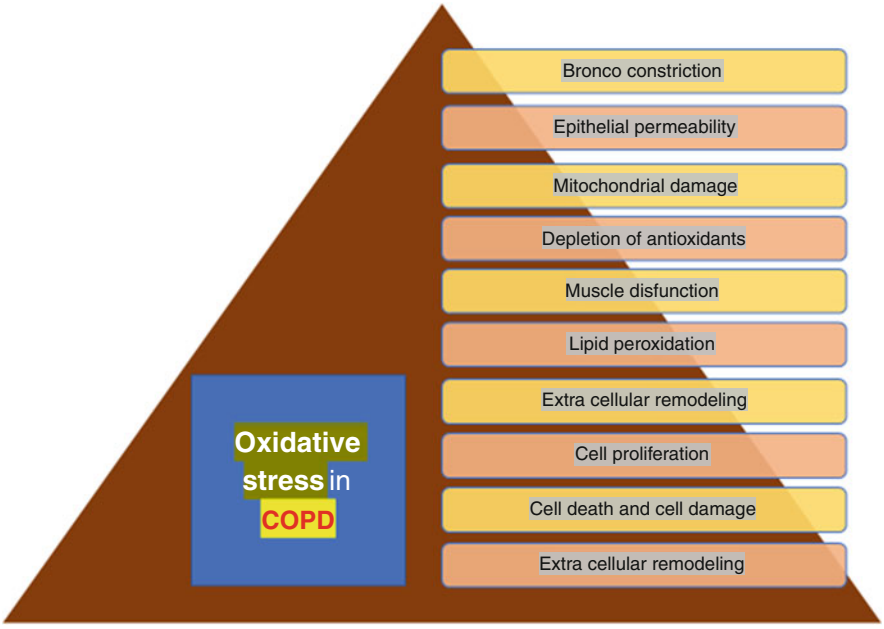


Fig. 12.1 ROS-meditated cellular feedback in COPD

transcription factors and gradually promotes the production of pro-inflammatory cytokines as well as chemokines. When induced, these chemo- and cytokines hold together to their respective receptors (epidermal growth factor receptors, platelet-derived growth factor receptor, VEGF receptor, etc.) that are well capable of producing ROS. Thus, ROS can be traced both up- and downstream during the cycle of inflammation (Fig. 12.2) [12].

An increased level of ROS results in apoptosis, which leads to successive increased inflammatory reactions. This eventuallly results in successions as well as in pathogenesis of human inflammatory diseases (HIDs) by virtue of antioxidant mechanism. In order to make sure the cellular importance of the fighting mechanism against infections and other inflammatory reactions, ROS levels are kept on checking under a normal physiological condition. Any disruption in this balance leads to oxidative stress and massive cellular destruction. One may infer naturally that adaptability between apoptosis, oxidants and inflammation performs a certain kind of dysfunction within the antioxidant system. It often accelerates the progression of HIDs [13].

12.3.3 Oxidative Stress and Neurodegenerative Disease

Neurodegeneration depicts an escalating dysfunction and neuronal loss in the central nervous system (CNS). This is eventually considered as a prime reason for cognitive

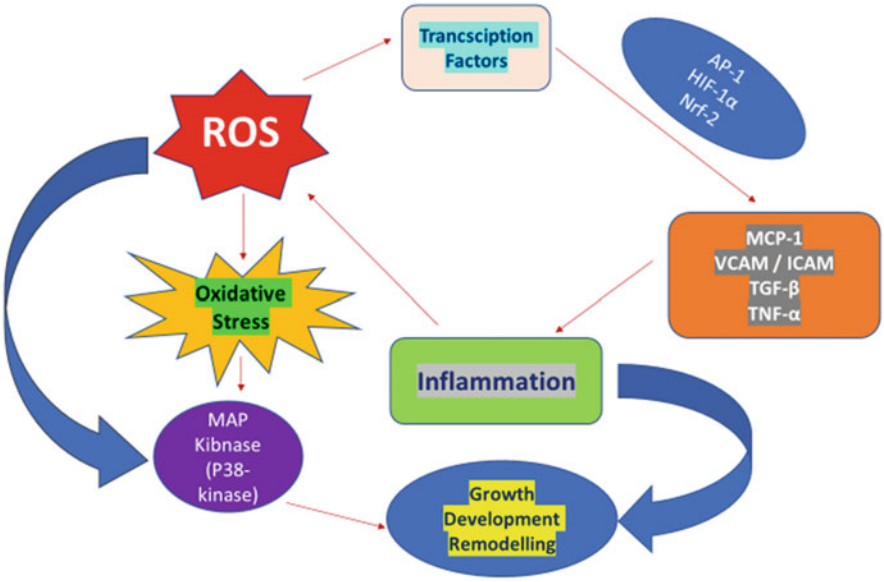


Fig. 12.2 Oxidative-stress-mediated redox-dependent signaling avenues and inflammation

as well as motor dysfunction. The blood-brain barrier separates the CNS from the remaining part of the body. The microglia are known to be the immune cells of the CNS, and studies suggest that in response to injury or infection, they release ROS and pro-inflammatory cytokines, which are linked to neurodegenerative diseases. Studies have shown that a salient feature of neurodegenerative diseases, like Parkinson's and Alzheimer's disease, is microglia activation. Deposition of amyloid plaque carried out by amyloid-β peptide (Aβ) chelating transition metal ions like Fe^{3+}, Zn^{2+} and Cu^{2+} can be taken as a featured character of Alzheimer's disease. The leap transition metals of Cu^{2+} and Fe^{3+} eventually facilitate some major chemical reactions, which often results in the altered oxidation state of both the metals. Parkinson's disease is brought about by α-synuclein (the accumulated aggregates of protein). Some of the major mutations in α-synuclein promote this disease. These mutations eventually result in mitochondria-mediated dysfunction and progressively lead to a massive production of ROS [12].

12.4 Role of HSPs in Neutralizing Diseases Inflicted by Oxidative Stress

Another prime necessity for cellular growth, cell functioning, and its further survival is the proper folding, degradation, and maturation of stressed proteins. These kinds of mechanism are taken up by molecular chaperones; out of them, few are heat shock proteins. The transcription of heat shock genes is hugely controlled by heat shock

factor 1 (HSF1). Evidence shows that some of the Hsp families play a crucial role as protein quality control machinery. They are ATP-independent small heat shock proteins (aA-crystallin, aB-crystallin, Hsp20), Hsp40, Hsp70, Hsp90, and Hsp110. These proteins are generally pretending to be a cochaperone for Hsp70. They also restrict the binding of ATP-dependent polypeptide to Hsp70, thus preventing the folding activity of the premature polypeptide of Hsp70. They contribute significantly to the prevention of diseases induced by oxidative stress [14].

12.4.1 Control of COPD Mediated by HSPs

Studies have shown that elevated HSPs levels are generally observed in COPD patients. Especially, HSP60 is said to have a role in COPD pathogenesis. Studies revealed that few HSPs are used as a possible serum marker in patients who developed COPD because of excessive smoking. Their studies elucidated a higher level of HSP27 in the serum of COPD patients. This can be attributed to the fact that elevated conditions of HSP27 are associated primarily with partial inflammation as well as oxidative stress. Several other studies have shown that inhaled toxins in smokers, causing immune response, often leads to distinctive pulmonary changes and also decreases the level of HSP27 in the pulmonary vascular network in COPD-sensitive patients [15]. It was concluded that serum HSP27 in elevated conditions may not be directly interconnected with smoking and can be found elevated after the development of COPD.

12.4.2 Control of Inflammatory Diseases Mediated by HSPs

HSPs are found to have an elevated state in several inflammatory diseases. In chronic allograft nephropathy and other inflammatory disorders such as acute coronary syndrome, a distinct serum elevation of HSP27 was reported. It is also noted that in the highly inflammatory regions of human atherosclerotic plaque, an elevated level of HSP90 immunostaining was predominantly observed [16–18]. Those that are able to control the folding and further processing of denatured and damaged proteins are often considered as highly conserved molecular chaperones. By executing significant anti-inflammatory actions, they are able to modulate inflammation through several mechanisms [19].

It is found that models of inflammation and stroke show a reduced expression of production of nitric oxide and COX-2 when they are exposed to upregulation as well as in preconditioning stress of Hsp70. These anti-inflammatory actions of Hsp70 are facilitated by the binding of Hsp70 to NF kappa B and followed by its gradual inhibition. It has been elucidated that Hsp70 hinders the nuclear translocation of NF kappa B, and this effect is mediated by the prevention of upstream events, which leads to NF kappa B activation. On the contrary, inflammation acts as a predominant stimulus for the upregulation of Hsp70 construction [20].

12.4.3 Prevention of Neurodegenerative Diseases by HSPs

In several neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), the deposition of plaques and protein misfolding are mediated by oxidative stress, thus promoting the formation of aggregates [21]. The protein aggregates are resulted from highly instructed filamentous insertion with the proper conformation of β-sheet in the core. These accumulations or deposits are of two types: fibrillar and insoluble [22, 23].

Alzheimer's disease is usually contemplated as one of the most common state of dementia, which eventually influences the aging population. In this disease, oxidative stress is incorporated with one of the earlier events. With regard to other neurodegenerative diseases that are associated with misfolded proteins, an analysis of Alzheimer disease brains revealed elevated levels of Hsps and their cochaperones, including Hsp27, Hsp70, and STI1 [24–26]. Studies have shown that there are a number of Hsp70 and Hsp90 cochaperones that possess a distinctive influence on neurodegenerative diseases. High-molecular weight FKBP51 and FKBP52 are able to induce a variety of effects on tau (an abundant CNS protein) structure and function, which play key roles in AD.

The relation between molecular chaperones in this particular disease was first suggested after observing Hsp27, Hsp40, Hsp60, and Hsp70, which were found to be confined in Lewy bodies [6]. A plethora of evidence suggests that a protective action of molecular chaperones against α-synuclein-induced toxicity is present both inside and out. It is also noticed that the recombinant human Hsp70 was very much capable of binding α-synuclein filaments. It also attenuates its inhibitory activity, just like chymotrypsin. Further, it can be added that heat shock in fibroblasts that expresses α-synuclein subsequently leads to a distinct elevated condition in Hsp70, which is also able to decrease the hindered effects of α-synuclein on the proteasome [26].

12.4.4 Deregulation of Hsp Genes and Cancer

It has been observed that tissues or cells from a vivid area of tumors exhibit levels of one or more Hsps [27, 28]. These observations have led to suggestions that some of the Hsps can be treated as a possible candiate for biomarkers. In breast cancer, the expression of Hsp is associated with bad forecast. Moreover, it is also resistant to radiation therapy and chemotherapy [28]. In endometrial, leukemia, and breast cancers, increased levels of Hsp27, as compared to those in nontransformed cells, are being diagnosed. Additionally, a distinct phosphorylation pattern is being observed in the Hsp27 of tumor cells [29].

It is found in the case of breast tumors that the expression of Hsp70 is related to short-term disease-free survival and metastasis among diseased patients who have been treated with combined hyperthermia, radiation therapy, and chemotherapy [30–32]. Several other Hsp family members (Hsp60, Hsp90α, and Hsp90β) show their overexpression in leukemia, breast tumors, Hodgkin's disease, and lung cancer [33–

35]. Overexpression of Hsp 70 gene is also observed in the adenovirus-transformed human embryonic kidney 293 cell line, which is related to the influential presence of adenovirus trans-activator E1a [36–38].

12.4.5 Bronchial Epithelial Carcinogenesis and Its Relationship to Hsps

It is found that the overexpression of Hsp60 and Hsp10 confer protection on the cells against ischemia/hypoxic/oxidative injury, and as a result, apoptosis is completely inhibited [39, 40]. During prostatic, exocervical, and colorectal carcinogenesis, overexpressive Hsp10 and Hsp60 were highly observed [41–43]. Further studies also confirmed these results. The relation between the prognosis of tumoral patients and Hsp60 levels is being reported in the case of acute myeloid leukemia [44] and bladder carcinoma. Hsp60 overexpression was found to be correlated with adverse prognosis. It was evident that Hsp60 is involved in oxidative-stress-mediated necrotic cell death or apoptosis. Based on the findings on the relation between smoking and lung cancer (LC), a higher frequency of adenocarcinoma was previously reported in patients suffering from COPD. The main focus of the study was to find out the expression of Hsp 60 and Hsp10 in bronchial biopsies from lung cancer patients and smokers with COPD and to show the interconnection between the carcinogenic steps of lung cancer and elevated expression of Hsps. The results showed conclusive evidence proving that the loss of Hsp10 and Hsp60 immunopositivity is correlated with progression to bronchial cancer in smokers having COPD [42, 45].

12.4.6 Oxidative Stress in Lungs and Hsps

It is a well-documented fact that oxidative stress is responsible for the pathogenesis of various kinds of diseases and that it is an important mediator in different biological processes like atherosclerosis, aging, carcinogenesis, ischemia-perfusion tissue injury, and chronic and acute inflammatory disorders. Major toxic and harmful reactive oxygen species (ROS) like superoxide, hydroxyl peroxide, and hydrogen radicals, which are produced during normal cellular respiration and aerobic metabolism, may turn to be fatal agents in destroying a cell's viability. This is facilitated by the oxidation of nucleic acids, membrane lipids, and proteins [46, 47].

In the case of bacteria, several antioxidant enzyme (AOE) genes have been found that are under the control of some genetic regulons. They are able to protect the cells against ROS. In *Escherichia coli*, oxyR gene products are found to facilitate the transcription of different genes, which include peroxide-destroying catalase, glutathione reductase, and alkyl hydroperoxide reductase [48].

Superoxides, on the other hand, can promote the induction of soxRS gene products, which in turn promotes the transcription of several genes, which encodes specific proteins like manganese-containing superoxide dismutase (MnSOD) and

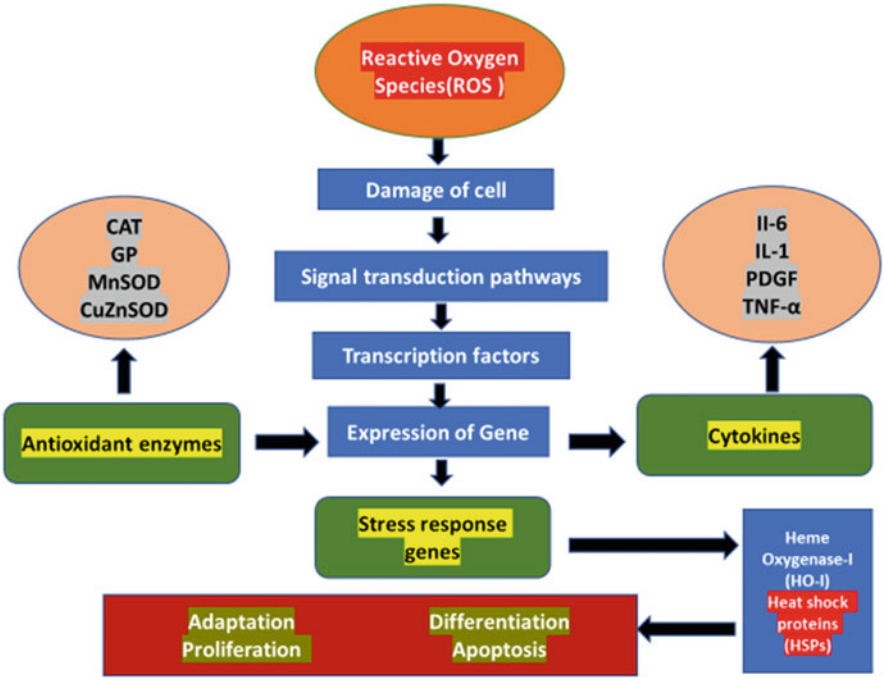


Fig. 12.3 The figure depicts molecular and cellular level responses to oxidative-stress-related factors

glucose-6-phosphate dehydrogenase. If eukaryotes are taken into consideration, it is observed that along with enzymatic agents, they do contain several nonenzymatic features such as protection against oxidative stress. Nonenzymatic defense agents include vitamins, sulfhydryl-containing glutathione, albumin, transferrin-lactoferrin, and ceruloplasmin, whereas enzymatic agents are glutathione peroxidase (GP), superoxide dismutase, and catalase (CAT) [49] (Fig. 12.3).

Lungs can be treated as a major target organ for injury caused by ROS. Researchers found that both chronic as well as acute inflammatory diseases like asthma, adult respiratory distress syndrome (ARDS), and pulmonary fibrosis are caused by ROS [47]. When oxidative stress is being imposed on cultured cells, it is found that they elicit several AOE genes, which include CAT, MnSOD, copper-zinc SOD (CuZnSOD), and GP [48, 49]. A notable function of these enzymes is scavenging ROS, as well as helping attenuate the "pro-oxidant" state of the cell in order to sustain regular homeostasis of cells [46, 47].

A substantial amount of research that was performed to decipher the function as well as the regulation of AOEs in lung injury mediated by oxidation is a great source of our understanding in this particular field. Presumably, the expression of various genes also upregulated right after oxidant lung injury. Notable genes among them are heme oxygenase-l (HO-I), heat shock proteins (HSP), and metallothionein (MT).

Investigators have shed light on the fact that transcriptional regulators like Egr-l and *NF-KB* take active roles in the molecular event of cellular response to oxidative stress during early phase [48–50].

12.5 Conclusion

Since their discovery, heat shock proteins have gained substantial amount of interests from researchers. They contribute to various physiological functions, say for example preventing the misfolding of proteins and protein-protein interactions. They are often found to influence the redox state and hydration of cells. On the other hand, oxidative stress is principally responsible for the pathogenesis of several diseases and is involved in biological processes like ischemia, aging, atherosclerosis, respiratory disease carcinogenesis, and other inflammatory disorders. In cell signaling, reactive oxygen species (ROS) often possess an antioxidant defense protective mechanism to prevent further cell damage, which can be responsible for the occurrence of various diseases. Heat shock proteins have an inevitable correlation with ROS and oxidative stress in several human diseases. Owing to their significant role in propagation of several human diseases, human heat shock proteins have gained much importance in research as well as in the medical field in recent years. Therefore, giving suitable emphasis on Hsps especially on oxidative stress-mediated diseases, respiratory diseases and most importantly human inflammatory diseases (HIDs) will serve as a potential tool to facilitate their treatment. Further research works may shed some light on the field of drug delivery system, which may result in developing some significant candidate drugs that can serve as a potential and consequential outcome in the early detection of these diseases.

Acknowledgement The author acknowledges DBT-funded BIF, University of Kalyani, for providing infrastructure facilities, and the Department of Science and Technology and Biotechnology of West Bengal Govt. for financial support (Project SA. No./ST/P/S&T/1-G14/2018).

References

1. Jaattela M (1999) Heat shock proteins as cellular lifeguards. Ann Med 31:261–271
2. Jolly C, Morimoto RI (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 92(19):1564–1572
3. Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10(2):86–103
4. Milarski KL, Morimoto RI (1986) Expression of human HSP70 during the synthetic phase of the cell cycle. Proc Natl Acad Sci U S A 83:9517–9521
5. Hang H, He L, Fox MH (1995) Cell cycle variation of Hsp70 levels in HeLa cells at 37°C and after a heat shock. J Cell Physiol 165:367–375
6. Knauf U, Bielka H, Gaestel M (1992) Over-expression of the small heat-shock protein, hsp25, inhibits growth of Ehrlich ascites tumor cells. FEBS Lett 309:297–302
7. Galea-Lauri J, Latchman DS, Katz DR (1996) The role of the 90-kDa heat shock protein in cell cycle control and differentiation of the monoblastoid cell line U937. Exp Cell Res 226:243–254

8. Samali A, Orrenius S (1998) Heat shock proteins: regulators of stress response and apoptosis. Cell Stress Chaperones 3:228–236
9. Shukla V, Mishra S, Pant H (2011) Oxidative stress in neurodegeneration. Adv Pharmacol Sci 2011:572634. https://doi.org/10.1155/2011/572634
10. Ünver R, Deveci F, Kırkıl G, Telo S, Kaman D, Kuluöztürk M (2016) Serum heat shock protein levels and the relationship of heat shock proteins with various parameters in chronic obstructive pulmonary disease patients. Turk thorac J 17(4):153–159. https://doi.org/10.5152/TurkThoracJ.2016.003
11. Rahman I (2005) Oxidative stress in pathogenesis of chronic obstructive pulmonary disease. Cell Biochem Biophys 43:167–188. https://doi.org/10.1385/CBB:43:1:167
12. Chatterjee S (2016) Oxidative stress, inflammation, and disease. Oxid Stress Biomater:35–58. https://doi.org/10.1016/b978-0-12-803269-5.00002-4
13. Ikwegbue P, Masamba P, Oyinloye B, Kappo A (2018) Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11:2. https://doi.org/10.3390/ph11010002
14. Lackie ER, Maciejewski A, Ostapchenko GV, Marques-Lopes J, Choy W, Duennwald ML, Prado FV, Prado M (2017) The Hsp70/Hsp90 chaperone machinery in neurodegenerative diseases. Front Neurosci 11:254. https://doi.org/10.3389/fnins.2017.00254
15. Jan Ankersmit H, Nickl S, Hoeltl E, Toepker M, Lambers C, Mitterbauer A, Kortuem B, Zimmermann M, Moser B, Bekos C, Steinlechner B, Hofbauer H, Klepetko W, Schenk P, Dome B (2012) Increased serum levels of HSP27 as a marker for incipient chronic obstructive pulmonary disease in young smokers. Respiration 83:391–399
16. Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, Tudev M, Ko YH, Choi YH, Kim S, Kim DI, Kim YW, Lee BB, Yoon JB, Park JE (2006) Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. Circulation 114:886–893
17. Djamali A, Reese S, Oberley T, Hullett D, Becker B (2005) Heat shock protein 27 in chronic allograft nephropathy: a local stress response. Transplantation 79:1645–1657
18. Madrigal-Matute J, López-Franco O, Blanco-Colio LM, Muñoz-García B, Ramos-Mozo P, Ortega L, Egido J, Martín-Ventura JL (2010) Heat shock protein 90 inhibitors attenuate inflammatory responses in atherosclerosis. Cardiovasc Res 86:330–337
19. Sevin M, Girodon F, Garrido C, de Thonel A (2015) HSP90 and HSP70. Implication in inflammation processes and therapeutic approaches for myeloproliferative neoplasms. Mediators Inflamm 2015:970242
20. Kalmar B, Greensmith L (2009) Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 61:310–318. https://doi.org/10.1016/j.addr.2009.02.003
21. Knowles TP, Vendruscolo M, Dobson CM (2014) The amyloid state and its association with protein misfolding diseases. Nat Rev Mol Cell Biol 15:384–396. https://doi.org/10.1038/nrm3810
22. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333–366. https://doi.org/10.1146/annurev.biochem.75.101304.123901
23. Han TW, Kato M, Xie S, Wu LC, Mirzaei H, Pei J et al (2012) Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell 149:768–779. https://doi.org/10.1016/j.cell.2012.04.016
24. Renkawek K, Bosman GJ, de Jong WW (1994) Expression of small heat shock protein hsp 27 in reactive gliosis in Alzheimer disease and other types of dementia. Acta Neuropathol 87:511–519. https://doi.org/10.1007/BF00294178
25. Perez N, Sugar J, Charya S, Johnson G, Merril C, Bierer L et al (1991) Increased synthesis and accumulation of heat shock 70 proteins in Alzheimer's disease. Brain Res Mol Brain Res 11:249–254. https://doi.org/10.1016/0169-328X(91)90033-T
26. Ostapchenko VG, Beraldo FH, Mohammad AH, XieY F, Hirata PH, Magalhaes AC et al (2013) The prion protein ligand, stress-inducible phosphoprotein 1, regulates amyloid-beta oligomer toxicity. J Neurosci 33:16552–16564. https://doi.org/10.1523/JNEUROSCI.3214-13.2013
27. Morimoto RI (1991) Heat shock: the role of transient inducible responses in cell damage, transformation, and differentiation. Cancer Cells 3:295–301

28. Fuller KJ, Issels RD, Slosman DO, Guillet JG, Soussi T, Polla BS (1994) Cancer and the heat shock response. Eur J Cancer 30A:1884–1891
29. Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA (1993) Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. J Natl Cancer Inst 85:1558–1570
30. Kaur J, Ralhan R (1995) Differential expression of 70-kDa heat shock-protein in human oral tumorigenesis. Int J Cancer 63:774–779
31. Ralhan R, Kaur J (1995) Differential expression of Mr 70,000 heat shock protein in normal, premalignant, and malignant human uterine cervix. Clin Cancer Res 1:1217–1222
32. Santarosa M, Favaro D, Quaia M, Galligioni E (1997) Expression of heat shock protein 72 in renal cell carcinoma: possible role and prognostic implications in cancer patients. Eur J Cancer 33:873–877
33. Jameel A, Skilton RA, Campbell TA, Chander SK, Coombes RC, Luqmani YA (1992) Clinical and biological significance of HSP89 alpha in human breast cancer. Int J Cancer 50:409–415
34. Yufu Y, Nishimura J, Nawata H (1992) High constitutive expression of heat shock protein 90 alpha in human acute leukemia cells. Leuk Res 16:597–605
35. Hsu PL, Hsu SM (1998) Abundance of heat shock proteins (hsp89, hsp60, and hsp27) in malignant cells of Hodgkin's disease. Cancer Res 58:5507–5513
36. Nevins JR (1982) Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell 29:913–919
37. Wu BJ, Hurst HC, Jones NC, Morimoto RI (1986) The E1A 13S product of adenovirus 5 activates transcription of the cellular human HSP70 gene. Mol Cell Biol 6:2994–2999
38. Williams GT, McClanahan TK, Morimoto RI (1989) E1a transactivation of the human HSP70 promoter is mediated through the basal transcriptional complex. Mol Cell Biol 9:2574–2587
39. Cappello F, Di Stefano A, David S, Rappa F, Anzalone R, La Rocca G, D'Anna SE, Magno F, Donner CF, Balbi B, Zummo G (2006) HSP60 and HSP10 Down-regulation predicts bronchial epithelial carcinogenesis in smokers with chronic obstructive pulmonary disease. Cancer 107 (10):2417–2424
40. He L, Lemasters JJ (2003) Heat shock suppresses the permeability transition in rat liver mitochondria. J Biol Chem 278:16755–16760
41. Cappello F, Bellafiore M, David S et al (2003) Ten kilodalton heat shock protein (HSP10) is overexpressed during carcinogenesis of large bowel and uterine exocervix. Cancer Lett 196:35–41
42. Cappello F, Rappa F, David S et al (2003) Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. Anticancer Res 23:1325–1331
43. Cappello F, Bellafiore M, Palma A et al (2003) 60KDa chaperonin (HSP60) is over-expressed during colorectal carcinogenesis. Eur J Histochem 47:105–110
44. Thomas X, Campos L, Mounier C et al (2005) Expression of heatshock proteins is associated with major adverse prognostic factors in acute myeloid leukemia. Leuk Res 29:1049–1058
45. Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S (1999) Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J 18:2040–2048
46. Choi AM, Alam J (1996) Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. Am J Respir Cell Mol Biol 15:9–19
47. Kinnula VL, Crapo JD, Raivio KO (1995) Biology of disease. Generation and disposal of reactive oxygen metabolites in the lung. Lab Inv *73:3–19*
48. Camhi S, Lee P, Choi AMK (1995) The oxidative stress response. New Horiz 3:170–182
49. Jornot L, Junod AF (1992) Response of human endothelial cell antioxidant enzymes to hyperoxia. Am. J. Respir. Cell Mol. Biol. 6:107115
50. Choi AMK, Sylvester SL, Otterbein L, Holbrook NJ (1995) Molecular responses to hyperoxia *in vivo:* relationship to increased tolerance in aged rats. Am J Respir Cell Mol Biol 13:74–82

Oxidative Stress in Liver Diseases

13

Lubhan Singh, Sagarika Majhi, Kavita Pabreja, Poonam Negi, Rohit Goyal, Gaurav Gupta, Dinesh Kumar Chellappan, and Kamal Dua

Abstract

Liver, being the second largest organ, maintains homeostasis by undergoing a number of risk factors that include alcohol, drugs, environmental pollutants, and radiation. All these factors are capable of inducing oxidative stress by generating free radicals that eventually result in various forms of severe liver diseases. In this chapter, the consequences of oxidative stress are studied, along with its pathophysiology, its effects on organelles, physiological alterations, and the common

L. Singh (✉)
Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

S. Majhi
I. T. S College of Pharmacy, Ghaziabad, India

K. Pabreja
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

P. Negi · R. Goyal
School of Pharmaceutical Sciences, Shoolini University, Bajhol, Solan, Himachal Pradesh, India

G. Gupta
School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India

D. K. Chellappan
Department of Life Sciences, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia

K. Dua
School of Pharmaceutical Sciences, Shoolini University, Bajhol, Solan, Himachal Pradesh, India

Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Ultimo, NSW, Australia

Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute (HMRI) & School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia

Centre for Inflammation, Centenary Institute, Sydney, NSW, Australia

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_13

diseases that occur due to oxidative stress. The progression of various liver diseases primarily involves lipid peroxidation, deoxyribonucleic acid (DNA) damage, signaling of inflammatory mediators, and ultimately generation of free radicals. The inarguable role of prooxidants in hepatic pathogenesis can be evidenced by an increase in the levels of biomarkers of oxidative stress, namely, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), to name a few. These markers are paralleled by utilizing endogenous antioxidant mechanisms, thus decreasing the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH).

This challenge was overcome by a diverse and rapid development in the field of biomarkers and antioxidants. Newer advances in the field of biomarkers outlined strategies to identify diseases at an early stage so that the treatment procedure could be both clinically useful and cost-effective. Advanced research on antioxidants, to treat liver disease, resulted in the emergence of natural substances that contain common natural herbal extracts, vitamins, and other compounds. Antioxidant use, either as a single compound or in combination, has become key molecules today for counteracting our stressed system and to achieve healthy homeostasis. However, new research should be carried out at cellular and molecular pharmacology levels in combination with drug targeting systems so as to get innovative ideas for the therapeutics of hepatic disease, which are not known enough.

Keywords

Liver/hepatic diseases · Biomarkers · Antioxidants · Prooxidants · Free radical

13.1 Introduction

Liver is a major and second largest organ for survival of the human body. Its main function is to metabolize food and synthesize, excrete, and detoxify endogenous molecules, drugs, and chemicals from the environment. Some other functions include storage and synthesis of glucose, red blood cell degradation, synthesis of various plasma proteins like albumin and globulin, hormone production, and bile acid formation. It also maintains homeostasis and host defense of our body.

As per anatomical perusal, liver lies under the diaphragm, anterior to the stomach. It consists of two varying blood supplies, namely, portal vein (conveys blood with digested nutrients from the stomach, spleen, pancreas) and hepatic artery (which helps in hepatic respiration by carrying oxygenated blood from the lungs to the liver). The liver is divided into four lobes, and microscopically each lobe consists of many small hexagonal lobules with a central vein. The lobules assist as the blood enters from the portal vein and hepatic artery and make its way down the cords of hepatic cells. The lobules are distinguished as per their perfusion capacity into three areas: *zone 1/periportal* > *zone 2/midzonal* > *zone 3/centrilobular* (in a row from highest to lowest oxygenation).

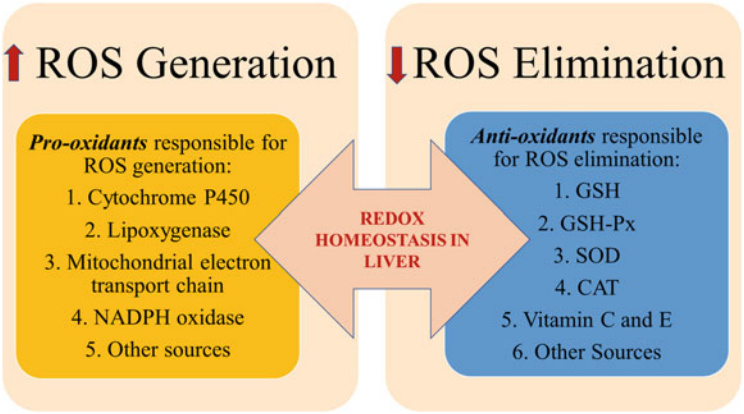


Fig. 13.1 Redox homeostasis in the liver

During the metabolism process in hepatic cells, a huge number of free radicals are generated, which are counterbalanced by our endogenous antioxidant defense system. These free radicals are known to induce oxidative stress to produce reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS). The free radicals are generated due to a disparity between prooxidants and antioxidants in the human system [1]. As the ratio of free radical generation increases, oxidative stress increases. This will ultimately lead to dysregulation and altered homeostasis of the liver, causing hepatic trauma, as shown in Fig. 13.1 [2].

As per various previously reported studies, oxidative stress is one of the initiators for numerous liver diseases, viz., (1) alcoholic liver disease (ALD), (2) nonalcoholic fatty liver disease (NAFLD), (3) liver fibro-proliferative diseases (fibrosis), (4) hepatic encephalopathy (HE), (5) cirrhosis, (6) hepatitis C, and (7) hepatocellular carcinoma (HCC) [3]. Long-term oxidative stress to hepatic cells can irreversibly vandalize lipids, proteins, and DNA, which take part in numerous pathways to control protein expression, gene transcription, hepatic stellate cell (HSC) activation, and cell apoptosis. This in turn modulates normal biological pathways, leading to hepatic injury described in Fig. 13.2.

In spite of the fact that antioxidants are yet to prove their efficacy in clinical trials, nevertheless, the applications of antioxidants could play a major role in the prevention of liver diseases due to oxidative stress. Antioxidants from natural origin are abundantly found in our daily diet (e.g., fruits, vegetables, and cereals), medicinal plants, and predominantly in flavonoid-containing products (such as green tea, milk thistle, coffee, naringenin, quercetin, curcumin, and resveratrol) [4]. Thus, in future, specific antioxidants can be identified for their preventive and therapeutic *in vivo* effects and to abolish oxidative stress, which results in various liver diseases.

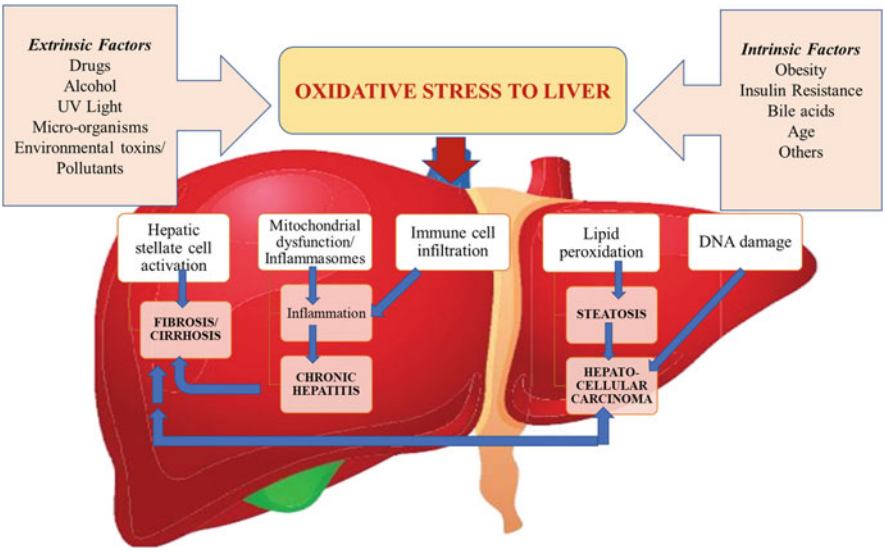


Fig. 13.2 General mechanism of oxidative-stress-induced liver disease

13.2 General Consequences of Oxidative Stress

13.2.1 Mitochondrial Dysfunction

The imbalance between pro- and antioxidants can alter the working dynamics of the mitochondria. It blocks fatty acid beta-oxidation and leads to a rise in nonmetabolized fatty acids in the cytosol, followed by free radical production. This is evident as the many microstructural changes in the mitochondria are observed under electron microscopy, such as gigantic mitochondria, dropping of cristae, and para-crystalline inclusion bodies in the matrix. Furthermore, when the electron transport chain is deteriorated, it results in "electron leakage," along with lipid peroxidation, which produces reactive aldehydes (MDA, 4-HNE), and the generation of mutated DNA. Buildup of ceramides (Cer) and diacylglycerols (DAGs) is observed due to deficient beta-oxidation of acylcarnitine, which is an inflammatory mediator altering insulin signaling. Increased activity of CYP2E1, depleted GSH, reduced GPx and manganese superoxide dismutase (MnSOD) activity, and impaired activity of cytochrome C also leads to ROS generation, as shown in Fig. 13.3.

13.2.2 Lipid Peroxidation

The cell membrane is comprised of polyunsaturated fatty acids that react with oxygen to generate peroxyl radicals. Peroxyl radicals are the initial free radical

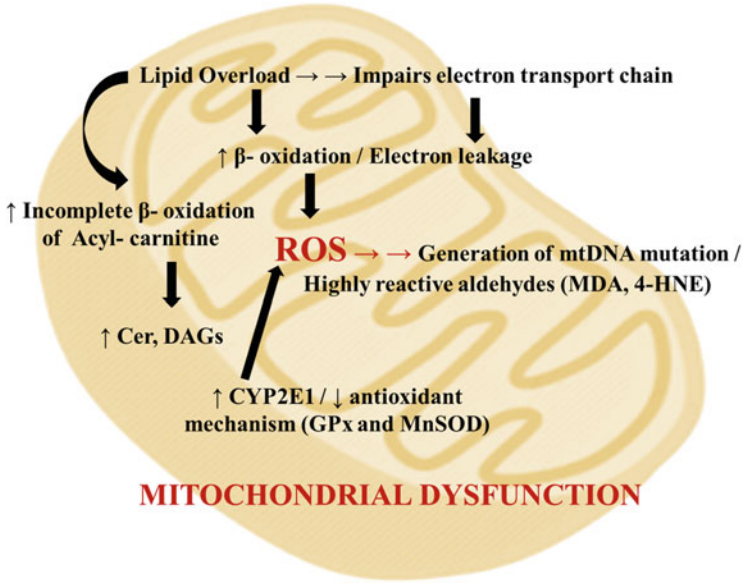


Fig. 13.3 Mechanism of mitochondrial dysfunction by ROS generation

intermediate produced through lipid peroxidation. This free radical can alter the membrane structure of lipids, membrane fluidity, and its functions. It alters ion transport, receptor recognition, receptor signaling, and osmotic gradients. The chain reaction is first initiated by a hydroxyl radical, which removes hydrogen molecule from lipids. Thus, a free radical can react with the free oxygen, forming a new peroxyl radical. Peroxyl radicals further reacts with aldehyde, which forms reactive end products such as malondialdehyde (mutagenic). Also, aldehyde adducts with proteins are formed that are proinflammatory and profibrogenic in alcohol-induced liver disease. Lipid peroxidation (LPO) also increases thiobarbituric acid reactive substances and 8-isoprostane levels in diseases like nonalcoholic fatty liver disease or hepatitis C.

13.2.3 DNA Damage

ROS attack and modification of particular DNA bases (thymine) lead to base pair modifications, cross-linking of DNA, strand breaks, and even mutations. These alterations for a prolonged duration might promote uncontrolled growth (i.e., cancer).

13.2.4 Protein Damage

Free radicals produced by oxidative stress modifies endogenous proteins and impairs the functioning of cells but may stimulate the host immune system, causing autoimmune diseases. Reactive oxygen species can also oxidize amino acids and enzymes making them inactive or antigenic, which can alter the normal protein structure and its function, leading to altered intracellular signaling cascades.

13.2.5 Altered Redox State

The redox state of any system is altered by changes in the second messenger signaling pathway. Oxidative stress produces reactive oxygen species, which intracellularly change signal transduction by second messengers. This influences many activities such as cell function, gene expression, apoptosis/necrosis, and cell death.

13.2.6 Iron Metabolism Derangements

The main prooxidizing mechanism altered metabolism of iron is via Fenton reaction, where an increased production of hydroxyl radical is observed. Lipid peroxidation is observed in various organelle membranes by hydroxyl radicals. This in turn impairs mitochondrial metabolism by producing mPTP (mitochondrial permeability transition pore) and mtDNA (mutated DNA), thereby increasing proapoptotic activity and MDA production. In addition, if iron load is continued for a prolonged period, it may lead to the production of iNOS (inducible nitric oxide synthase) via NF-κB activation. The escalated nitric oxide concentration reacts with superoxide anion to produce reactive nitrogen species. This produces reactive aldehydes (MDA) through lipid peroxidation, along with the activity of iron, which acts as a direct competitive antagonist of antioxidant enzymes.

13.2.7 Insulin Resistance and Endothelial Dysfunction

Insulin activity influences nitric oxide (NO) production, which causes vasodilatation, and induces anti-inflammatory, antithrombotic and antifibrogenic properties of the endothelium. Obesity and insulin resistance increase the expression of proinflammatory cytokines (tumor necrosis factor alpha (TNF-α), interleukin-1 (IL1), and interferon gamma (IFN-γ)), which in turn increases iNOS, decreases endothelial nitric oxide synthase (eNOS), and upregulates the Ras/mitogen-activated protein kinase (MAPK) pathway. The worsening of insulin resistance is proportional to the extremity of endothelial dysfunction (ED), observed in various models of liver disease.

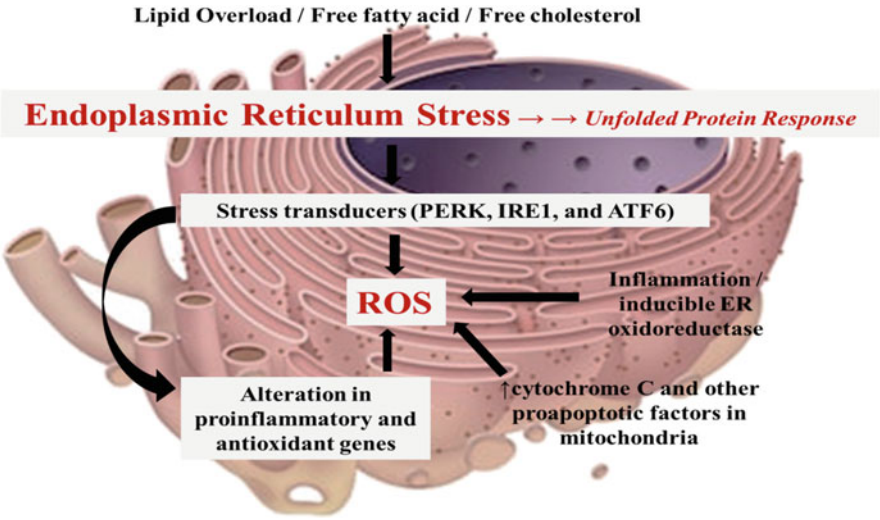


Fig. 13.4 Mechanism of endoplasmic reticulum stress for ROS generation

13.2.8 Endoplasmic Reticulum (ER) Stress

To maintain ER homeostasis, metabolism of the lipids is essential. Due to altered metabolism and glut of lipids, free fatty acids and cholesterol initiate ER stress. The stress further produces "unfolded protein response" (UPR) to regain ER homeostasis, as shown in Fig. 13.4. But extended and routine stimulation of UPR activates the proapoptotic and inflammatory pathway, leading to oxidative stress. The activity of UPR is arbitrated through various transducers like PERK (protein kinase RNA-like ER kinase), IRE1 (inositol-requiring signaling protein 1), and ATF6 (activating transcription factor 6). They regulate the balance of proinflammatory and antioxidant genes. Other factors like overexpression of inducible ERO1 (ER oxidoreductin 1) also increases the production of reactive oxygen species. ER stress induces SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) inactivity, depletion of calcium stores, and blockade of ETC (electron transport chain). All these cause an increased amount of cytochrome C and other proapoptotic factors in the cytosol, which can ultimately increase the level of reactive oxygen species.

13.2.9 Altered Gut-Liver Axis

Qualitative and quantitative alteration of normal intestinal microflora, namely, dysbiosis, may be involved in the growth and development of hepatic diseases like NAFLD (nonalcoholic fatty liver disease). Inappropriate inflammatory response due to bacteria and its product translocation, induces upregulation of pro-inflammatory mediators and NADPH oxidase system . Also, there are some endogenous alcohol

production by alcohol-producing bacteria. Normally, lack of inhibition of inflammatory response by NPRL-3 and -6 shows a decreased inflammatory response, inhibiting IL1β and IL18, whereas the activation of inflammasomes results in the cleavage of cytokine precursors (pro-IL1β, pro-IL18). Fecal microbiota transplantation (donor fecal solution administered to the intestinal tract of a recipient in order to alter gut microbial composition to confer a health benefit) can be the therapeutic option, along with coadministration of pre- and probiotics for intestinal dysbiosis and NAFLD.

13.3 Sources of Oxidative Stress and Their Physiological Outcomes

A huge number of oxidative stress inducers are inked till date. To list some of them, a brief description of the oxidative stress source, its stress induction pathway, the consequences, the effect on various cell organelles, and the liver disease produced is presented in Table 13.1 below.

13.4 Clinical Biomarkers of Liver Toxicity

Biomarkers have become a clinically essential diagnostic tool for various studies in the field of medical science, research, and clinical trial. It is appropriate and justified and provides a clinically relevant outcome for various treatments and populations, which can be used commonly and repeatedly. They can efficiently evaluate and measure normal biological processes (heart rate, temperature, blood pressure) and pharmacologic and pathogenic processes or act as a therapeutic indicator. In drug discovery, from preclinical to each stage of clinical trials, the role of the biomarker has been increasing drastically. As per the Food and Drug Administration (FDA) guidelines [11], a list of *conventional biomarkers* measured in serum/urine is used in preclinical and clinical screening for hepatotoxicity.

13.4.1 Alanine Aminotransferase (ALT)

It is primarily confined to liver. Its level in blood increases due to liver necrosis, heart injury, and skeletal muscle injury (necrosis). It is frequently used to estimate hepatocellular injury/necrosis.

13.4.2 Aspartate Aminotransferase (AST)

It is primarily found in the heart, brain, skeletal muscle, and liver. Due to its similarity with ALT, AST (less specific) is accountable for the metabolism (transamination) of aspartate. To distinguish DILI (drug-induced liver injury) from

Table 13.1 Source of oxidative stress and its consequences, system involved, and liver diseases

Source of oxidative stress	Pathways	Consequences	Effect on systems involved	Liver diseases	References
Alcohol	1. In hepatic cells, ethanol is oxidized to acetaldehyde with the help of NAD+- requiring enzyme. Further, in mitochondria, acetaldehyde is oxidized to acetate by aldehyde dehydrogenases (ALDH) 2. The microsomal ethanol oxidizing system (MEOS), CYP2E1 for the metabolism of alcohol 3. Catalase in peroxisomes also oxidize ethanol by utilizing hydrogen peroxide (H_2O_2)	↑ Level of ROS, ↓ level of SOD, GSH-Px, GRD, GSH, and GST	DNA damage, mitochondrial dysfunction, lipid peroxidation, protein adducts	Steatosis, steatohepatitis, liver cancer, alcoholic liver disease (ALD), fibrosis, cirrhosis	[5]
Obesity, diabetes, dyslipidemia, jejunoileal bypass, drugs, and parenteral nutrition	↑ Angiotensin II produces hepatic ROS generation; ↑ mitochondrial CYP2E1 expression causes redox state. Impaired β-oxidation may lead to the accumulation of fatty acids within hepatocytes and development of hepatic diseases	↑ in ALT, AST, ALP, MDA, and bilirubin;↓ in SOD, GSH, CAT, and GSH-Px	Altered uptake, synthesis, oxidation, and export of fatty acids →→ leads to excessive fat accumulation in liver	Nonalcoholic fatty liver disease (NAFLD), hepatic steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis with portal hypertension, hepatocellular carcinoma (HCC)	[6]

(continued)

Table 13.1 (continued)

Source of oxidative stress	Pathways	Consequences	Effect on systems involved	Liver diseases	References
Radiation, temperature, mobile phone, cold stress	Hepatic cells decrease in a concentration-dependent manner	↑ in MDA and total nitric oxide, ↓ in SOD, myeloperoxidase, and GSH-Px	Hepatic antioxidant status and ATPases were affected, ↑ in DNA adduct	Autoimmune cholestatic liver diseases (AC), autoimmune hepatitis (AIH), chronic liver disease	[7]
High-fat diet	↓ Expression of antioxidant defense genes, such as GSH-Px-1, Cu/Zn-SOD, and paraoxonase enzymes; ↑ COX-2 inhibitor and CDK4a inhibitor; and ↓ cyclin D1 and phosphorylation of retinoblastoma protein	↓ in CAT, SOD, and GSH-Px			
Ammonia	Astrocytes stimulated by ammonia activate NMDA receptors and calcium-dependent processes →→lower antioxidant enzyme activity →→astrocytic swelling in the brain	↑ Amounts of glutamine, neutrophil activation; ↑ production of ROS, oxidation of RNA; ↑ levels of protein tyrosine-nitrated proteins, heat shock protein-27, and 8-hydroxyguanosine	Mitochondria dysfunction, local and systemic inflammation/infection, impaired protein synthesis, and molecular disruptions in the brain	Hepatic encephalopathy (HE)	[8]

Alcohol, hepatitis B/C virus, cholestasis, iron overload	Injury of hepatocytes →→→ transformation of hepatic stellate cells to activated collagen-I-producing cells →→ characterized by scar accumulation and nodule formation	↑ TGF beta expression, ↑ release of profibrogenic GF, cytokines, and prostaglandins	Activation of the membrane-bound enzyme NADPH oxidase and alteration of complex IV in the mitochondrial respiratory chain (increased expression of the mitochondrial chaperone prohibitin)	Liver fibro-proliferative diseases	[9]
Hypoxia/ reoxygenation	Ischemia/reperfusion (I/R) →→ redox state →→ rise in xanthine oxidase and NADPH oxidase →→ leads to injury to liver cells	↑ ROS, rise in xanthine oxidase and NADPH oxidase	Altered mitochondrial electron transport chain, Kupffer cells, and neutrophils potentiate oxidative stress	Liver injury	[10]

extrahepatic organ injury and diagnose acute alcoholic hepatitis and cirrhosis, the ratio between serum ALT and AST is useful. It is frequently used to estimate hepatocellular injury/necrosis.

13.4.3 Alkaline Phosphatase (ALP)

It is found in liver and is a partially predictive marker of hepatobiliary injury, cholestasis, conventional biliary injury, along with xenobiotic-generated cholestasis in individuals. To diagnose cholestatic DILI, more than twofold independent elevation of serum ALP or an ALT:ALP ratio of not more than 2 is admissible.

13.4.4 Total Bilirubin (TBL)

It is taken up by liver, where it gets conjugated and secreted into bile. Its level indicates the rates of enzyme degradation and is a marker of hepatobiliary injury and liver function. It also increases due to hemolysis.

13.4.5 Gamma-Glutamyl Transferase (GGT)

It is localized in the kidney, liver, and pancreas. It gets elevated due to conventional biliary injury, alcohol intake, or heart disease. Gamma-glutamyl transferase value is proportional to ALP elevations, which are markers of hepatobiliary injury and cholestasis.

13.4.6 Albumin

It is one of main serum total proteins and decreases with chronic hepatic disease where liver fails to synthesize enough albumin, whereas the other serum protein, i.e., globulin, increases due to the production of acute phase proteins.

13.4.7 Ammonia

Liver usually converts ammonia to the less toxic urea, and its injury may lead to the elevation of ammonia in blood. End-stage hepatic disease and a high-risk coma in liver disease is indicated by an increased level of ammonia.

13.4.8 Cholesterol/Triglycerides

Cholesterol/Triglycerides increase in the blood due to failure of bile elimination as the liver fails to remove them from the bile ducts.

13.4.9 Clotting Time (CT)

It is not designated as a favorable and subtle biomarker. It increases with severe liver injury wherein liver fails to produce coagulation factors, leading to increased clotting time.

13.4.10 Urobilinogen

It is a colorless product of bilirubin reduction, similar to bilirubin. Its low level in urine may be due to biliary obstruction.

The biomarkers used may have varied pros and cons depending on their type, use, and availability. But to list some of the advantages, they are as follows: least invasiveness, minimal morbidity/ mortality, less cost, easy application and availability, and uncomplicated reproducibility. In addition, optimized biomarkers with scores are useful for treatment monitoring. On the other hand, the disadvantages are that some markers are not organ specific and have low sensitivity, making them incapable of distinguishing intermediate stages; the clearance rate of a biomarker can be influenced by impaired biliary and renal excretion; and most of the biomarkers need further validation.

13.4.11 Recent Advances

Apart from conventional and commonly used biomarkers, recent advances had provided us with novel therapeutics. They not only overcome hepatotoxicity, but they are also useful for the treatment and prevention of liver diseases. After the identification of hepatic diseases, few therapies of natural origin can be used for treatment. Some of them are discussed below.

13.4.11.1 Graft-Derived Cell-Free DNA

This can detect liver injury and can contribute to more effective, less toxic personalized immune suppression. Guanine-cytosine (Gc) DNA is one of the markers that can detect transplant injury (liquid biopsy) in early stages, which can in turn provide efficient therapeutic interventions.

13.4.11.2 MicroRNA

MicroRNAs (miRNAs) are endogenous and noncoding ribonucleic acids (RNAs) that are useful as a biomarker in both patho- and physiological processes. According

to some studies for viral-, alcohol-, hepato-carcinoma-, and xenobiotic-induced hepatic injury-, miRNA-122 (in plasma/serum) is an biomarker. Similarly, for xenobiotic-induced hepatic injury, biomarkers like miRNA-192 (in plasma/serum) and miR-291a-5p (in urine) are studied.

13.4.11.3 Alpha-Fetoprotein (AFP)

AFP is not only produced in the liver but is also found in the brain and kidney. This biomarker is used for the management of hepatocellular carcinoma (HCC)/necrosis. Its level rises up to 70% in HCC patients.

13.4.11.4 Cortisol

It is a metabolite of cholesterol estimated in urine as a cirrhosis biomarker. Adrenal cortex releases cortisol from the adrenal cortex, where the hypothalamus-pituitary-adrenal (HPA) axis monitors its secretion. It is bound to transcortin in the blood and maintains homeostasis. It is a predictive biomarker of oxidative stress and liver damage.

13.4.11.5 CD133 and EpCAM

CD133 and epithelial cell adhesion molecule (EpCAM) are advanced biomarkers for stem/progenitor cells in the hepatic cells. CD133 is recommended for intercellular communication. EpCAM has complex physiological functions like regulation of proliferation, cell-cell adhesion, migration, differentiation, and survival of cells.

13.4.11.6 Hydroxyproline and 5-Oxoproline

These are the intermediates in the synthesis of glutathione. These are the biomarkers of oxidative stress and glutathione status in diseases like viral hepatitis, bilharzia, metabolic disorders, and toxicity induced by drugs and chemicals. These are predominantly detected in urine and serum and are responsible for hepatic fibrosis, which is the most dangerous disorder of damaged tissues.

13.4.11.7 Ophthalmic Acid

It is a serum analog of glutathione produced along a similar biosynthetic route as glutathione. Hepatic toxicity due to oxidative stress followed by glutathione depletion can alter the levels of ophthalmic acid.

13.4.11.8 Pentraxin 3

Pentraxin (PTX3) is a prototype for the protein family that has a long pentraxin group (multifunctional pattern-identification proteins). It facilitates pathogen recognition and opsonic activity by the mechanism of innate immunity. Long-term rise in PTX3 is linked to increased morbidity and severity in various diseases such as psoriasis, atherosclerosis, angina pectoris, and ischemic heart disorders.

13.4.11.9 Fibrinogen α-Chain

Liver fibrosis is due to the assembling of extracellular matrix proteins. If the fundamental reason is not cured or eradicated, it may give progress to the ailment. Further, this leads to many medical complications, viz., hepatocellular carcinoma or

death. Thus, recognition, execution, as well as investigation of liver fibrosis are the major concerns in the detection as well as the healing of patients with chronic liver disease.

13.4.11.10 Soluble CD163

Kupffer cells get activated in various inflammatory circumstances that affect the liver. During inflammation/necrosis, due to oxidative stress in hepatic cells, the Kupffer cells get activated and can be identified by a specific serum biomarker, CD163. CD163 in high levels is observed in acute and chronic liver failure, as well as alcoholic hepatitis.

13.4.11.11 YKL-40

Patients with alcoholic liver disease and chronic hepatitis C show increased concentration of YKL-40 protein. It also plays an important role in cancer cell development, as well as survival. It participates in the inflammatory process of tumor, as well as angiogenesis. Patients with metastatic tumor (deficient diagnosis) have the highest serum level of YKL-40.

Some other biomarkers are cytokines, interleukin-1 (in plasma, produced by all liver cells but primarily by Kupffer cells and increases in cellular stress), glutathione *S*-transferase P-form (in serum, produced in the hepatocytes and found during hepatocellular injury), cytokeratin-18 (in serum, expressed by epithelial cells and found during apoptosis or necrosis), high-mobility group box protein I (in serum, found in a wide range of tissues and increases in necrosis and inflammation). Also, there are glutamate dehydrogenase (GLDH) in serum (found in the liver, kidney, and skeletal muscle; increases in hepatocellular necrosis), malate dehydrogenase in serum (found in mitochondria and the extra-mitochondrial compartment of hepatic cells, increases in hepatocellular necrosis), purine nucleoside phosphorylase in serum (present in the liver, mainly in the cytoplasm of endothelial cells and Kupffer cells; altered in hepatocellular necrosis), and paraoxanase-1 in serum (produced primarily in the liver but also found in the kidney, brain, and lungs; altered in hepatocellular necrosis).

13.5 Antioxidants for Liver Diseases

Antioxidants are endogenous or exogenous molecules that can prevent the oxidation process of any substrate, thus reducing the free radicals generated in our body. They maintain prooxidant/antioxidant cellular balance by donating electrons to free radicals. Many natural antioxidants are demonstrated for various liver diseases till date [12]. Antioxidants show their health benefits by various mechanisms, which include scavenging free radicals that can start the oxidation process, transition metal ion chelation that participates in redox reactions, O_2 dismutation, and breaking oxidative chain reactions. Antioxidants from plants are taken through diet and can maintain liver homeostasis. Below are the natural antioxidants discussed briefly for their putative benefits.

13.5.1 Green Tea

It is obtained from *Camellia sinensis* leaves and is used as a beverage because of its flavor and aroma. It possesses anti-inflammatory and antioxidant properties. Its antioxidant property is due to chemical constituent, viz., (−)-epigallocatechin-3-gallate, quercetin, caffeine, and chlorogenic acid in huge quantity.

13.5.2 Silymarin

It is obtained from Silybum marianum (mixture of flavonolignans and flavanoid), and has been used for the treatment of liver diseases from ages. Silybinin is hepatoprotective due to its iron-chelating properties.

13.5.3 Quercetin

Quercetin, mainly found in apples and onions, is a flavonoid with hepatoprotective, anti-inflammatory, heavy metal chelation, and antioxidant properties. Quercetin prevents transforming growth factor beta (TGF-b), connective tissue growth factor (CTGF), and collagen type I alpha 1 (Col-1a) expressions; decreases liver enzymes; and inhibits nuclear factor kappa B (NF-kB) and HSC activation. Also, SOD and CAT activity is improved by quercetin.

13.5.4 Naringenin

Naringenin mainly found in citrus fruits and tomato skin have anti-inflammatory, metal ion reductor, hepatoprotective, and antioxidant activities by increasing the level of SOD, catalase, and GSH.

13.5.5 Curcumin

It is obtained from the rhizomes of *Curcuma longa*, with anticarcinogenic, anti-inflammatory, and antioxidant properties. It is hepatoprotective in liver injury as it increases GSH, hydroxyl-1, SOD, CAT, and nuclear factor erythroid 2-related factor 2 (Nfr2), therefore strengthening the antioxidant system.

13.5.6 Coffee

It principally contains chlorogenic acid for health benefits such as chemoprotective, anticholestatic, and antioxidant properties. A dose-dependent inverse relationship was observed for coffee intake and liver cirrhosis.

13.5.7 Resveratrol

A phytoalexin is principally obtained from berries, grapes, and red wine and has anticarcinogenic, anti-inflammatory, hepatoprotective and antioxidant properties. It decreases TGF-b, NF-kB, LPO and reduces oxidative stress.

13.5.8 Melatonin

Although melatonin is an endogenous molecule, its use as an antioxidative, anti-inflammatory, and antiapoptotic has been explored now. It is a serotonin derivative with an ability to target both ROS and reactive nitrogen species. It has antioxidant power, which is much greater than that of vitamin E or C or glutathione. Melatonin at once can bind with two hydroxyl radicals to form cyclic 3-hydroxymelatonin (excreted in urine). It amplifies messenger RNA levels of superoxide dismutase and gamma-glutamylcysteine synthase to form glutathione and glutathione peroxidase [13].

13.5.9 Current Antioxidative Therapy in Clinical Trials

For advances in hepatic injury therapy, many antioxidants have been currently under clinical trial so as to develop a better antioxidative therapy. The information is obtained from the http://www.ClinicalTrials.gov website, as shown in Table 13.2. Dietary supplements like vitamin E, nutritional antioxidants (zinc and coenzyme Q10), plant flavonoids, and xenobiotics like silymarin, quercetin, Siliphos, selenium, resveratrol, metadoxine, ginger, green tea extract, *N*-acetylcysteine, methionine, propofol, alpha lipoic acid, mitoquinone mesylate, and chocolate are studied for numerous liver diseases. Sometimes natural source antioxidants that are highly effective in animal models do not show therapeutic benefits in humans. Thus, in the near future, continuous studies are required for all endogenous substances or bioactive compounds isolated from natural sources with huge patient number and long-time duration.

Table 13.2 Current antioxidative therapy for various liver diseases in clinical trial

Sr. no.	Condition/disease	Antioxidants	Location
1	Nonalcoholic fatty liver disease	MEDOX (natural purified anthocyanin)	China
2	Nonalcoholic fatty liver disease	Chinese bayberry juice	China
3	Liver cirrhosis, liver cancer	Dietary supplement: vitamin B-6, glutathione, and dextrins	Taiwan
4	NAFLD, prediabetes	Drug: metadoxine	Mexico
5	Nonalcoholic fatty liver disease	Dietary supplement: Mastiha	Greece
6	HIV infections, fatty liver	Antioxidant vitamin E, along with weight reduction and exercise	Canada
7	Hepatocellular carcinoma	Dietary supplement: vitamin B-6, coenzyme Q10, vitamin B-6 + coenzyme Q10	Taiwan
8	Hepatitis C, oxidative stress	Dietary supplement: antioxidant supplementation (vitamin E 800 mg, C 500 mg, and zinc 40 mg) for 24 weeks	Brazil
9	Nonalcoholic steatohepatitis	Dietary supplement: Protandim	United States
10	Chronic hepatitis C	Dietary supplement: Viusid (immunomodulator)	Cuba
11	Alcoholic hepatitis	Dietary supplement: omega-5 fatty acid supplement	Mexico
12	NAFLD in children	Dietary supplement: lycopene-enriched tomato juice and energy-restricted diet	Italy
13	Fatty liver in obesity	Long-lifestyle follow-up (FLiO diet) and diet control	Spain
14	Fatty liver disease in obese children	Dietary supplement: *N*-acetyl cysteine 600 mg once/day or twice/day	United States
15	Chronic hepatitis C and oxidative stress	Drug: silymarin Dietary supplement: green tea extract (EGCG)	United States
16	Liver and periportal fibrosis, oxidative stress	Dietary supplement: praziquantel + antioxidant	Ethiopia
17	Severe alcoholic hepatitis	Granulocyte-colony-stimulating factors (G-CSF), *N*-acetylcysteine	India
18	Cirrhosis	Dietary supplement: vitamin E supplement (tocofersolan)	Switzerland
19	Alcoholic hepatitis	*N*-acetylcysteine	Belgium
20	Chronic hepatitis C	Drug: mitoquinone mesylate (MitoQ)	New Zealand
21	Chronic hepatitis C	Orange juice	Brazil
22	Severe alcoholic hepatitis	Drug: prednisone plus metadoxine/ pentoxifylline plus metadoxine	Mexico
23	Nonalcoholic fatty liver disease	Drug: Lovaza	United States

(continued)

Table 13.2 (continued)

Sr. no.	Condition/disease	Antioxidants	Location
24	Fatty liver and nonalcoholic steatohepatitis	Drug: metformin Dietary supplement: Siliphos+ selenium − methionine + alpha lipoic acid	Mexico
25	Nonalcoholic steatohepatitis	Drug: vitamin D and lifestyle counseling	Italy

13.6 Conclusions and Prospects

Considering today's scenario of excess mental stress, environmental factors, and unhealthy lifestyle, oxidative stress is one of the main sinners inducing a variety of hepatic diseases in humans. To overcome all these problems, a better diagnostic tool is being used as intervention in the form of biomarkers, apart from our conventional diagnostic tools. These biomarkers can diagnose a disease with an effective and safe dose so as to provide very early and effective treatment. Also, in the near future, antioxidants of natural and synthetic origin that, through investigation, are proven to show efficacious absorption and bio-availability can be utilized. Natural antioxidants are cheap and are effective for many hepatic diseases, but more basic and clinical investigation is needed to identify the best compound by developing strategies to improve bio-disposition and toxicity research in this field.

References

1. Jones DP (2006) Redefining oxidative stress. Antioxid Redox Signal 8:1865–1879
2. Muriel P (2007) Some experimental models of liver damage. In: Sahu SC (ed) Hepatotoxicity: from genomics to in vitro and in vivo models. Wiley, West Sussex, p 119e137
3. Halina CL, Agata M (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
4. Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB (2014) Resources and biological activities of natural polyphenols. Nutrients 6:6020–6047
5. Valente MJ, Carvalho F, Bastos M, de Pinho PG, Carvalho M (2012) Contribution of oxidative metabolism to cocaine-induced liver and kidney damage. Curr Med Chem 19:5601–5606
6. Nguyen V, Huang J, Doan V, Lin X, Tang X, Huang Y, Tang A, Yang X, Huang R (2015) Hepatoprotective effects of Yulangsan polysaccharide against nimesulide-induced liver injury in mice. J Ethnopharmacol 172:273–280
7. Dornas WC, de Lima WG, dos Santos RC, Guerra JF, de Souza MO, Silva M, Souza e Silva L, Diniz MF, Silva ME (2013) High dietary salt decreases antioxidant defences in the liver of fructose-fed insulin-resistant rats. J Nutr Biochem 24:2016–2022
8. Skowronska M, Albrecht J (2013) Oxidative and nitrosative stress in ammonia neurotoxicity. Neurochem Int 62:731–737
9. Paik YH, Kim J, Aoyama T, De Minicis S, Bataller R, Brenner DA (2014) Role of NADPH oxidases in liver fibrosis. Antioxid Redox Signal 20:2854–2872
10. Elias-Miro M, Jiménez-Castro MB, Rodés J, Peralta C (2013) Current knowledge on oxidative stress in hepatic ischemia/reperfusion. Free Radic Res 47:555–568

11. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S (2008) The current state of serum biomarkers of hepatotoxicity. Toxicology 245:194–205
12. Casas-Grajales S, Muriel P (2015) Antioxidants in liver health. World J Gastrointest Pharmacol Ther 6:59–72
13. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z (2003) Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 50:1129–1146

Oxidative Stress and Inflammation Can Fuel Cancer

14

Foram U. Vaidya, Abu Sufiyan Chhipa, Nibha Sagar, and Chandramani Pathak

Abstract

Both oxidative stress and inflammation are interdependent cellular consequences of a biological defense system, which can fuel cancer and other pathophysiological provenience. In recent past, several emerging evidences showed that prevalence of oxidative stress and inflammation promotes multiple oncogenic events, including cell proliferation, angiogenesis, migration, metabolic reprogramming, and evasion of regulated cell death in cancer cells. Oxidative stress and chronic inflammation contribute to the progression of cancer in a unanimous pattern with significant cellular signaling response and outcomes. However, both oxidative stress and inflammation are also associated with the pathogenesis of several other diseases. The oxidative stress is an imbalance between oxidant and antioxidant defense system, which in turn damaging the macromolecules and dysregulation of complex casacde of cell signaling. Sustained oxidative stress can trigger chronic inflammation by activating the number of transcription factors such as NF-κB, AP-1, β-catenin/Wnt, p53, PPAR-γ, HIF-1α, and Nrf2. The activation of these transcription factors leads to altered expression of various genes and proteins including growth factors, cell cycle regulatory molecules, oncogenes, tumor suppressor genes, pro-inflammatory cytokines, and chemokines etc. Reprogramming of the cellular signaling cascade for self-survival is one of the key characteristics of cancer cells. In this chapter, we delineate the current knowledge and mechanistic interplay between oxidative stress and inflammation, which can fuel cancer.

F. U. Vaidya · A. S. Chhipa · C. Pathak (✉)
Cell Biology Laboratory School of Biological Sciences and Biotechnology, Indian Institute of Advanced Research, Gandhinagar Gujarat India
e-mail: cmpathak@iiar.res.in

N. Sagar
Clinical Genomics Center Mount Sinai Hospital, Toronto ON Canada

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_14

Keywords

Cancer · Oxidative stress · Inflammation · Cancer metabolism · Oxidative stress · Cell proliferation

14.1 Introduction

Oxidative stress and inflammation are both important physiological processes. The body has an endogenous defense system to combat both processes to maintain the homeostasis of cells. However, prolonged oxidative stress and inflammation are fatal components of a cellular defense system, and both are key causative factors for the onset of several diseases, including diabetes, cancer, cardiovascular and neurological disorders, premature aging, etc. Mechanistically, oxidative stress is a cellular imbalance of oxidants and anti-oxidants molecules , which are the causative factors for the origin of various pathophysiological consequences [1–3]. It was assumed that reactive oxygen species (ROS) like superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO•) are oxidants molecules those are generated during normal cellular physiological processes and metabolism [4, 5]. However, ROS are also generated intracellularly by intracellular enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOXs), family proteins as a primary product [6]. NADPH oxidases (NOXs) are one of the evolutionary conserved families of enzymes, which reduces molecular oxygen to free oxygen radical t [7]. At normal physiological condition, ROS generated by NOX participate in various normal cellular functions and are consequently metabolized by antioxidant enzymes. However, unmetabolized ROS leads to the perturbation of oxidative stress. Many reports suggest that a low concentration of ROS helps in coordination of various physiological processes, including immunity, cell growth, cellular signal transduction, regulation of cell proliferation, cell differentiation, and balanced gene expression for the maintenance of cellular homeostasis [8, 9]. On the contrary, accumulation of ROS tends to cellular damage and progression of various pathophysiological consequences has been suggested [10].

The steady-state formation of free radicals is balanced by cellular antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase [11]. These enzymes are known as antioxidant defense systems that balance the level of ROS. Imbalance between ROS production and its consumption by antioxidants enzymes culminates oxidative stress, which leads to the progression of various pathological conditions, including metabolic dysfunctions, cardiovascular diseases, neurodegenerative diseases, cancer, and premature aging [1, 4, 12]. It has been found that the accumulation of ROS inhibits the activities of antioxidant enzymes, which promotes tumorigenesis [13]. Accumulation of oxidative stress perturbates damage of biological macromolecules, including deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, and lipids, by oxidation and nitration reaction [14]. These events play crucial role in the progression of cancer [15, 16].

Since the recent past, several reports highlighted that antioxidants and oxidant scavenging systems play key role in the prevention of several diseases [17]. Interestingly, it is remarkable that most of the antioxidants have anti-inflammatory and other biological activities. Oxidative stress and inflammation, both have close connections to modulate cellular, physiological, immunological, and pathological response. Although, initially inflammation attributes ROS production for invasion of infections or unwanted cells, but prolonged inflammation contributes to the setting up tumor microenvironment for cell survival through the activation of transcription factors and multiple downstream signaling cascades that trigger the activation of pro-inflammatory genes and the onset of inflammation. Thus, oxidative stress and inflammation have an interdependent link to the progression of cancer. In this review, we provided insights of oxidative stress and inflammation derived molecular mechanisms that fuel progression of cancer.

14.2 Mechanism of ROS Generation: The Concept Behind Oxidative Stress and Cancer

In a broad context, oxidative stress is an outcome of disbalance between prooxidant and antioxidant mechanisms in that case the threshold level of free radicals progressively increases, which contributes damaging of cellular components and functions of the various genes, as well as cellular response that progresses pathophysiological consequences [18, 19]. The term ROS encompasses free radicals and their nonreactive intermediates. Free radicals are reactive species that consist of one or more unpaired electrons. The prevalence of these unpaired electrons makes free radicals highly reactive [19]. Reactive species are broadly classified into four types: reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive sulfur species (RSS), and reactive chloride species (RCS) [20]. The superoxide ion that is produced as a result of the reduction of molecular oxygen by one electron is dismutated to H_2O_2 by the enzyme superoxide dismutase (SOD) [21]. Subsequent reactions further result in the generation of hydroxyl ions (OH^-) in the presence of certain metal ions. In addition, molecular oxygen also reacts with nitric oxide (NO) to generate peroxynitrite ($ONOO^-$), which is a known oxidant, and the consequent loss of protective NO resulted emergence of pathological conditions[22]. Superoxide ions are the most commonly produced oxygen free radical under physiological conditions. The inefficient transfer of electrons across the respiratory chain allows the leakage of these electrons, and their subsequent interaction with molecular oxygen results in the formation of superoxide ions (O_2^-). These highly reactive radicals are most commonly found in cancer cells. Oxidative stress promotes hypoxic environment in the cells favor an impaired gene regulation and functions. Hypoxic conditions (low intracellular oxygen) also result in an overwhelming production of ROS inside the cells, which is an evident from the extensively studied in cancer cells [23]. This paradoxical increase in ROS in hypoxic cells occurs due to the accumulation of electrons in the mitochondria under low oxygen concentrations, which fail to accept the electrons in complex IV during the electron transport chain [24]. The major

internal sources of oxidative stress are peroxisomes and other enzymes, including xanthine oxidase and NADPH oxidase. Along with these, oxidative stress can also be generated through external sources, which include ultraviolet (UV) radiation, chemicals, unhealthy lifestyle, and environmental factors [25].

An emerging evidence suggests that reactive species are capable of causing nicks in DNA molecules and negatively influence the normal process of DNA repair [26]. Furthermore, the oxidation of DNA by free radicals aids in the accumulation of mutation, which promotes carcinogenesis. In addition, elevated levels of reactive species in cancer cells significantly affect intracellular proteins, causing their structural deformities and compromised integrity [27]. Moreover, the chronically elevated levels of reactive species (ROS) also act as secondary messengers to modulate multiple signaling pathways in cancer cells [28]. A key feature of malignant cells is their adaptation to a chronically elevated oxidative stress, which has been utilized for self survival and proliferation. Interestingly, the biology of these events is largely governed by the activation of oncogenes and inactivation of tumor supressor genes, which phenomenon direct cancer cells toward the survival signaling pathways. Generally, cancer cells are known to possess low level of antioxidant enzymes, which tend to accumulate a relatively higher amount of ROS as compared to the normal cells, which apparently aid in modulating multiple signaling pathways for cell prolifeartion and survival [29]. Elevated oxidative stress in cancer cells has been shown in angiogenesis, migration, metabolic reprogramming, and evasion of cell death [30]. ROS have been considered as a double-edged sword that acts as both protumorigenic and antitumorigenic [31]. An increased level of ROS above the sublethal stage drags cancer cell toward programmed cell death. Cancer cells cautiously maintain a relatively higher oxidative stress below the lethal levels to sustain their proliferative signaling pathways [32]. The consequence of elevated oxidative burden in cancer cells allows to maintain an intracellular tumor microenvironment, which supports invasion and metastasis [27]. Therefore, the regulation of oxidative stress in cancer cells may be advantageous for cancer prevention and therapy.

Metabolic reprogramming is one of the hallmarks of cancer cells [33]. The bizarre behavior of cancer cells of shifting toward the less energy-efficient aerobic glycolysis pathway and sidelining an energy-efficient oxidative phosphorylation pathway, known as "Warburg Effect," for its energy production still remains a large enigma in cancer biology [33, 34]. Metabolically, cancer cells furnish hypoxic environment and progress anaerobic metabolism to produce lactate even in the presence of sufficient oxygen that may liberate to utilize as an energy source [35, 36]. An elevated level of lactate and lactate dehydrogenase are most common in many types of tumors [37]. Metabolic enzymes lactate dehydrogenase A, and pyruvate kinase M2 has been considered as poor prognosis markers of cancer [38]. Pyruvate kinase (PK) is a glycolytic enzyme that catalyzes the conversion of phosphoenol pyruvate into pyruvate. It has two isoforms: PKM1 and PKM2; PKM1 exists as a tetramer, while PKM2 exists as tetramer or dimer.The tetrameric and dimeric form of PKM2 is allosterically regulated by various enzymes and metabolites in the cancer cells [39]. Cancer cells preferably express the M2 isoform of PK. [40]. In the event of cancerous signaling, the PKM2 isoform is converted from its active tetrameric form

to an inactive dimeric form and acts as a transcription factor to execute a number of nonmetabolic functions, such as cell proliferation, cell cycle regulation, and evasion of cell death in tumors [41]. The inactivation of PKM2 through its conversion from tetrameric to dimeric form is an oncogenic event. Oxidative stress is also known to regulate the inactivation of PKM2 in cancer cells [42]. Moreover, dysfunctional mitochondria are also associated with altered metabolism and oxidative stress in cancer. As an example, mitochondrial SIRT3 is one of the negative regulators of aerobic glycolysis. SIRT3 destabilizes hypoxia inducible factor 1α (HIF1α) and prevents glycolytic switch in cancer cells. Loss of SIRT3 in cancer cells augment intracellular ROS. These escalated levels of ROS stabilize HIF1α and promotes aerobic glycolysis to reprogram glucose metabolism in cancer cells [43]. Moreover, the increased rate of pentose phosphate pathway (PPP) as a result of increased glycolytic flux allows the scavenging of ROS through NADPH produced during PPP, thus preventing sublethal ROS accumulation in the cancer cells [44]. As a result, increased oxidative stress provides a favorable environment for metabolic reprogramming without causing any severe cellular damage per se.

14.2.1 Oxidative Stress and Cell Proliferation

Cell proliferation is the complex process by which cells increase their number. Cancer cells retain their ability to consistently proliferate by alteration of complex signal transduction pathways of programmed cell death (apoptosis, autophagy and necrosis). The intricate reprogramming of cellular events in the transformed cells occurs to mainatin limitless proliferative potential and evasion of a programmed cell death. Although, high oxidative stress is also responsible for stimulation of oncogenic signaling in cancer cells. In the context of cell proliferation, escalated oxidative stress provide an ambient environment for invasion and metastsis in the cancer cells [45, 46]. In recent past, several reports advocate that antioxidants inhibit the proliferation of cancer cells [47, 48]. Moreover, mitochondria are the principal source of ROS and are acknowledged to regulate both cell proliferation and quiescence [49]. It has been shown that the levels of mitochondrial superoxide dismutase (SOD) determine the regulatory functions of mitochondrial ROS. Low levels of SOD allows the proliferation of cancer cells [50]. The levels of ROS vary significantly during the different phases of cell cycle [51]. In addition, ROS are also reported to increase the expression of various cyclins (cyclin B2, cyclin D3, cyclin E1, and cyclin E2) and hasten the G1 to S phase transition during the cell cycle in cancer cells [52–54]. Supportively, the treatment of cancer cells with antioxidants decreases the levels of different cyclins and subsequently delays the G1 to S phase transition [55]. Furthermore, concentrations of cyclin D1 further maintain ROS to sublethal levels, seemingly to sustain their own levels in cancer cells [56]. On the contrary, higher levels of endogenous antioxidant molecules (MnSOD, Cu/ZnSOD, catalase, and glutathione peroxidase) negatively regulate cell proliferation [57]. The levels of ROS are also associated with the mutation of various proto-oncogenes. Ataxia telangiectasia mutated (ATM) is a tumor suppressor gene, which gets

activated in response to high oxidative stress [58]. Mutations in ATM genes substantiate the presence of a higher degree of oxidative stress in cancer cells, while treatment with antioxidants has been reported to prevent cell proliferation in ATM knockout conditions [10]. Similarly, mutations in P53, a widely acknowledged tumor suppressor gene, results in the loss of its tumor-suppressing properties. Moreover, the mutant p53 was further observed to have an additional capacity to promote cancer cell proliferation [59]. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a regulatory protein that governs the expression of various antioxidant proteins to protect cells from excessive damage from oxidative stress [60]. Mutant p53 interacts with NRF2 to partly activate its transcriptional functions to maintain a sustained high oxidative stress (below lethal levels) to promote cancer cell proliferation [61, 62]. Another protein, Bcl-2 (a regulator of apoptosis), inhibits cell proliferation by reducing the G1 to S phase transition [63]. Under oxidative stress, the M2 isoform of pyruvate kinase (PKM2) translocates to mitochondria and phosphorylates the Bcl2 protein, followed by the subsequent stabilization of its phosphorylated form. These interim alterations at molecular level allows the cancer cell to evade apoptosis and preserve its proliferative capacity under high oxidative stress [64].

Although, functional relationship of ROS in context to cell death and survival signaling is an emerging and seeking extensive efforst to resove the molecular mechanism. It has been assumed that disparity in the functions of ROS (pro- or antitumorigenic) may depend up on their intracellular concentrations, which ultimately define the fate of a cancer cell in adopting either a cell survival or a cell death pathway [59]. Adaptation to high oxidative stress is a key characteristic of a cancer cell. However, these high levels of oxidants are meticulously maintained to ensure the ultimate presence of ROS below lethal levels. These scrupulously maintained levels of ROS (sublethal levels) alters the function of many genes and proteins in cancer cells, which helps in continous cell prolifeartion and reprogramming of cancer cell metabolism [10]. Thefrefore, shifting ROS concentration toward their lethal levels was employed by using number of anticancer agents and regulatory proteins to induce apoptosis in cancer cells [60]. Since the last decade, several synthetic and natural derivatives have been identified as anticancer agents. Many reports revealed that antiapoptotic and proapoptotic proteins have an imperative role in oxidative stress and cell death signaling. It is important to note that as P21 is a cyclin-dependent kinase (CDK) inhibitor that regulates cell cycle arrest. Apart from this, recent reports suggest that p21 also induces cell death in a P53-independent manner [62]. However, P21 induces apoptosis by increasing mitochondrial ROS in cancer cells [61, 63]. Chronic exposure to favorable ROS levels promotes p21 and TP53 expression to induce cell senescence [64]. Furthermore, high oxidative stress further cooperates with P53 to switch senescence to apoptosis [32].

14.2.2 Oxidative Stress in Invasion and Metastasis

Invasion and metastasis are the two events that propel the migration of cancer cells from their site of origin. While invasion accounts for the spread of cancer cells in surrounding tissues and metastasis is the movement of cancer cells toward distant

organs [65, 66]. Cancer cell migration progress the advanced stages of cancer and metastsis has been generally accounted for the highiest mortality rate in population [67]. Cancer cell migration is the result of intricate signaling pathways and involves complex dysregulated communication between different malignant and nonmalignant cells [68]. The mechanisms of cancer migration are governed by various transcription factors, including (but not limited to) NF-kB, Zeb, HIF-1α c-MYC, STAT, E2F1, Snail, ETS-1, Twist, AP-1, metalloproteases (MMP-9 and MMP-2), chemokines or cytokines like transforming growth factor beta (TGF-β), etc. [23]. A recent report from Wang and Unternaehrer suggested that epithelial to mesenchymal transition (EMT) is an important mechanism by which solid tumors become metastatic and also acquire self renewal capabilities like stem cells (known as cancer stem cells) that induce resistance, replicative immortality and invasiveness.[69–71]. However, metastasis itself is a complex phenomenon, which consist changes in a cancer cell and the stromal cells those events help to create tumor micro environment by elevating the level of pro-inflammatory cytokines and chemokines, which has been released by cancer cells or by the tumors themselves [72]. Several reports advocate that ROS and oxidative stress play a major role in the process of EMT [73, 74]. ROS influence the activities of a majority of transcription factors (mentioned above) involved in cancer metastasis. ROS induced oxidative stress is associated with TGF-β-induced regulation of MMP-9 and urokinase-type plasminogen activator to promote cancer cell migration [23]. The expression of MMPs is also increased by hypoxia signaling, which has been modulated by oxidative stress in cancer cells [75]. Hypoxia signaling also regulates the expression of vimentin, a protein largely responsible for the initiation and progression of cancer cells and it is substantially involved in the cancer cell migration [76]. Although, ROS are also responsible for the epigenetic changes in the promoter region of a number of tumor suppressor genes and EMT markers including E-cadherin and Snail [77]. Thus, oxidative stress significantly contributes to the progression of malignancy in cancer patients.

Angiogenesis is the formation of new blood vessels from an existing vasculature [78]. An accumulation of ROS turns oxidative stress and constitutive tumor microenvironment favor cancer cells to release various angiogenic factors [79]. Vascular endothelial growth factor (VEGF) is a key protein that regulates the formation of new blood vessels. It stimulates the permeability and tube formation of endothelial cells through its receptor protein (VEGFR2). The stimulation of VEGF expression is mediated by a number of signaling pathways [80]. NADPH oxidases are a group of enzymes, which are known to involve in the production of ROS in neutrophils and macrophages. They catalyze the production of ROS in response to infection and help in providing a first line of defense to infection [81]. ROS play a significant role as a secondary messenger for regulation of various cellular signaling and physiological process in the cells. The deliberate production of ROS attribute to sustain these pathways is undoubtedly perceivable in cancer cells. For instance dysregulated function of NOX family proteins in cancer cells, serve as a reservoir of ROS to augment cell survival signaling [81]. Recent report suggests that ROS regulate angiogenesis through VEGF [82]. Endothelial cell proliferation via VEGF and angiopoietin-1 (Ang1) signaling considerably depends upon the increased levels of

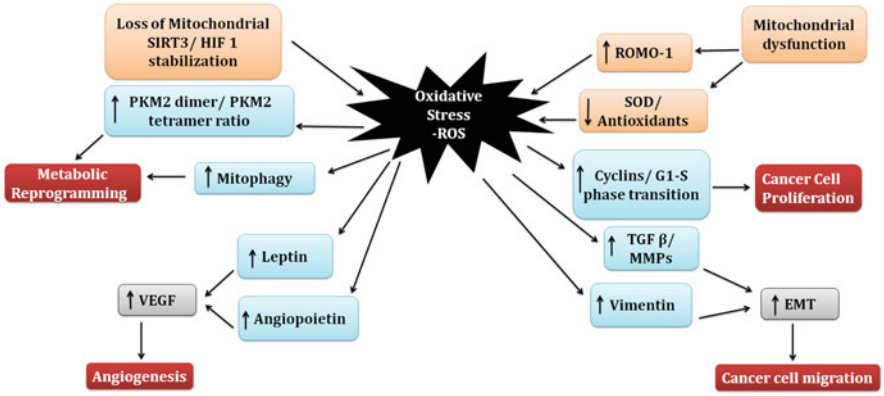


Fig. 14.1 Schematic representation of cellular events mediated by ROS signaling in cancer cells. *Metabolic reprogramming*: loss of mitochondrial SITR3 functions leads to HIF1 stabilization, which tends to increased ROS generation in cancer cells. Elevation in intracellular ROS results in increased mitophagy, leading to decreased oxidative phosphorylation and increased glycolytic flux. High ROS also increase the dimeric PKM2 in cancer cells, thus further supporting aerobic glycolysis (Warburg effect). *Cell proliferation*: compromised mitochondrial integrity results in low levels of SOD or other antioxidants, along with manifested reactive oxygen species modulator 1 (ROMO1) expression allow to increase ROS levels in cancer cells. Sustained oxidative stress increases the expression of various cyclins involved in cell proliferation. *Cancer migration*: ROS increase the expression of MMPs through TGF β. Furthermore, ROS also mediate the expression of vimentin. The expression of MMPs and vimentin increase EMT and promote cancer cell migration. *Angiogenesis*: oxidative stress increases the expression of leptin and angiopoietin, which further increase VEGF expression (a nodal protein responsible for angiogenesis)

ROS in tumor cells [22]. Leptin is another angiogenic factor that stimulates VEGF expression through a ROS signaling pathway [83]. Taken together, ROS direct diverse sets of signaling pathways in cancer cells to induce the expression and secretion of VEGF and other angiogenic factors to facilitate the process of angiogenesis and invasion in cancer. The schematic representation of different cancerous events mediated by oxidative stress has been shown Fig. 14.1.

14.3 Inflammation and Cancer

The link between inflammation and cancer was established for the first time in 1863, when Rudolf Virchow suggested that the "lymphoreticular infiltrate" reflected the origin of cancer at sites of chronic inflammation [84]. Since then, inflammation has been considered for provenience of cancer. However, its definitive roles remain to be elucidated. Inflammatory mediators can trigger cell proliferation and invasion by providing bioactive fuel molecules such as (1) growth factors to sustain proliferative signaling, (2) survival factors (downregulation of proapoptotic and upregulation of antiapoptotic) that restrict programmed cell death, and (3) extracellular matrix-modifying enzymes and proangiogenic factors that facilitate invasion, angiogenesis,

and metastasis. Tumor inflammatory microenvironment subsequently promote epithelial to mesenchymal transition for invasion and angiogenesis [85, 86]. Since the recent past, several studies have demonstrated that during the progression of cancer, inflammatory mediators such as pro-inflammatory cytokines and chemokines directly or indirectly play significant role in the activation of various sets of genes and regulatory proteins to evade regulated cell death signaling and compromise immune response for continuous cell proliferation [86–89]. Although , activated immune cells, i.e., macrophages, can release reactive molecules, especially reactive oxygen species (ROS), which are actively mutagenic for the neighboring cancer cells and accelerating their genetic evolution toward states of heightened malignancy [10, 90]. An inflammation associated cancer caused by microorganisms, e.g., *Helicobacter pylori*, triggers chronic inflammation and heightened the risk of colon cancer [91, 92]. Chronic hepatitis caused by hepatitis B and C viruses can progresses hepatocellular carcinoma, which is the third leading cause of cancer and has high mortality rate globally [93, 94]. Schistosomiasis, human herpes virus, and Kaposi sarcoma are also causative agents of inflammation-associated cancer [95, 96]. Considerable evidence suggests that more than 20 types of cancers arise from chronic inflammation. Inflammatory diseases increase the risk of development of various types of cancers, such as cervical, gastric, bladder, esophageal, intestinal, thyroid, ovarian, and prostate cancers [97, 98]. Altered expresion of various genes and proteins, including TNF-α, TGF-β, IL-1α, IL-1β, IL-6, IL-8, IL-18, MMP-2, MMP-7, MMP-9, VEGF, COX-2, iNOS, prostaglandins, and chemokines, are associated with cellular signaling of inflammation and cancer [99]. The expression of all these molecules is mainly regulated by transcription factors NF-κB and STAT3, which are constitutively activated in many types of tumors [100]. NF-κB, in turn, is activated by inflammatory stimuli like TNF-α and IL-1 or through the sensing of "danger signals" (pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)) by Toll-like receptors (TLRs) or nucleotide oligomerization domain 1/2 (NOD1/2) [101]. Further, the members of a NOD-like receptor (NLR) family assemble into a multiprotein complex called inflammasome and activate caspase-1 to cleave pro-IL-1β and pro-IL-18 into their mature form, which is highly secreted by the cancer cells [102]. The cellular components involved in chronic inflammation that fuel cancer are depicted in Fig. 14.2. An interplay between oxidative stress and inflammation may have a close connection in reprogramming of cellular events in cancer cells.

14.4 Oxidative Stress and Inflammation

Oxidative stress can trigger inflammation, and simultaneously, the inflammatory mediators also aid to percieve oxidative stress. In order to evade the pathogen, inflammatory cells such as macrophages and neutrophils produce an excessive amount of reactive oxygen and recative nitrogen species (ROS and RNS) such as hydrogen peroxide, superoxide, hypochlorous acid, hydroxyl free radical, peroxynitrite, and nitric oxide. Further, during a pathological inflammatory

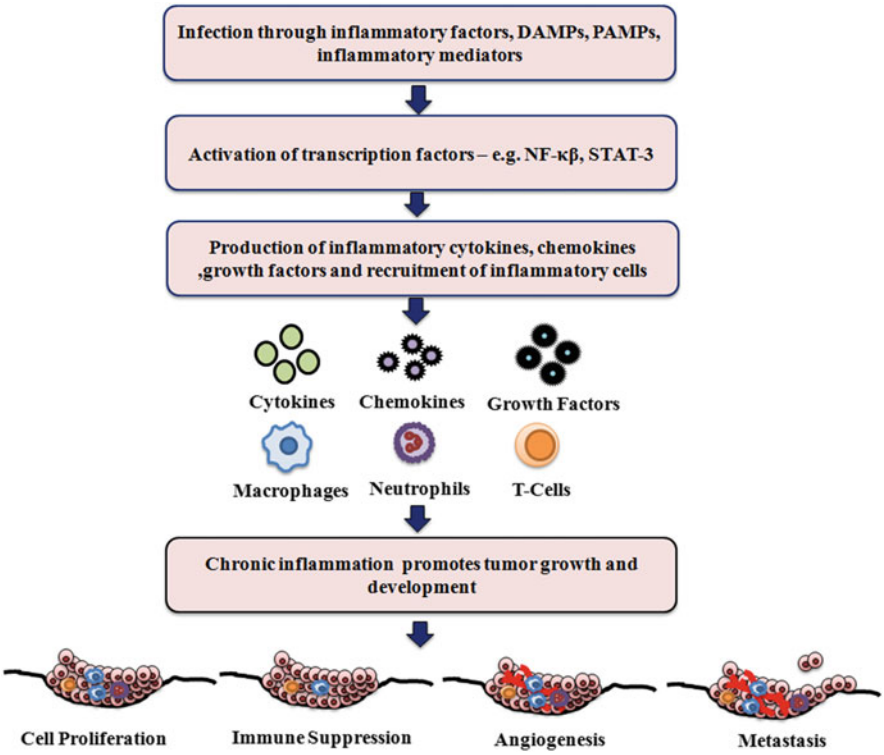


Fig. 14.2 Schematic representation of cellular components involved in chronic inflammation and cancer

condition, the release of reactive species from phagocytic cells can stimulate localized oxidative stress [103]. An excessive ROS production during the process of oxidative metabolism has been reported to contribute synthesis and secretion of pro-inflammatory cytokines by activating nuclear factor-kappa B/active protein-1 (NF-κB/AP-1), as well as the production of tumor necrosis factor-alpha (TNF-α) [104]. Oxidative stress can activate transcription factors such as NF-κB, AP-1, β-catenin/Wnt, HIF-1α, p53, PPAR-γ, and Nrf2. The activation of these transcription factors results in the expression of a number of different genes associated with inflammatory cytokines, growth factors, cell cycle regulatory molecules, chemokines, and anti-inflammatory molecules. Oxidative stress also plays a vital role in the activation of NOD-like receptor protein 3 (NLRP3) inflammasome [105]. The NLRP3 inflammasome is a multimeric molecular complex that initiates innate immune response by releasing pro-inflammatory cytokines such as IL-18 and IL-1β [106]. Moreover, a number of studies reported the role of mitochondrial ROS in the regulation of inflammatory signaling. The impaired mitochondria function is also associated with the release of ROS, which leads to the activation of the NLRP-3 inflammasome complex and subsequent pro-inflammatory cytokine secretion, that

event is associated with localized inflammation [107, 108]. Apart from these, the level of antioxidants also plays a significant role in regulation of inflammatory response. Several natural anti-oxidants i.e. curcumin, quercetin are known to have anti-inflammatory response and biological activites agisnt inflammatory diseases and cancer. Recent report revealed that Glutathione (GSH) is an intracellular tripeptide, which is a master antioxidant that protects the cells from oxidative damage. Upon downregulation of GSH, ROS production gets increased, which results in inflammation, imbalanced immune response, as well as higher susceptibility to infection was reported [109]. Further, studies also suggest that DNA-based modification induced by ROS also triggers inflammation by the activation of the NF-kB pathway [110]. Importantly to understand the insght molecular mechanism it is an important to identify the key molecules and pathways those can connect oxidative stress and inflammation in cancer. Here we have emphasized key molecules and associated molecular mechanisms, which can help to understand cellular consequences of inflammation and oxidative stress in cancer.

14.4.1 Cytokines

Cytokines are considered as important biological molecules of the immune system. Earlier they were considered as secretory proteins thoese were involved in the regulation of immune and cellular response to maintain the homeostasis of cells [111]. Later on, they were considered as key players in inflammation and various pathological conditions [112]. Cytokines are low molecular weight proteins those are responsible for modulating immune response and cellular signaling network to define the fate of cells [113]. They are synthesized by immune cells and stromal cells such as fibroblasts and endothelial cells. Cytokines are involved in the regulation of proliferation, differentiation, cell migration, cell survival, immune cell activation, and programmed cell death [114]. Cytokines stimulate antitumoral response at initial stage; however, prolonged secretion favors chronic inflammation, which leads to the development of a tumor microenvironment that promotes cell transformation and malignancy [86]. Thus, they are linked to tumor-promoting inflammation. Inflammatory cytokines include tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), IL-6, IL-10, IL-1β, IL-17, and IL-23, and the pathways utilized by these cytokines are NF-κB and STAT3 signaling pathways [115]. There are several types of pro-inflammatory cytokines (TNF-α, IL 1β, IL-6, IL-18) and anti-inflammatory cytokines (TGF β, IL-10, etc.), which display distorted expression levels at tumor sites [116]. The emerging reserach revealed that the altered expression of cytokines are associated with the progression of malignancies [117]. Moreover, the interplay between ROS and cytokines also significantly contributes to oxidative stress and tumor progression. As aforementioned, the chronic perpetuation of oxidative stress is a prerequisite for the survival of cancer cells. Cytokines are integral components secreted by immune cells and play crucial roles in maintaining this oxidative stress by regulating the nuclear transcription factors that are largely responsible for activation of various genes and proteins as well as generation of

reactive species in normal and cancer cells. Interferon γ (INF γ), a pro-inflammatory cytokine, which has been found to induce the activation of NOX and dual oxidase 2 (DUOX-2) enzymes (the intrinsic enzyme systems that produce ROS) in cancer cells [118]. In addition, inflammatory cytokines inhibit NAD(P)H:quinine oxidoreductase-1 (NQO1) enzyme in cancer cells to induce the consequent oxidative stress [119]. High levels of TNF α and ROS are also positively associated with incidences of cachexia in cancer patients [120]. Taken together, cytokines act as regulatory molecules to ensure sustained oxidative stress in cancer cells.

14.4.1.1 Interleukins

Interleukins are a diversified multifunctional group of proteins that facilitate communication between different immune cells and regulate various cellular functions, including inflammatory response, cell proliferation and differentiation, as well as gene expression. More than 38 interleukins have been identified and considered as pro- and anti-inflammatory cytokines. Some interleukins may reflect pro- and antitumorigenic response. The most considered pro-inflammatory interleukins are IL-1β, IL-3, IL-6, IL-8, IL-17, and IL-19 [121, 122]. In agreement, IL-1β participates in inflammation and cell death signaling processed by caspase-1 mediated activation of inflammasome [106]. Recent reports suggest that IL-1β is associated with the progression of cancer, angiogenesis, pro-inflammatory and pyrogenic activities, as well as inducible nitric oxide synthase (iNOS)-dependent stimulation of NO generation [112, 123]. IL-1β is secreted by immune cells to amplify the immune response. .Next, IL-3 is a monomeric cytokine that has an imperative role in regulating the production and differentiation of hematopoietic stem cells and myeloid progenitor cells [124]. This interleukin is primarily secreted by activated T cells, eosinophils, basophils, and mast cells. A recent report highlighted that IL-3 promotes cell proliferation and the migration of endothelial cells and vascular smooth muscle cells [125]. In addition, it has been shown to inhibit osteoclast and osteoblast formation [126, 127].

Interleukin-6 is a pro-inflammatory cytokine that promotes cell proliferation and inhibits apoptosis. It has protumorigenic effect [128]. IL-6 binds to its receptor IL-6Rα and coreceptor glycoprotein 130 (gp 130), which activates the JAK (Janus kinases)/STAT (signal transducers and activators of transcription) signaling pathway [129]. STAT is a transcription factor, which has been found activated during progression of cancer IL-6 induces the phosphorylation of both STAT1 and STAT3 for tumorigenesis. Several reports suggest the involvement of IL-6 and STAT3 in the survival and continous proliferation of cancer cells [130, 131]. Recent report also suggest that the IL-6/JAK/STAT signaling pathway contributes to cancer initiation and progression [132]. Previous report demostrate IL-6 is predominantly produced by stromal fibroblasts in a gastric cancer mouse model [133], and it can provoke tumorigenesis through hypermethylation of tumor suppressor genes and hypomethylation of retrotransposon long interspersed nuclear element-1 (LINE-1) in oral squamous cell cancer lines in vitro [134]. IL-6 protects tumor cells from apoptosis by activating the genes those are involved in the progression of cell cycle and the suppression of apoptosis. IL-6 has also been

shown to act as an autocrine growth factor for tumors [135]. IL-6 has been attributed an essential role in the initiation and maintenance of chronic inflammation in the colon cancer [136]. Trans-signaling of IL-6 is associated with JAK/STAT and the MAPK pathways and has an important role in development of inflammation-induced colon cancer [137].

Further, interleukin-23 and IL-12 are also key players in tumor formation. They are pro-inflammatory cytokines involved in tumor progression. The receptors for both were primarily expressed in natural killer T cells and T cells. They are also present in macrophages, monocytes, and dendritic cells. Upon bacterial infection, IL-23 and IL-12 are produced primarily by activated antigen-presenting cells. IL-23 can locally suppress the presence of CD8T cells and thus reduces antitumor immunosurveillance in the local tumor microenvironment [138].

IL-17 is yet another cytokine that has been implicated recently in pro-inflammatory and tumorigenic response. The production of IL-17 is triggered downstream of IL-23 via the STAT3 pathway [139]. IL-17 in turn induces the production of TNF α and IL-6, which are known protumorigenic effector molecules [140, 141]. Recent report highlighted that cervical cancer display an elevated levels of IL-17 and IL-6, as well as inflitration of neutrophils and recruitment of macrophages at the tumor sites [142]. Thus, IL-17 and IL-6, both might work together in promoting tumor development. Moreover, IL-17 provokes angiogenesis and potentiates tumor growth [143]. IL-17 also regulates neutrophil chemotaxis [144] and produces CD8 and CD4+ T cells, those are largely found in mouse and human tumor microenvironments [144, 145]. Recent report suggest that IL-17 has a key role in the immunosurveillance of tumors, as shown in immunocompetent mice [146]. The interplay between interleukins and ROS also has a pivotal role in cancer progression by modulating a complex cascade of cellular signaling. Interleukins regulate the level of ROS, and consecutively oxidative stress also modulates the expression of interleukins [147]. Oxidative stress is also known to regulate the expression of cell surface protein ICAM-1 (intracellular adhesion protein 1, CD54) and interleukin-8 (IL-8). A current report suggests that H_2O_2 activates the NFκB-inducing kinase (NIK) and subsequent NIK-mediated phosphorylation of IKK, which extensively influences IL-1β-mediated NF-kB activation, which facilitates cell survival and tumor progression [148].

Interleukins direct cellular response in an autocrine and paracrine manner [149]. Most of them communicate their signals by Janus kinase-signal transducer and activator of transcription signaling pathway [150]. Most of the interleukins direct signal by binding their own receptors (IL-R), those are expressed on the surface of the target cells and participates in signal transduction directly [151]. Therefore, inhibition of interaction between ILs and their receptors could be potentail target for cancer therapeutics.

14.4.1.2 Tumor Necrosis Factor-α

TNF-α is one of the pleiotropic inflammatory cytokines that is expressed by monocytes, macrophages, and T cells [152, 153]. The activity of TNF-α is associated with various cellular responses, including cell proliferation, differentiation, cell

survival, and cell death [154]. More commonly, it has been shown to play a significant role in pathophysiological conditions, including chronic inflammatory diseases, angiogenesis, and cancer [155]. TNF-α can stimulate pro- and antiapoptotic signals in tumor cells, macrophages, endothelial cells, and the majority of other cells inside the tumor microenvironment [152]. It defines the fate of cell death or survival through its receptor-mediated signaling. TNF-α directs various cellular response by interacting with their receptor, known as tumor necrosis factor (TNF) receptor (TNFR). Binding these cytokines to their receptors leads to the activation of multiple signal transduction pathways [156]. TNF-α mediates its effects via two different receptors, that is, TNF-α receptor I (also called p55 or p60) and TNF-α receptor II (also called p75 or p80). TNF-α receptor I is ubiquitously expressed, while TNF- α receptor II is expressed specifically in endothelial and immune cells [157, 158]. Even though both receptors bind to the TNF, TNFR-1 is the prime receptor that mediates its cellular effects in a majority of cell types [159]. The binding of TNF to TNFR-1 forms a homotrimer and recruits TNFR-associated death domain (TRADD) by the homologous binding of the DDs of both proteins. TRADD recruits downstream adaptor proteins such as TNFR-associated factor 2 (TRAF-2), receptor-interacting protein (RIP), and Fas-associated death domain (FADD), which further recruit key molecules that are responsible for intricate signaling response to activate mitogen-activated protein kinases (MAPKs), NF-κB, and cell death, respectively [160]. The binding of ligand to TNFR1 activates an array of growth factors and inflammatory mediators by the activation of the AP1 transcription factors or IκB kinases (IKKs), which ultimately activate nuclear factor-κB (NF-κB). The activation of the inhibitor of κB (IκB) kinase (IKK) initiates the TNF-induced NF-κB activation. TNFR-1 recruits the IKK, which is activated by a RIP-dependent mechanism that involves TAK1, MEKK3, and TAB2 [161, 162]. The activated IKK phosphorylates IκB, which holds NF-κB in the cytoplasm and triggers polyubiquitination and, subsequently, degradation by the 26S proteasome. This results in the translocation of NF-κB in the nucleus. NF-κB is an inducible transcription factor, and after translocation into the nucleus, it initiates the transcription of its target genes such as IEX-1L, A20, cIAP-1, Bcl-xL, cIAP-2, and XIAP, which are the negative regulators of apoptosis [163]. NF-κB also induces antioxidant manganese superoxide dismutase (MnSOD) [164]. The transcriptional activity of NF-κB is further regulated by acetylation and phosphorylation, which modulate DNA binding by NF-κB and interaction with transcriptional coactivators and/or corepressors [159]. TNF potentiates its biological functions through the stimulation of distinct signaling pathways such as c-Jun N-terminal kinase (JNK) and nuclear factor κB (NF-κB) [160, 165, 166]. Moreover, TNF-α stimulates the production of mitochondrial ROS in tumor cells and facilitates ROS-dependent cell migration by the activation of NF-κB [110]. Sustained activation of NF-κB contributes to cell survival, whereas the activation of JNK leads to cell death [160, 167]. TNF-α is known to stimulate the expressions of inflammatory genes such as cyclooxygenase-2 (COX-2), inflammatory cytokines, chemokines, inducible nitric oxide synthase (iNOS), lipoxygenase-2 (LOX-2), cell adhesion molecules, and antiapoptotic proteins [168]. TNF-α promotes cell survival by

triggering the activation of various set of genes and protein directed by NF-κB-dependent activation of anti-apoptotic molecules [100].

In addition, TNF-α promotes the adhesion of a range of tumor cells to the mesothelium and potentiates tumor migration and metastasis in vivo, partially via NF-κB-dependent induction of the chemokine receptor CXCR4, IL-8, and intercellular adhesion molecule-1 and the upregulation of monocyte chemoattractant protein-1 (MCP-1) in cancer cells [169–175]. TNF-α upregulates lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1) in endothelial cell, which facilitates the adhesion and trans-endothelial migration of cancer cells [113]. TNF-α is closely linked to tumor-induced cachexia, which is an inflammatory multiorgan failure during the later stage of cancer, and with inflammatory paraneoplastic syndromes, which has been noticed in pancreatic cancer [152].

14.4.1.3 TGF-β

TGF-β is one of the key cytokine that has an important roles in immunological tolerance, cell proliferation, and differentiation. This cytokine exhibits regulatory response in inflammation, which is linked to the progression of chronic inflammation and cancer [176]. Primarily, it was considered as a anti-inflammatory cytokine, but later on reports revealed that it is also involved in tissue remodeling, tumor initiation, progression and metastasis, and suppression of antigen-specific CD8T cell function [177, 178]. It is an important pleotropic cytokine that has an impact on immunological and nonimmunological functions. The binding of TGF-β to the heterodimeric receptor pair of TGF-β receptors I and II (TβRI/II) phosphorylates and translocates the transcription factors SMAD2 and SMAD3 in the nucleus to induce transcription [179]. The alteration of TGF-β signaling has been observed in various types of cancer. The upregulation of TGF-β1 ligand has been observed in colon, breast, esophageal, lung, pancreatic, liver, gastric, and prostate cancers. However, in most types of cancer that occur in the biliary tract, bladder, stomach, esophagus, brain, ovary, pancreas, and prostate, the mutations and loss of type I and type II TGF-β receptor expressions are noticed [180, 181]. Moreover, recent evidence suggests that the alteration in single allele of *TGFBR1* increases the risk of cancer [182, 183]. It has been noticed that during an early development of cancer and premalignant lesions, TGF-β coordinates function of tumor suppression. However, in a genetic mouse model for human cancer, genetic deletion of the TGF-β receptor results in tumor incidence and progression was noticed [184]. TGF-β regulates tissue remodeling and inflammation in a paracrine manner. It alters the functions of fibroblasts, endothelial cells, T cells, and macrophages to stimulate an inflammatory milieu related to chronic inflammatory diseases. After vascular lesion, TGF-β releases from platelets and induces angiogenesis [178]. It is the potent chemoattractant for monocytes and granulocytes [185, 186]. Moreover, TGF-β is known to limit the opsonizing and phagocytic activities of innate responders. It inhibits T-cell-mediated rejection of carcinoma cells. TGF-β inhibits natural killer (NK) cells and neutrophil-associated tumor rejection. In a tumor microenvironment, TGF-β is responsible for immune suppression. It inhibits the antitumor efficacy of macrophages, monocytes, NK cells, dendritic cells, and T cells; simultaneously

enhances motility and stimulates their recruitment [187–190]. These result in the secretion of cytokines, chemokines, growth factors, extracellular matrix-modifying enzymes, and proteases, which promote tumorigenesis and stimulate cell growth, invasion, and motility [191].

Indeed, ROS play a pivotal role in TGF-β-mediated tumorigenic effects. ROS regulate, SMADs, NF-kB, and MAPKs, which lie downstream of TGF-β signal transduction and also promote cell motility [192, 193]. TGF-β upregulates ROS level by the activation of H_2O_2-generating NADPH oxidase. TGF-β induces Smad3-dependent expression of NOX4 gene, which is the primary source of ROS in pancreatic cancer, as well as p40phox subunit (NCF4)-dependent expression of NOX2 gene in HeLa cells. TGF-β also restricts vital function of antioxidant enzymes such as catalase, glutaredoxin, glutathione peroxidase (GPx), superoxide dismutase (SOD), and the major antioxidant glutathione. Consecutively, the upregulated ROS may promote TGF-β expression and increases the release of TGF-β in tumor microenviron, which may favors tumor progression [194].

14.4.2 Chemokines

Chemokines are a superfamily of small cytokines and have a chemotactic feature to regulate cell trafficking [195]. Chemokines activate transmembrane G protein-coupled chemokine receptors (GPCRs) and direct cellular response [196]. Chemokines direct the trafficking response of leukocytes into the tumor microenvironment to promote malignancy [197]. Chemokines were mainly defined as soluble factors involved in the regulation of the directional migration of leukocytes during the stages of inflammation [84]. Chemokines are involved in the chemotaxis of monocytes, neutrophils, lymphocytes, basophils, natural killer cells, eosinophils, endothelial cells, and dendritic cells [198]. Chemokines and their receptors are one of the key players in cancer-related inflammation (CRI) [199]. The secretion of chemokines, as well as the expression of chemokine receptors, promotes tumor growth and metastasis. CXCR4-CXCL12 and CCR7-CCL21 are commonly involved chemokine receptors and chemokines in a wide range of tumors. Moreover, the majority of tumors express an elevated level of CXCR4 [84, 200]. Recent report show that tumor microenvironment enriched with ROS significantly influences the expression and function of CXCR4. ROS are reported to upregulate CXCR4 expression in immune and cancer cells, which promotes metastasis in cancer [201].

A growing body of evidence suggests that chemokines and their receptors are direct targets of the activation of various sets of oncogenes [202]. Chemokines can aid cancer progression by intiation of cell proliferation and evasion of apoptosis [203]. Chemokines can activate NF-κB and STAT-3 signaling to promote cell proliferation and survival [204]. Moreover, the RAS-RAF signaling pathway stimulates NF-κB and produces inflammatory chemokine such as CXCL8 [205]. Chemokines directly promote cell proliferation and restrict programmed cell death in multiple ways. Chemokines can activate mitogen-activated protein kinase

(MAPK) and extracellular signal-regulated kinase (Erk) signaling pathways [206, 207]. Recent report revealed that chemokines can promote cell proliferation and survival of cancer cells by altering the expression of proapoptotic and antiapoptotic genes, as well as proteins such as elevated expression of MDM2, and the downregulation of Bcl-2 were reported in cancer cells [208]. Cancer cells have elevated expression of chemokine receptors and promote angiogenesis. Elevated expression of chemokines and their receptor on cancer cells favor to develop tumor microenvironment and invasion. There are four families of chemokines has been nomenclatured depending on the pattern of cysteine residues; they are C, CC, CXC, and CX3C, where C stands for cysteine and X stands for noncysteine amino acids [209, 210].

In many type of cancer, the levels of CCL2 and CCL5 were found elevated, which indicates high numbers of intratumor myeloid cells can differentiates into mature tumor-associated macrophages (TAM) inside the microenvironment [211, 212]. TAMs are key inflammatory components of the cancer stroma that affect the range of neoplastic transformation [213]. Several reports suggest that oncogenic fusion transcript FUS-CHOP trans-activates various chemokines such as CCL2, CCL5, and CXCL8 [214]. The transcription factor Myc overexpresses in many human tumors and promotes cell proliferation, as well as guides the remodeling of extracellular microenvironment with inflammatory mediators and inflammatory cells [215]. Chemokine Receptors such as CCR6 and CXCR1 are expressed in pancreatic and colorectal cancers [216, 217], CXCR2 in melanoma [218] and esophageal cancer cells [219], CCR7 in squamous cell carcinoma of the head and neck [220], CCR10 in melanoma [221], and CXCR6 in prostate cancer [219]. A recent report indicates that small CXCR4 antagonist CTCE-9908 restrict migration of ovarian cancer cells to CXCL12 and induces cell death by mitotic catastrophe by arresting cell cycle at G_2-M phase [222]. In recent years, cancer-associated fibroblasts (CAF) have been widely studied and investigated and were found to be a source of CCL5 and CXC chemokines [223]. Thus, these reports suggest that altered expression of chemokines and their receptors promote progression of cancer.

14.4.3 Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases have an important role in cancer cell invasion and metastasis. MMPs (matrix metalloproteinases) are zinc-dependent endopeptidase enzymes that degrade extracellular matrix (ECM). MMPs regulate tissue homeostasis and disease pathogenesis, including the remodeling of soluble ECM (extracellular matrix) components and cell-cell and cell-matrix adhesion molecules [224]. MMPs are one of the most thoroughly studied proteases due to their frequent overexpression in a variety of cancers. Leukocyte-derived MMPs help in the progression of neoplasm [225]. MMPs have been identified as key inflammatory cell-derived mediators of tumor-associated angiogenesis in many experimental mouse models of cancer. Report indicates that MMP-9 regulates angiogenesis by mobilizing ECM-sequestered VEGF and stimulates vascular endothelial cell proliferation and

angiogenesis [226]. MMPs also promote angiogenesis and cell growth by inhinition of apoptotic cell death. For example, MMP-7 degrades FasL, subsequently producing soluble FasL, which decreases the ability to trigger apoptosis via the death receptor pathway [227]. In addition to the proteolytic members of the MMP family, other classes of extracellular and intracellular enzymes released by leukocytes, like chymase and tryptase, are also associated with promotion of neoplastic transformation [228]. Moreover, oxidative stress also plays a critical role in the upregulation of subgroups of MMPs, such as MMP-13, MMP-3, MMP-10, MMP-2, and MMP-9. Reports suggest that under prolonged oxidative stress, MMP-2 and MMP-9 post-transcriptionally activate and degrade type IV collagen and contribute to tumor metastasis and invasion. Therefore, under persistent oxidative stress, the activation of MMPs progresses chronic inflammation and malignant transformation [229, 230].

14.4.4 Inflammasome

An assembly of multiprotein complex that forms after sensing DAMPs or PAMPs is known as inflammasome, which governs the maturation of inflammatory cytokines such as IL-18 and IL-1β. The complex comprises nucleotide-binding oligomerization domain (NOD)-like receptor containing pyrin domain 3 (NLRP3) protein, adaptor molecule apoptosis-associated speck-like protein containing CARD (caspase recruitment domain) (ASC), and cysteine protease caspase-1. The oligomerization of these three major molecules directs the maturation of inflammatory cytokines and initiates innate immune response. The activation of the complex and maturation of inflammatory cytokines promote pyroptotic cell death and host defense mechanism [231]. There are multiple members of the NLR family, such as NLRP1, NLRC4, AIM2 (absent in melanoma 2), and NLRP3 inflammasomes, out of which NLRP3 is the fully characterized and most widely studied complex [106]. A large growing body of evidence revealed that inflammasomes tend to potentiate carcinogenesis in distinct conditions, which depends upon the phase in which the complex gets activated. There are multiple approaches has been identified for the activation of the NLRP3 inflammasome complex. NLRP3 protein and other pattern recognition receptors like TLRs sense the DAMPs and PAMPs stimulated by pathogens such as bacteria and subsequently activate the NF-kB signaling pathway, which promotes the translation of pro-inflammatory cytokines. In addition, extracellular ATP-dependent activation of P2X purinoceptor 7 (P2X7) triggers ATP-gated ion channel and can alter potassium efflux, which directly allow to activate NLRP3 inflammasome complex. Moreover, crystals such as monosodium urate (MSU), transported via lipid vesicles, merge with lysosomes, which leads to lysosomal rupture and results in the activation of the NLRP3 inflammasome. Interestingly, an intensive oxidative stress also plays a pivotal role in the activation of the inflammasome complex. Lysosomal rupture and the recognition of DAMPs and PAMPs stimulate ROS production. As a result, an excessive oxidative stress activates the NLRP3 inflammasome complex [232]. Therefore, ROS act as an indispensable secondary messenger in inflammasome activation. Further, the

NLRP3 inflammasome complex cleaves the proform of cytokines with activated caspase 1 and subsequently releases the mature interleukin-18 (IL-18) and interleukin-1β (IL-1β), which induces inflammation and pyroptosis [233]. Moreover, reports suggest that ROS produced by H_2O_2 can also leads to the activation of inflammasome [105]. Further, ROS produced by NADPH oxidase has also been implicated in the activation of the NLRP3 inflammasome [234]. It has been shown that inflammation contributes to the pathogenesis of most acute and chronic liver diseases that are associated with inflammasome activation and NF-kB connection [235]. The activation of inflammasome via interleukins is associated with NF-kB-mediated cell survival signaling. Oxidative stress and mitochondrial ROS also influence inflammasome-mediated IL-1β secretion and inflammatory response in various pathophysiological conditions [236].

14.5 Conclusion

Generation of oxidative stress is the consequence of an imbalance between the production and elimination of reactive oxygen species (ROS). In the past decades, several reports have suggested that oxidative stress and inflammatory mediators have a significant contribution to the progression of various pathophysiological consequences. Cancer cells cautiously maintain a sublethal level of ROS intracellularly and generate oxidative stress to set up a tumor microenvironment with the aid of inflammatory mediators to sustain their survival and proliferative potential. The accumulation of intracellular oxidants augments oxidative burden to the cells, which is a prerequisite for oncogenic transformation for the survival of cancer cells. However, free radicals and ROS are widely acknowledged to contribute to oxidative stress, and the activation of inflammatory signaling leads to the onset of cell proliferation, invasion, angiogenesis, and metabolic reprogramming of cancer cells. The level of ROS and oxidative stress influences the activation of immune cells and cellular signaling cascades to define the fate of cells. Primarily, inflammation causes the recruitment of activated immune cells to eliminate unwanted substances as a defense mechanism. But prolonged inflammation constitutively activates immune cells such as macrophages, fibroblasts, and endothelial cells, which release numerous inflammatory mediators such as cytokines, chemokines, leukotrienes prostaglandins, and reactive oxygen species as well as nitric oxide for the perturbation of oxidative stress, which leads to the progression of pathological conditions. Therefore, sustained inflammation and oxidative stress may fuel cancer and other pathological conditions by altering normal cellular signaling cascades. The relationship between inflammation and cancer has been studied extensively, but cross talk and insights into the mechanism of cellular signaling remain elusive. In conclusion, both oxidative stress and inflammation are intricate events of cells that culminate in the progression of cancer, as shown in Fig. 14.3. Therefore, an improved understanding of cellular signagling and moeclar event asociated with oxidative stress and inflammation in cancer could be extremly benificial for prognosis and therapeutic value.

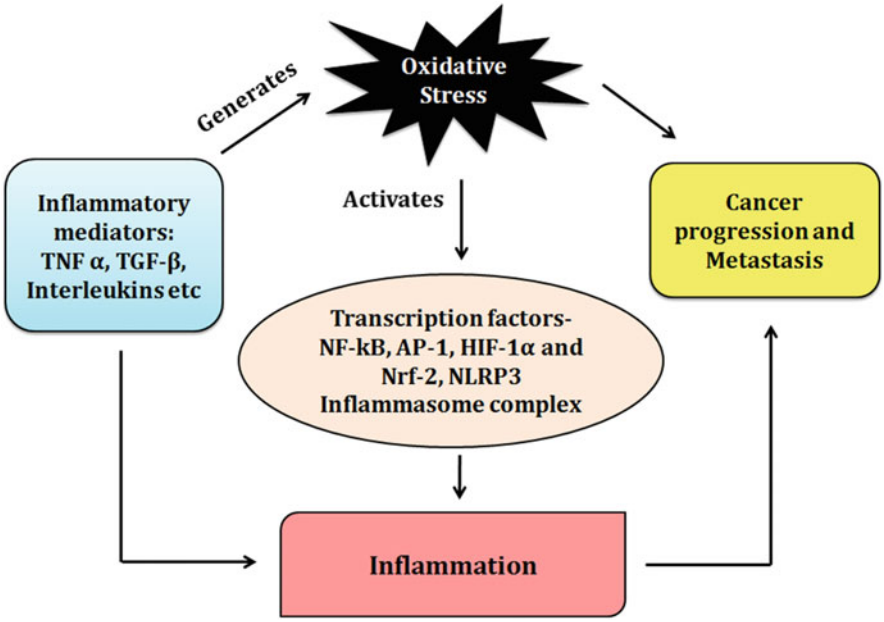


Fig. 14.3 Schematic representation of the interdependent link between oxidative stress and inflammation, which culminates in the progression of cancer

References

1. Alfadda AA, Sallam RM (2012) Reactive oxygen species in health and disease. J Biomed Biotechnol 2012:1–14. https://doi.org/10.1155/2012/936486
2. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D (1987) Oxygen radicals and human disease. Ann Intern Med 107:526–545
3. Manoharan S, Guillemin GJ, Abiramasundari RS, Essa MM, Akbar M, Akbar MD (2016) The role of reactive oxygen species in the pathogenesis of Alzheimer's disease, Parkinson's disease, and Huntington's disease: a mini review. Oxid Med Cell Longev 2016:8590578
4. Panieri E, Santoro M (2016) ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis 7:e2253
5. Zini R, Berdeaux A, Morin D (2007) The differential effects of superoxide anion, hydrogen peroxide and hydroxyl radical on cardiac mitochondrial oxidative phosphorylation. Free Radic Res 41:1159–1166
6. Holmström KM, Finkel T (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 15:411–421
7. Sumimoto H (2008) Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J 275:3249–3277
8. Finkel T (2011) Signal transduction by reactive oxygen species. J Cell Biol 194:7–15
9. Trachootham D, Lu W, Ogasawara MA, Valle NR-D, Huang P (2008) Redox regulation of cell survival. Antioxid Redox Signal 10:1343–1374
10. Liou G-Y, Storz P (2010) Reactive oxygen species in cancer. Free Radic Res 44:479–496

11. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9
12. McCord JM (2000) The evolution of free radicals and oxidative stress. Am J Med 108:652–659
13. Oberley T, Oberley L (1997) Antioxidant enzyme levels in cancer. Histol Histopathol 12:525–535
14. Reiter RJ, Acuña-Castroviejo D, Tan DX, Burkhardt S (2001) Free radical-mediated molecular damage. Ann N Y Acad Sci 939:200–215
15. Pollack M, Leeuwenburgh C (1999) Molecular mechanisms of oxidative stress in aging: free radicals, aging, antioxidants and disease. In: Handbook of oxidants and antioxidants in exercise. Elsevier Science BV, Amsterdam, pp 881–923
16. Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 313:17
17. Matés JM, Sánchez-Jiménez F (1999) Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 4:D339–D345
18. Breitenbach M, Eckl P (2015) Introduction to oxidative stress in biomedical and biological research. Biomol Ther 5:1169–1177
19. Burton GJ, Jauniaux EJ (2011) Oxidative stress. Best Pract Res Clin Obstet Gynaecol 25:287–299
20. Li R, Jia Z, Trush MA (2016) Defining ROS in biology and medicine. React Oxyg Species 1:9
21. Fukai T, Ushio-Fukai MJA (2011) Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal 15:1583–1606
22. Ushio-Fukai M, Nakamura Y (2008) Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Lett 266:37–52
23. Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, Varol M, Jain A, Khan M, Sethi GJB (2019) Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomolecules 9:735
24. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J (2000) Electron transport and oxidative phosphorylation. In: Molecular cell biology, 4th edn. WH Freeman, New York
25. Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 30:11–26
26. Bibov MY, Kuzmin AV, Alexandrova AA, Chistyakov VA, Dobaeva NM, Kundupyan OL (2018) Role of the reactive oxygen species induced DNA damage in human spermatozoa dysfunction. AME Med J 3:1–12
27. Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14
28. Yang H, Villani RM, Wang H, Simpson MJ, Roberts MS, Tang M, Liang X (2018) The role of cellular reactive oxygen species in cancer chemotherapy. J Exp Clin Cancer Res 37:266
29. Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, LLeonart ME (2013) Oxidative stress and cancer: an overview. Ageing Res Rev 12:376–390
30. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49:1603–1616
31. Acharya A, Das I, Chandhok D, Saha T (2010) Redox regulation in cancer: a double-edged sword with therapeutic potential. Oxid Med Cell Longev 3:23–34
32. Reczek CR, Chandel NS (2017) The two faces of reactive oxygen species in cancer. Annu Rev Cancer Biol 1:79–98
33. Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 21:297–308
34. Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit cancer cells? Trends Biochem Sci 41:211–218

35. Dhup S, Dadhich RK, Porporato PE, Sonveaux P (2012) Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. Curr Pharm Des 18:1319–1330
36. Goodwin ML, Pennington Z, Westbroek EM, Cottrill E, Ahmed AK, Sciubba DM (2019) Lactate and cancer: a "lactatic" perspective on spinal tumor metabolism (part 1). Ann Transl Med 7:220–220
37. Pathak C, Vinayak M (2005) Modulation of lactate dehydrogenase isozymes by modified base queuine. Mol Biol Rep 32:191–196
38. Mohammad GH, Damink SO, Malago M, Dhar DK, Pereira SP (2016) Pyruvate kinase M2 and lactate dehydrogenase A are overexpressed in pancreatic cancer and correlate with poor outcome. PLoS One 11:e0151635
39. Hsu M-C, Hung W-C (2018) Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. Mol Cancer 17:35
40. Gui DY, Lewis CA, Vander Heiden MG (2013) Allosteric regulation of PKM2 allows cellular adaptation to different physiological states. Sci Signal 6:pe7
41. Anastasiou D, Yu Y, Israelsen WJ, Jiang J-K, Boxer MB, Hong BS, Tempel W, Dimov S, Shen M, Jha A (2012) Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. Nat Chem Biol 8:839
42. Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang J-K, Shen M, Bellinger G, Sasaki AT, Locasale JW, Auld DSJS (2011) Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science 334:1278–1283
43. Finley LW, Carracedo A, Lee J, Souza A, Egia A, Zhang J, Teruya-Feldstein J, Moreira PI, Cardoso SM, Clish CB (2011) SIRT3 opposes reprogramming of cancer cell metabolism through HIF1α destabilization. Cancer Cell 19:416–428
44. Fiaschi T, Chiarugi P (2012) Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. Int J Cell Biol 2012:762825
45. Burdon RH, Gill V, Rice-Evans C (1990) Oxidative stress and tumour cell proliferation. Free Radic Res Commun 11:65–76
46. Kumari S, Badana AK, Malla R (2018) Reactive oxygen species: a key constituent in cancer survival. Biomark Insights 13:1177271918755391
47. Storz P (2005) Reactive oxygen species in tumor progression. Front Biosci 10:1881–1896
48. Yang Y, Fang E, Luo J, Wu H, Jiang Y, Liu Y, Tong S, Wang Z, Zhou R, Tong Q (2019) The antioxidant alpha-lipoic acid inhibits proliferation and invasion of human gastric cancer cells via suppression of STAT3-mediated MUC4 gene expression. Oxid Med Cell Longev 2019:3643715
49. Hamanaka RB, Chandel NS (2010) Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends Biochem Sci 35:505–513
50. Wang M, Kirk JS, Venkataraman S, Domann FE, Zhang HJ, Schafer FQ, Flanagan SW, Weydert CJ, Spitz DR, Buettner GR (2005) Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1 α and vascular endothelial growth factor. Oncogene 24:8154–8166
51. Patterson JC, Joughin BA, van de Kooij B, Lim DC, Lauffenburger DA, Yaffe MB (2019) ROS and oxidative stress are elevated in mitosis during asynchronous cell cycle progression and are exacerbated by mitotic arrest. Cell Syst 8:163–167.e162
52. Ranjan P, Anathy V, Burch PM, Weirather K, Lambeth JD, Heintz NH (2006) Redox-dependent expression of cyclin D1 and cell proliferation by Nox1 in mouse lung epithelial cells. Antioxid Redox Signal 8:1447–1459
53. Téphane Honoré S, Kovacic H, Éronique Pichard V, Briand C, Rognoni J-B (2003) α2β1-Integrin signaling by itself controls G1/S transition in a human adenocarcinoma cell line (Caco-2): implication of NADPH oxidase-dependent production of ROS. Exp Cell Res 285:59–71
54. Verbon EH, Post JA, Boonstra J (2012) The influence of reactive oxygen species on cell cycle progression in mammalian cells. Gene 511:1–6

55. Kelkel M, Schumacher M, Dicato M, Diederich M (2011) Antioxidant and anti-proliferative properties of lycopene. Free Radic Res 45:925–940
56. Laphanuwat P, Likasitwatanakul P, Sittithumcharee G, Thaphaengphan A, Chomanee N, Suppramote O, Ketaroonrut N, Charngkaew K, Lam EW-F, Okada S (2018) Cyclin D1 depletion interferes with oxidative balance and promotes cancer cell senescence. J Cell Sci 131:1–13
57. Kurutas EB (2015) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 15:71
58. Kozlov SV, Waardenberg AJ, Engholm-Keller K, Arthur JW, Graham ME, Lavin M (2016) Reactive oxygen species (ROS)-activated ATM-dependent phosphorylation of cytoplasmic substrates identified by large-scale phosphoproteomics screen. Mol Cell Proteomics 15:1032–1047
59. Mantovani F, Collavin L, Del Sal G (2019) Mutant p53 as a guardian of the cancer cell. Cell Death Differ 26:199–212
60. Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426
61. Haupt S, Raghu D, Haupt Y (2016) Mutant p53 drives cancer by subverting multiple tumor suppression pathways. Front Oncol 6:12
62. Lisek K, Campaner E, Ciani Y, Walerych D, Del Sal G (2018) Mutant p53 tunes the NRF2-dependent antioxidant response to support survival of cancer cells. Oncotarget 9:20508
63. Du X, Fu X, Yao K, Lan Z, Xu H, Cui Q, Yang E (2017) Bcl-2 delays cell cycle through mitochondrial ATP and ROS. Cell Cycle 16:707–713
64. Liang J, Cao R, Wang X, Zhang Y, Wang P, Gao H, Li C, Yang F, Zeng R, Wei P (2017) Mitochondrial PKM2 regulates oxidative stress-induced apoptosis by stabilizing Bcl2. Cell Res 27:329–351
65. Martin TA, Ye L, Sanders AJ, Lane J, Jiang WG (2013) Cancer invasion and metastasis: molecular and cellular perspective. In: Madame Curie bioscience database [Internet]. Landes Bioscience, Austin
66. Seyfried TN, Huysentruyt LC (2013) On the origin of cancer metastasis. Crit Rev Oncog 18:43
67. Lambert AW, Pattabiraman DR, Weinberg RA (2017) Emerging biological principles of metastasis. Cell 168:670–691
68. Baba AI, Câtoi C (2007) Tumor cell morphology. In: Comparative oncology. The Publishing House of the Romanian Academy, Bucharest
69. Heerboth S, Housman G, Leary M, Longacre M, Byler S, Lapinska K, Willbanks A, Sarkar S (2015) EMT and tumor metastasis. Clin Transl Med 4:6
70. Mittal V (2018) Epithelial mesenchymal transition in tumor metastasis. Annu Rev Pathol 13:395–412
71. Wang H, Unternaehrer JJ (2019) Epithelial-mesenchymal transition and cancer stem cells: at the crossroads of differentiation and dedifferentiation. Dev Dyn 248:10–20
72. Nagarsheth N, Wicha MS, Zou W (2017) Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol 17:559
73. Lee SY, Ju MK, Jeon HM, Lee YJ, Kim CH, Park HG, Han SI, Kang HS (2019) Reactive oxygen species induce epithelial-mesenchymal transition, glycolytic switch, and mitochondrial repression through the Dlx-2/Snail signaling pathways in MCF-7 cells. Mol Med Rep 20:2339–2346
74. Liao Z, Chua D, Tan NS (2019) Reactive oxygen species: a volatile driver of field cancerization and metastasis. Mol Cancer 18:65
75. Miyazaki Y, Hara A, Kato K, Oyama T, Yamada Y, Mori H, Shibata T (2008) The effect of hypoxic microenvironment on matrix metalloproteinase expression in xenografts of human oral squamous cell carcinoma. Int J Oncol 32:145–151
76. Zhang L, Huang G, Li X, Zhang Y, Jiang Y, Shen J, Liu J, Wang Q, Zhu J, Feng X (2013) Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor-1α in hepatocellular carcinoma. BMC Cancer 13:108

77. Nishida N, Kudo M (2013) Oxidative stress and epigenetic instability in human hepatocarcinogenesis. Dig Dis 31:447–453
78. Nishida N, Yano H, Nishida T, Kamura T, Kojiro MJ (2006) Angiogenesis in cancer. Vasc Health Risk Manag 2:213
79. Kim Y-W, Byzova TVJB (2014) Oxidative stress in angiogenesis and vascular disease. Blood 123:625–631
80. Carmeliet PJ (2005) VEGF as a key mediator of angiogenesis in cancer. Oncology 69:4–10
81. Babior BM (2004) NADPH oxidase. Curr Opin Immunol 16:42–47
82. Xia C, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, Jiang BH (2007) Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. Cancer Res 67:10823–10830
83. Sun X, Wei J, Tang Y, Wang B, Zhang Y, Shi L, Guo J, Hu F, Li X (2017) Leptin-induced migration and angiogenesis in rheumatoid arthritis is mediated by reactive oxygen species. FEBS Open Bio 7:1899–1908
84. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860
85. Blaylock RL (2015) Cancer microenvironment, inflammation and cancer stem cells: a hypothesis for a paradigm change and new targets in cancer control. Surg Neurol Int 6:92
86. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA (2014) Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014:149185
87. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
88. Kiraly O, Gong G, Olipitz W, Muthupalani S, Engelward BP (2015) Inflammation-induced cell proliferation potentiates DNA damage-induced mutations in vivo. PLoS Genet 11: e1004901
89. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454:436
90. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899
91. Kim SS, Ruiz VE, Carroll JD, Moss SF (2011) Helicobacter pylori in the pathogenesis of gastric cancer and gastric lymphoma. Cancer Lett 305:228–238
92. Wroblewski LE, Peek RM, Wilson KT (2010) Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev 23:713–739
93. Arbuthnot P, Kew M (2001) Hepatitis B virus and hepatocellular carcinoma. Int J Exp Pathol 82:77–100
94. El-Serag HB (2002) Hepatocellular carcinoma and hepatitis C in the United States. Hepatology 36:1206–1213
95. de Martel C, Franceschi S (2009) Infections and cancer: established associations and new hypotheses. Crit Rev Oncol Hematol 70:183–194
96. Mostafa MH, Sheweita S, O'Connor PJ (1999) Relationship between schistosomiasis and bladder cancer. Clin Microbiol Rev 12:97–111
97. Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357:539–545
98. Stark T, Livas L, Kyprianou N (2015) Inflammation in prostate cancer progression and therapeutic targeting. Transl Androl Urol 4:455
99. Shrihari T (2017) Dual role of inflammatory mediators in cancer. Ecancermedicalscience 11:721
100. Hoesel B, Schmid JA (2013) The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 12:86
101. Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22:240–273
102. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G (2009) The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 10:241–247

103. Fialkow L, Wang Y, Downey GP (2007) Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic Biol Med 42:153–164
104. Salzano S, Checconi P, Hanschmann E-M, Lillig CH, Bowler LD, Chan P, Vaudry D, Mengozzi M, Coppo L, Sacre S (2014) Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal. Proc Natl Acad Sci 111:12157–12162
105. Martinon F (2010) Signaling by ROS drives inflammasome activation. Eur J Immunol 40:616–619
106. Latz E, Xiao TS, Stutz A (2013) Activation and regulation of the inflammasomes. Nat Rev Immunol 13:397–411
107. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11:136
108. Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469:221–225
109. Ghezzi P (2011) Role of glutathione in immunity and inflammation in the lung. Int J Gen Med 4:105
110. Morgan MJ, Liu Z-G (2011) Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res 21:103–115
111. Blanco P, Palucka AK, Pascual V, Banchereau J (2008) Dendritic cells and cytokines in human inflammatory and autoimmune diseases. Cytokine Growth Factor Rev 19:41–52
112. Klampfer L (2011) Cytokines, inflammation and colon cancer. Curr Cancer Drug Targets 11:451–464
113. Liang M, Zhang P, Fu J (2007) Up-regulation of LOX-1 expression by TNF-α promotes trans-endothelial migration of MDA-MB-231 breast cancer cells. Cancer Lett 258:31–37
114. Zamarron BF, Chen W (2011) Dual roles of immune cells and their factors in cancer development and progression. Int J Biol Sci 7:651
115. Lin W-W, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Investig 117:1175
116. Knüpfer H, Preiss R (2010) Serum interleukin-6 levels in colorectal cancer patients—a summary of published results. Int J Colorectal Dis 25:135–140
117. Tanaka T, Bai Z, Srinoulprasert Y, Yang B, Hayasaka H, Miyasaka M (2005) Chemokines in tumor progression and metastasis. Cancer Sci 96:317–322
118. Yu JH, Kim H (2014) Oxidative stress and cytokines in the pathogenesis of pancreatic cancer. J Cancer Prev 19:97
119. Prawan A, Buranrat B, Kukongviriyapan U, Sripa B, Kukongviriyapan V (2009) Inflammatory cytokines suppress NAD (P) H: quinone oxidoreductase-1 and induce oxidative stress in cholangiocarcinoma cells. J Cancer Res Clin Oncol 135:515–522
120. Fortunati N, Manti R, Birocco N, Pugliese M, Brignardello E, Ciuffreda L, Catalano MG, Aragno M, Boccuzzi GJ (2007) Pro-inflammatory cytokines and oxidative stress/antioxidant parameters characterize the bio-humoral profile of early cachexia in lung cancer patients. Oncol Rep 18:1521–1527
121. Esquivel-Velázquez M, Ostoa-Saloma P, Palacios-Arreola MI, Nava-Castro KE, Castro JI, Morales-Montor J (2015) The role of cytokines in breast cancer development and progression. J Interferon Cytokine Res 35:1–16
122. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014:561459
123. Ravichandran K, Tyagi A, Deep G, Agarwal C, Agarwal R (2011) Interleukin-1-induced iNOS expression in human lung carcinoma A549 cells: involvement of STAT and MAPK pathways
124. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S (2011) The pro-and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta Mol Cell Res 1813:878–888
125. Brizzi MF, Formato L, Dentelli P, Rosso A, Pavan M, Garbarino G, Pegoraro M, Camussi G, Pegoraro L (2001) Interleukin-3 stimulates migration and proliferation of vascular smooth muscle cells. Circulation 103:549–554

126. Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S, Rizzoli V, Roodman GD, Giuliani N (2005) IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. Blood 106:1407–1414
127. Gupta N, Barhanpurkar AP, Tomar GB, Srivastava RK, Kour S, Pote ST, Mishra GC, Wani MR (2010) IL-3 inhibits human osteoclastogenesis and bone resorption through downregulation of c-Fms and diverts the cells to dendritic cell lineage. J Immunol 185:2261–2272
128. Heikkilä K, Ebrahim S, Lawlor DA (2008) Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. Eur J Cancer 44:937–945
129. Hodge DR, Hurt EM, Farrar WL (2005) The role of IL-6 and STAT3 in inflammation and canicer. Eur J Cancer 41:2502–2512
130. Aggarwal BB, Sethi G, Ahn KS, Sandur SK, Pandey MK, Kunnumakkara AB, Sung B, Ichikawa H (2006) Targeting signal-transducer-and-activator-of-transcription-3 for prevention and therapy of cancer. Ann N Y Acad Sci 1091:151–169
131. Rao V, Dyer C, Jameel J, Drew P, Greenman J (2006) Potential prognostic and therapeutic roles for cytokines in breast cancer. Oncol Rep 15:179–185
132. Sansone P, Bromberg J (2012) Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol 30:1005–1014
133. Kinoshita H, Hirata Y, Nakagawa H, Sakamoto K, Hayakawa Y, Takahashi R, Nakata W, Sakitani K, Serizawa T, Hikiba Y (2013) Interleukin-6 mediates epithelial–stromal interactions and promotes gastric tumorigenesis. PLoS One 8:e60914
134. Gasche JA, Hoffmann J, Boland CR, Goel A (2011) Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells. Int J Cancer 129:1053–1063
135. Baffet G, Braciak T, Fletcher R, Gauldie J, Fey G, Northemann W (1991) Autocrine activity of interleukin 6 secreted by hepatocarcinoma cell lines. Mol Biol Med 8:141–156
136. Atreya R, Mudter J, Finotto S, Müllberg J, Jostock T, Wirtz S, Schütz M, Bartsch B, Holtmann M, Becker C (2000) Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. Nat Med 6:583
137. Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S (2004) TGF-β suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity 21:491–501
138. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ (2007) TGF-[beta] and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell-mediated pathology. Nat Immunol 8:1390
139. Onishi RM, Gaffen SL (2010) Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology 129:311–321
140. Chen X-W, Zhou S-F (2015) Inflammation, cytokines, the IL-17/IL-6/STAT3/NF-κB axis, and tumorigenesis. Drug Des Devel Ther 9:2941
141. Maniati E, Soper R, Hagemann T (2010) Up for Mischief? IL-17/Th17 in the tumour microenviroment. Oncogene 29:5653
142. Vidal AC, Skaar D, Maguire R, Dodor S, Musselwhite LW, Bartlett JA, Oneko O, Obure J, Mlay P, Murphy SK (2015) IL-10, IL-15, IL-17, and GMCSF levels in cervical cancer tissue of Tanzanian women infected with HPV16/18 vs. non-HPV16/18 genotypes. Infect Agent Cancer 10:10
143. Numasaki M, Fukushi J-I, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT (2003) Interleukin-17 promotes angiogenesis and tumor growth. Blood 101:2620–2627
144. Kolls JK, Lindén A (2004) Interleukin-17 family members and inflammation. Immunity 21:467–476
145. Kryczek I, Wei S, Zou L, Altuwaijri S, Szeliga W, Kolls J, Chang A, Zou W (2007) Cutting edge: Th17 and regulatory T cell dynamics and the regulation by IL-2 in the tumor microenvironment. J Immunol 178:6730–6733
146. Swann JB, Smyth MJ (2007) Immune surveillance of tumors. J Clin Investig 117:1137

147. Meier B, Radeke H, Selle S, Younes M, Sies H, Resch K, Habermehl G (1989) Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-α. Biochem J 263:539–545
148. Li Q, Engelhardt JF (2006) Interleukin-1β induction of NFκB is partially regulated by H2O2-mediated activation of NFκB-inducing kinase. J Biol Chem 281:1495–1505
149. Hsu S, Hsu P (1994) Autocrine and paracrine functions of cytokines in malignant lymphomas. Biomed Pharmacother 48:433–444
150. Bellido T, Borba VZ, Roberson P, Manolagas SC (1997) Activation of the Janus kinase/STAT (signal transducer and activator of transcription) signal transduction pathway by interleukin-6-type cytokines promotes osteoblast differentiation. Endocrinology 138:3666–3676
151. Yuzhalin A, Kutikhin A (2014) Interleukins in cancer biology: their heterogeneous role. Academic, Cambridge
152. Szlosarek P, Charles KA, Balkwill FR (2006) Tumour necrosis factor-α as a tumour promoter. Eur J Cancer 42:745–750
153. Szlosarek PW, Balkwill FR (2003) Tumour necrosis factor α: a potential target for the therapy of solid tumours. Lancet Oncol 4:565–573
154. Leek R, Landers R, Fox S, Ng F, Harris A, Lewis C (1998) Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 77:2246
155. Sethi G, Sung B, Aggarwal BB (2008) TNF: a master switch for inflammation to cancer. Front Biosci 13:5094–5107
156. Dempsey PW, Doyle SE, He JQ, Cheng G (2003) The signaling adaptors and pathways activated by TNF superfamily. Cytokine Growth Factor Rev 14:193–209
157. Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 3:745
158. Aggarwal BB, Shishodia S, Ashikawa K, Bharti AC (2002) The role of TNF and its family members in inflammation and cancer: lessons from gene deletion. Curr Drug Targets Inflamm Allergy 1:327–341
159. Wajant H, Pfizenmaier K, Scheurich P (2003) Tumor necrosis factor signaling. Cell Death Differ 10:45
160. Wang X, Lin Y (2008) Tumor necrosis factor and cancer, buddies or foes? Acta Pharmacol Sin 29:1275–1288
161. Devin A, Cook A, Lin Y, Rodriguez Y, Kelliher M, Liu Z-G (2000) The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. Immunity 12:419–429
162. Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, Liao W, Chen Z, Zheng-gang L, Su B (2001) The essential role of MEKK3 in TNF-induced NF-[kappa] B activation. Nat Immunol 2:620
163. Karin M, Yamamoto Y, Wang QM (2004) The IKK NF-[kappa] B system: a treasure trove for drug development. Nat Rev Drug Discov 3:17
164. Kamata H, Honda S-I, Maeda S, Chang L, Hirata H, Karin M (2005) Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120:649–661
165. Reinhard C, Shamoon B, Shyamala V, Williams LT (1997) Tumor necrosis factor α-induced activation of c-jun N-terminal kinase is mediated by TRAF2. EMBO J 16:1080–1092
166. Song HY, Régnier CH, Kirschning CJ, Goeddel DV, Rothe M (1997) Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-κB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. Proc Natl Acad Sci 94:9792–9796
167. Liu J, Lin A (2005) Role of JNK activation in apoptosis: a double-edged sword. Cell Res 15:36
168. Shih R-H, Wang C-Y, Yang C-M (2015) NF-kappaB signaling pathways in neurological inflammation: a mini review. Front Mol Neurosci 8:77
169. Alkhamesi NA, Ziprin P, Pfistermuller K, Peck DH, Darzi AW (2005) ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exp Metastasis 22:449–459

170. Choo MK, Sakurai H, Koizumi K, Saiki I (2006) TAK1-mediated stress signaling pathways are essential for TNF-α-promoted pulmonary metastasis of murine colon cancer cells. Int J Cancer 118:2758–2764
171. Kitakata H, Nemoto-Sasaki Y, Takahashi Y, Kondo T, Mai M, Mukaida N (2002) Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells. Cancer Res 62:6682–6687
172. Kulbe H, Hagemann T, Szlosarek PW, Balkwill FR, Wilson JL (2005) The inflammatory cytokine tumor necrosis factor-α regulates chemokine receptor expression on ovarian cancer cells. Cancer Res 65:10355–10362
173. Mochizuki Y, Nakanishi H, Kodera Y, Ito S, Yamamura Y, Kato T, Hibi K, Akiyama S, Nakao A, Tatematsu M (2004) TNF-α promotes progression of peritoneal metastasis as demonstrated using a green fluorescence protein (GFP)-tagged human gastric cancer cell line. Clin Exp Metastasis 21:39–47
174. Van Grevenstein W, Hofland L, Van Rossen M, Van Koetsveld P, Jeekel J, Van Eijck C (2007) Inflammatory cytokines stimulate the adhesion of colon carcinoma cells to mesothelial monolayers. Dig Dis Sci 52:2775–2783
175. Ziprin P, Ridgway PF, Pfistermüller KL, Peck DH, Darzi AW (2003) ICAM-1 mediated tumor-mesothelial cell adhesion is modulated by IL-6 and TNF-α: a potential mechanism by which surgical trauma increases peritoneal metastases. Cell Commun Adhes 10:141–154
176. Balkwill F (2009) Tumour necrosis factor and cancer. Nat Rev Cancer 9:361
177. Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, Sporn MB, Fauci AS (1986) Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 163:1037–1050
178. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH (1986) Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci 83:4167–4171
179. Letterio JJ (2005) TGF-[beta] signaling in T cells: roles in lymphoid and epithelial neoplasia. Oncogene 24:5701
180. Bierie B, Moses HL (2006) TGF-β and cancer. Cytokine Growth Factor Rev 17:29–40
181. Levy L, Hill CS (2006) Alterations in components of the TGF-β superfamily signaling pathways in human cancer. Cytokine Growth Factor Rev 17:41–58
182. Valle L, Serena-Acedo T, Liyanarachchi S, Hampel H, Comeras I, Li Z, Zeng Q, Zhang H-T, Pennison MJ, Sadim M (2008) Germline allele-specific expression of TGFBR1 confers an increased risk of colorectal cancer. Science 321:1361–1365
183. Zeng Q, Phukan S, Xu Y, Sadim M, Rosman DS, Pennison M, Liao J, Yang G-Y, Huang C-C, Valle L (2009) Tgfbr1 haploinsufficiency is a potent modifier of colorectal cancer development. Cancer Res 69:678–686
184. Bierie B, Moses HL (2006) Tumour microenvironment: TGF [beta]: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 6:506
185. Brandes M, Mai U, Ohura K, Wahl S (1991) Type I transforming growth factor-beta receptors on neutrophils mediate chemotaxis to transforming growth factor-beta. J Immunol 147:1600–1606
186. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB (1987) Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci 84:5788–5792
187. Gruber BL, Marchese MJ, Kew RR (1994) Transforming growth factor-beta 1 mediates mast cell chemotaxis. J Immunol 152:5860–5867
188. Li MO, Wan YY, Sanjabi S, Robertson A-KL, Flavell RA (2006) Transforming growth factor-β regulation of immune responses. Annu Rev Immunol 24:99–146
189. Maghazachi A, Al-Aoukaty A (1992) Transforming growth factor-beta 1 is chemotactic for interleukin-2-activated natural killer cells. Nat Immun 12:57–65
190. Wrzesinski SH, Wan YY, Flavell RA (2007) Transforming growth factor-β and the immune response: implications for anticancer therapy. Clin Cancer Res 13:5262–5270

191. Bierie B, Moses HL (2010) Transforming growth factor beta (TGF-β) and inflammation in cancer. Cytokine Growth Factor Rev 21:49–59
192. Liu R-M, Pravia KG (2010) Oxidative stress and glutathione in TGF-β-mediated fibrogenesis. Free Radic Biol Med 48:1–15
193. Tochhawng L, Deng S, Pervaiz S, Yap CT (2013) Redox regulation of cancer cell migration and invasion. Mitochondrion 13:246–253
194. Krstić J, Trivanović D, Mojsilović S, Santibanez JF (2015) Transforming growth factor-beta and oxidative stress interplay: implications in tumorigenesis and cancer progression. Oxid Med Cell Longev 2015:654594
195. Zlotnik A (2006) Chemokines and cancer. Int J Cancer 119:2026–2029
196. Stone MJ, Hayward JA, Huang C, Huma ZE, Sanchez J (2017) Mechanisms of regulation of the chemokine-receptor network. Int J Mol Sci 18:342
197. Dubinett SM, Lee J, Sharma S, Mulé JJ (2010) Chemokines: can effector cells be re-directed to the site of tumor? Cancer J 16:325
198. Martins-Green M, Petreaca M, Wang L (2013) Chemokines and their receptors are key players in the orchestra that regulates wound healing. Adv Wound Care 2:327–347
199. Proost P, Wuyts A, Van Damme J (1996) The role of chemokines in inflammation. Int J Clin Lab Res 26:211–223
200. Rossi D, Zlotnik A (2000) The biology of chemokines and their receptors. Annu Rev Immunol 18:217–242
201. Chetram MA, Hinton CV (2013) ROS-mediated regulation of CXCR4 in cancer. Front Biol 8:273–278
202. Salazar N, Castellan M, Shirodkar SS, Lokeshwar BL (2013) Chemokines and chemokine receptors as promoters of prostate cancer growth and progression. Crit Rev Eukaryot Gene Expr 23:77–91
203. Strieter RM, Gomperts BN, Keane MP (2007) The role of CXC chemokines in pulmonary fibrosis. J Clin Investig 117:549
204. Clarke CN, Kuboki S, Tevar A, Lentsch AB, Edwards M (2009) Roles of NF-kappaB and STAT3 in CXC chemokine–mediated hepatocyte proliferation and cell death. J Am Coll Surg 209:S59. https://doi.org/10.1016/j.jamcollsurg.2009.06.141
205. Richmond A (2002) NF-κB, chemokine gene transcription and tumour growth. Nat Rev Immunol 2:664
206. Bonacchi A, Romagnani P, Romanelli RG, Efsen E, Annunziato F, Lasagni L, Francalanci M, Serio M, Laffi G, Pinzani M (2001) Signal transduction by the chemokine receptor CXCR3 activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt controls cell migration and proliferation in human vascular pericytes. J Biol Chem 276:9945–9954
207. Fang WB, Jokar I, Zou A, Lambert D, Dendukuri P, Cheng N (2012) CCL2/CCR2 chemokine signaling coordinates survival and motility of breast cancer cells through Smad3 protein-and p42/44 mitogen-activated protein kinase (MAPK)-dependent mechanisms. J Biol Chem 287:36593–36608
208. Su H, Najul EJS, Toth TA, Ng CM, Lelievre SA, Fred M, Tang CK (2011) Chemokine receptor CXCR4-mediated transformation of mammary epithelial cells by enhancing multiple RTKs expression and deregulation of the p53/MDM2 axis. Cancer Lett 307:132–140
209. Murphy PM, Baggiolini M, Charo IF, Hébert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA (2000) International union of pharmacology. XXII Nomenclature for chemokine receptors. Pharmacol Rev 52:145–176
210. Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity 12:121–127
211. Balkwill F (2004) Cancer and the chemokine network. Nat Rev Cancer 4:540
212. Bottazzi B, Polentarutti N, Acero R, Balsari A, Boraschi D, Ghezzi P, Salmona M, Mantovani A (1983) Regulation of the macrophage content of neoplasms by chemoattractants. Science 220:210–212
213. Pollard JW (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4:71

214. Germano G, Frapolli R, Simone M, Tavecchio M, Erba E, Pesce S, Pasqualini F, Grosso F, Sanfilippo R, Casali PG (2010) Antitumor and anti-inflammatory effects of trabectedin on human myxoid liposarcoma cells. Cancer Res 70:2235–2244
215. Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI (2007) Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med 13:1211
216. Ghadjar P, Coupland SE, Na I-K, Noutsias M, Letsch A, Stroux A, Bauer S, Buhr HJ, Thiel E, Scheibenbogen C (2006) Chemokine receptor CCR6 expression level and liver metastases in colorectal cancer. J Clin Oncol 24:1910–1916
217. Marchesi F, Piemonti L, Fedele G, Destro A, Roncalli M, Albarello L, Doglioni C, Anselmo A, Doni A, Bianchi P (2008) The chemokine receptor CX3CR1 is involved in the neural tropism and malignant behavior of pancreatic ductal adenocarcinoma. Cancer Res 68:9060–9069
218. Singh S, Nannuru K, Sadanandam A, Varney M, Singh R (2009) CXCR1 and CXCR2 enhances human melanoma tumourigenesis, growth and invasion. Br J Cancer 100:1638
219. Wang B, Hendricks DT, Wamunyokoli F, Parker MI (2006) A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. Cancer Res 66:3071–3077
220. Wang J, Seethala RR, Zhang Q, Gooding W, Van Waes C, Hasegawa H, Ferris RL (2008) Autocrine and paracrine chemokine receptor 7 activation in head and neck cancer: implications for therapy. J Natl Cancer Inst 100:502–512
221. Murakami T, Cardones AR, Finkelstein SE, Restifo NP, Klaunberg BA, Nestle FO, Castillo SS, Dennis PA, Hwang ST (2003) Immune evasion by murine melanoma mediated through CC chemokine receptor-10. J Exp Med 198:1337–1347
222. Kwong J, Kulbe H, Wong D, Chakravarty P, Balkwill F (2009) An antagonist of the chemokine receptor CXCR4 induces mitotic catastrophe in ovarian cancer cells. Mol Cancer Ther 8:1893–1905
223. Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. Nat Rev Cancer 6:392
224. Gialeli C, Theocharis AD, Karamanos NK (2011) Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 278:16–27
225. Van Lint P, Libert C (2007) Chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation. J Leukoc Biol 82:1375–1381
226. Deryugina EI, Quigley JP (2015) Tumor angiogenesis: MMP-mediated induction of intravasation-and metastasis-sustaining neovasculature. Matrix Biol 44:94–112
227. Almendro V, Ametller E, García-Recio S, Collazo O, Casas I, Augé JM, Maurel J, Gascón P (2009) The role of MMP7 and its cross-talk with the FAS/FASL system during the acquisition of chemoresistance to oxaliplatin. PLoS One 4:e4728
228. de Souza Junior DA, Santana AC, da Silva EZM, Oliver C, Jamur MC (2015) The role of mast cell specific chymases and tryptases in tumor angiogenesis. Biomed Res Int. 2015:142359
229. Mori K, Shibanuma M, Nose K (2004) Invasive potential induced under long-term oxidative stress in mammary epithelial cells. Cancer Res 64:7464–7472
230. Westermarck J, KÄHÄRI V-M (1999) Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J 13:781–792
231. Swanson KV, Deng M, Ting JP-Y (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 19:477–489
232. Lin C, Zhang J (2017) Inflammasomes in inflammation-induced cancer. Front Immunol 8:271
233. Abais JM, Xia M, Zhang Y, Boini KM, Li P-L (2015) Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? Antioxid Redox Signal 22:1111–1129
234. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 320:674–677
235. Szabo G, Petrasek J (2015) Inflammasome activation and function in liver disease. Nat Rev Gastroenterol Hepatol 12:387
236. Zhong Z, Zhai Y, Liang S, Mori Y, Han R, Sutterwala FS, Qiao L (2013) TRPM2 links oxidative stress to NLRP3 inflammasome activation. Nat Commun 4:1–11

Role of Oxidative Stress in Chronic Kidney Disease

15

Kiran Dahiya, Rakesh Dhankhar, Priya Dahiya, Raunak Ahlawat, and Navdeep Hooda

Abstract

Kidneys are important organs of the renal system. Besides being one of the principal excretory organs of the body, these perform many important biological functions. Any impairment or dysfunction of the kidney persisting for more than a 3-month duration is termed as chronic kidney disease (CKD). There are many causes of CKD, the most important ones being diabetes and hypertension. The grading of CKD is done depending on the changes in the glomerular filtration rate (GFR). Oxidative stress is a common accompaniment to CKD. Increased production of oxidants, including reactive oxygen species (ROS), in CKD may be due to associated inflammation, abnormality of iron metabolism, or disturbed high-density lipoprotein (HDL) metabolism besides other causes. The most common cause of mortality in CKD or end-stage renal disease (ESRD) is cardiovascular disease, and oxidative stress plays the main culprit in that. Various consequences of increased oxidative stress include endothelial dysfunction, left ventricular hypertrophy, and cardiac fibrosis. Mounting of inappropriate defense system involving nuclear factor erythroid 2-related factor 2 (Nrf2) and other antioxidants also adds up to the condition. This chapter is an attempt to throw light on these aspects, as well as important enzymatic markers for this disease. The therapeutic role of various measures to counter this oxidative stress in CKD will also be discussed.

K. Dahiya (✉) · R. Dhankhar
Pt. BD Sharma PGIMS, Rohtak, Haryana, India

P. Dahiya · N. Hooda
Rajshree Medical Research Institute and Hospital, Bareilly, Uttar Pradesh, India

R. Ahlawat
Maulana Azad Medical College, New Delhi, India

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_15

Keywords

Chronic kidney disease · Oxidative stress · Reactive oxygen species · Antioxidants · Enzymatic markers · Cardiovascular disease

15.1 Kidney and Its Normal Physiology

The kidneys are the primary functional organs of the renal system. These are a pair of bean-shaped organs covered by the renal capsule present in the abdominal cavity behind the peritoneum. The right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen. Internally, each kidney is divided into three major regions, viz., cortex, medulla, and pelvis. The renal cortex is a space between the medulla and the outer capsule. The renal medulla contains the majority of the nephrons. The renal pelvis connects the kidney to the circulatory and nervous systems of the body. The basic structural and functional unit of the kidney that filters the blood in order to regulate chemical concentrations and produce urine is known as a nephron [1].

A nephron is the smallest functional unit of the kidney. Each kidney is made up of more than one million nephrons. Each nephron is composed of glomerulus, proximal convoluted tubule (PCT), loop of Henle (LOH), distal convoluted tubule (DCT), and a series of collecting ducts. Kidneys filter blood in a three-step process. First, the nephrons filter blood that runs through the capillary network in the glomerulus. Almost all solutes, except for proteins, are filtered out into the glomerulus by a process called glomerular filtration, producing an ultrafiltrate consisting of the other smaller circulating elements. Second, the filtrate is collected in the renal tubules. Most of the solutes get reabsorbed in the PCT by a process called tubular reabsorption. In the loop of Henle, the filtrate continues to exchange solutes and water with the renal medulla and the peritubular capillary network. Water is reabsorbed during this step, and additional solutes are secreted into the kidney tubules during tubular secretion. The collecting ducts collect filtrate coming from the nephrons and fuse in the medullary papillae. From here, the papillae deliver the filtrate (now called urine) into the minor calyces, which eventually connect to the ureters through the renal pelvis. Ureters finally drain the urine into the urinary bladder [1].

The main physiological functions of the kidney include excretion of waste products, e.g., urea, creatinine, drugs, etc. (excretory function); regulation of electrolytes, serum osmolality, and acid-base balance within narrow limits (homeostatic function); and formation of erythropoietin, renin–angiotensin system, and activation of vitamin D (endocrine function) [1].

15.2 Chronic Kidney Diseases

Chronic kidney disease, also known as chronic renal failure, chronic renal disease, or chronic kidney failure, is defined as an abnormality of the kidney structure or function, present for more than 3 months, with implications for health. It is not unusual for people to realize that they have chronic kidney failure only when their kidney function is down to 25% of normal. Chronic kidney failure, unlike acute kidney failure, is a slow and gradually progressive process. Even if one kidney stops functioning, the other can carry out normal functions. It is not usual for signs and symptoms to be noticeable until the disease is fairly well advanced and the condition has become severe, by which time most of the damage is irreversible [2].

Further, persons with CKD are defined as all individuals with markers of kidney damage or those with an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 on at least two occasions, 90 days apart (with or without markers of kidney damage). The markers of kidney disease may include albuminuria (albumin creatinine ratio ACR $>$ 3 mg/mmol), hematuria of renal origin, electrolyte abnormalities due to renal tubular dysfunction, histological abnormalities of renal tissue, structural abnormalities of the kidneys detected by imaging, or a history of kidney transplantation [3].

15.2.1 Causes

The most common causes of CKD are diabetes and hypertension, termed as diabetic and hypertensive nephropathies, respectively. Other causes include [3]:

- Polycystic kidney disease (PKD)
- Renal artery stenosis
- Infection
- Systemic autoimmune diseases such as systemic lupus erythematosus (lupus nephritis), Goodpasture's syndrome, etc.
- IgA glomerulonephritis
- Drugs or toxins
- Alport syndrome, rare genetic condition
- Hemolytic uremic syndrome in children
- Henoch–Schönlein purpura

15.2.2 Clinical Features

The common signs and symptoms of chronic kidney disease include [3]:

- Anemia
- Hematuria/proteinuria
- Discolored urine

- Abnormal urine output
- Edema
- Easy fatigability
- Disorientation/decreased mental alertness/unexplained headache
- Hypertension
- Insomnia
- Loss of appetite
- Erectile dysfunction
- Polyuria
- Persistent itching
- Muscle cramps/twitches
- Nausea/vomiting
- Pain in the abdomen radiating to the lower back
- Shortness of breath
- Sudden change in bodyweight

15.2.3 Grading of CKD

The grading of chronic kidney disease may be done by noting changes in the GFR rate as follows [3]:

GFR category	GFR (mL/min/1.73 m^2)	Terms used for the grading of CKD
G1	≥90	Normal or high
G2	60–89	Mildly decreased
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	<15	Kidney failure

15.2.4 Diagnosis [4]

- Blood tests: serum levels of creatinine, urea, uric acid, electrolytes, and estimated GFR (eGFR), calculated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation—$186 \times (\text{creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203} \times (0.742$ if female$) \times (1.210$ if black)
- Urine tests: urine albumin to creatinine ratio (UACR) (normal is 30 mg/g or less)
- Imaging tests like ultrasound, computerized tomography (CT) scan, magnetic resonance imaging (MRI), etc.
- Kidney biopsy

15.2.5 Treatment [4]

Treatment usually includes curing the cause and employing measures to control signs and symptoms, reduce complications, and slow the progression of the disease. It includes following a diet chart with low-protein diet. Different drugs used include:

- Antihypertensive
- Anti-inflammatory
- Antihyperlipidemics
- Antihistaminics
- Antiemetics
- Erythropoietin
- Calcium and vitamin D supplements

For end-stage renal disease (ESRD), treatment options include:

- Dialysis
- Renal transplant
- Regenerative medicine approaches

15.2.6 Mortality and ESRD

A very high rate of mortality has been reported in patients with end-stage renal disease, approaching approximately 9% per year. It is also notable that most prominent causes of death (approximately 50%) have been attributed to cardiovascular disease (CVD) [5].

15.3 Oxidative Stress and CKD

Oxidative stress (OS) is defined as an imbalance between oxidant molecules and the antioxidant systems of the body in favor of oxidants. Oxidant compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are continually formed under physiological conditions and get removed by several antioxidant defense mechanisms. OS leads to certain metabolic derangements and/or oxidation of macromolecules like lipids, deoxyribonucleic acid (DNA), and proteins, bringing about oxidative damage in cells, tissues, or organs, culminating in several disorders [6].

The kidney is a metabolically active organ and is thus quite vulnerable to damage caused by perpetrators of OS. Several complications of CKD, such as inflammation and CVD, are also linked to enhanced OS. This association between CKD and its complications is achieved through several mechanisms like induction of activities of certain enzymes responsible for producing oxidants and less availability of antioxidants due to dietary restrictions, diuretic use, protein energy wasting, and/or decreased intestinal absorption. Patients with CKD are otherwise also more predisposed to oxidative stress because of increased propensity of the presence of

other comorbid conditions like diabetes mellitus and hypertension. These patients generally belong to advanced age, which is again associated with oxidative stress. Further, with end-stage disease, because of the risk of hyperkalemia, patients are advised to have a restricted intake of fresh fruits that are a rich source of minerals and vitamins with antioxidant activity, especially vitamin C. The human body is unable to synthesize vitamin C by itself. In addition, treatment in the form of hemodialysis aggravates the generation of ROS by the activation of polymorphonuclear leucocytes (PMNLs), as well as loss of antioxidants during the procedure. Low levels of antioxidants (both enzymatic and nonenzymatic) have been observed in this disease. In addition to vitamin C, plasma vitamin E concentrations are also reported to be lower in these patients, making the defense against oxidation of low-density lipoprotein (LDL) particles still poorer. Oxidized LDL particles are one of the major culprits in the pathogenesis of CVD. Plasma levels of major scavengers of ROS, like superoxide dismutase, glutathione peroxidase, etc., have also been reported to be low due to the downregulation seen in patients with CKD [7].

Inflammation is the natural defense mechanism of the body against any kind of insult. It is a common accompaniment of CKD and contributes substantially to morbidity and mortality associated with this disease. It involves the recruitment of immune cells and the release of inflammatory mediators in the form of cytokines and interleukins. CKD has been found to lead to the activation of PMNLs, important component of the immune system, as well as different mediators and markers of inflammation, such as CRP, IL-6, TNF-α, fibrinogen, etc., suggesting their strong association. Inflammation enhances the oxidative status of the body by a number of mechanisms. In this process, an enzyme, myeloperoxidase, is activated by PMNLs to combat any pathogen invasion by generating ROS, adding further to oxidative stress [2].

15.3.1 Mechanism of Increased Oxidative Stress in CKD

Different mechanisms may be enumerated as responsible for the increased production of oxidants and the decreased concentration of antioxidants in patients suffering from CKD [8].

1. Mechanisms of Increased Oxidant Production
   (a) Increased expression of ROS-producing enzymes (e.g., nicotinamide adenine dinucleotide phosphate (NADPH oxidase), cyclooxygenase, lipoxygenase, etc.)
   (b) Decreased generation of NO by reduced activity of NO synthase (NOS) (via NOS inactivation, depletion of tetrahydro-biopterin (BH4), accumulation of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NOS)
   (c) Leakage from the mitochondrial respiratory chain
   (d) Respiratory burst with the help of myeloperoxidase
   (e) Recruitment and activation of PMNLs and other immune cells

(f) Oxidation of important macromolecules of the body, including lipids, proteins, LDL particles, etc. and initiating a chain reaction

2. Impaired Antioxidant Defense System
   (a) Decline in the generation of endogenous antioxidants, including antioxidant enzymes, reduced glutathione (GSH), uric acid, etc.
   (b) Defective or insufficient expression of genes encoding different antioxidant molecules
   (c) Improper activation or mounting of antioxidant enzymes
   (d) Depletion, by way of consumption, of antioxidant molecules to counter increased ROS levels
   (e) Impaired activity of good cholesterol carrier, i.e., high-density lipoprotein (HDL)
   (f) Insufficient inclusion of fresh fruits and vegetables in routine diet
   (g) Loss of water-soluble antioxidant molecules during hemodialysis procedure
   (h) Anemia leading to compromised antioxidant status

15.3.1.1 Role of HDL Disturbances in Oxidative Stress of CKD Patients

HDL is also termed as "good cholesterol" as it transports cholesterol from the peripheral organs to the liver (reverse cholesterol transport) in contrast to low-density lipoprotein (LDL), also known as "bad cholesterol," which transports cholesterol from the liver to the peripheral tissues, including the coronary arteries. Any defect or decline of HDL molecules is going to contribute to CVD. Various antioxidant and antiatherogenic actions of HDL are well known. These may be enumerated as follows [8]:

- Reverse cholesterol transport by HDL
- Endothelial cell migration followed by repair (via scavenger receptor B1)
- Inactivation of platelet-activating factor (PAF) and PAF-like phospholipids by action of PAF acetyl hydrolase
- Various antioxidant and anti-inflammatory actions:
  - ApoA-I-mediated scavenging of oxidized phospholipids present in lipoproteins and cell membrane
  - Lecithin cholesterol acyl transferase (LCAT)-mediated hydrolysis of oxidized phospholipids
  - Prevention of LDL oxidation
  - Elimination of oxidized phospholipids by action of paraoxonase-1 and glutathione peroxidase (GPX)

Patients with CKD tend to have alterations in both HDL quantity and HDL quality. Even a mildly impaired GFR is associated with low HDL cholesterol (HDL-C) concentration, which becomes progressively worse through ESRD. Moreover, in CKD, HDL particles tend to be smaller and denser due to metabolic defect. It has been observed that patients with renal dysfunction have significant disturbances in lipoprotein metabolism, and HDL in these patients becomes dysfunctional. Patients with CKD have lower plasma levels of HDL-C and reduced ability of

HDL to bind to ATP-binding cassette transporter A1 (ABCA1), resulting in slowing down the reverse cholesterol transport and disturbances in HDL maturation due to decreased LCAT. Studies have demonstrated that the HDL of CKD patients loses its vasoprotective, antioxidative, and anti-inflammatory properties and turns into a noxious particle that promotes endothelial dysfunction via stimulating superoxide production and limiting NO bioavailability. Alterations of HDL at the molecular and functional levels have also been observed in renal transplant recipients, even in those with excellent graft function [9].

15.3.2 Role of Iron Metabolism in Oxidative Stress in CKD

Iron is a redox-active transition metal. As a part of iron and heme containing proteins, which perform variety of important functions in the body, it is highly essential for body. Besides this, free or active iron catalyzes the formation of highly reactive and toxic ROS in the body. Thus, iron homeostasis is brought about by cells through a tightly regulated mechanism making use of iron regulatory protein (IRP) and hepcidin. Increased intracellular iron has been demonstrated in polymorphonuclear leucocytes isolated from patients on hemodialysis and has been associated with vascular calcification. Vascular calcification induces stiffness of vessel walls, decreasing their vascular compliance. Iron-induced calcification is found to be mediated by interleukin 24 (IL-24) and is, hence, linked to oxidative stress. CKD patients are frequently found to suffer from anemia and are treated with erythropoiesis-stimulating agents (ESA) and iron (oral or parenteral). Excessive iron treatment has a risk of overloading the cells with free iron. The accumulation of iron in mitochondria, a highly redox-active place in the cell, may potentiate the leakage of ROS from the electron transport chain and the production of highly reactive and damaging hydroxyl radicals ($OH^{\bullet}$) through Fenton and Haber–Weiss reactions (Fig. 15.1).

Intravenous iron has been shown to increase biologic markers of oxidative stress in cell cultures, animal models, and ESRD patients on hemodialysis. It has been reported that intravenous administration of iron sucrose in dialysis patients results in an increase in total peroxide, free iron, and markers of lipid peroxidation, which gets significantly improved with administration of the antioxidant vitamin E. Intravenous

Fig. 15.1 Fenton and Haber–Weiss reactions

Fenton Reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + OOH^{\bullet} + H^+$$

Haber-Weiss Reaction:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^-$$

iron has been reported to add to cytotoxicity and tissue injury, as well as to exacerbate oxidative stress, promoting endothelial dysfunction, inflammation, and the progression of both CKD and cardiovascular disease. This generation of oxidative stress, tubular injury, glomerular permeability, and renal inflammation may be mitigated by therapy with the antioxidant *N*-acetyl cysteine (NAC). It is also possible that the transient injury that may be caused by intravenous iron sucrose is outweighed by the benefits of iron repletion and repair of anemia.

15.3.3 Inflammation in CKD and Oxidative Stress [10]

It is quite a known fact that inflammation plays a key role in CKD progression and its outcome. Inflammation is another important cause of increased oxidative stress observed in patients with advanced renal disease, with malnutrition, chronic volume overload, and autonomic dysfunction being among some of the factors implicated in the increased inflammatory state seen in renal impairment. Systemic or intrarenal inflammation contributes to the deregulation of microvascular response to its regulators and sustains the production of an array of tubular toxins, including ROS, leading to tubular injury, nephron damage, and the onset of CKD. Circulating pro-inflammatory cytokines activate intrarenal microvasculature, including endothelial cells and leukocytes, resulting in the local amplification of pro-inflammatory factors and ROS. These processes affect cell surface adhesion molecules and disrupt the glycocalyx layer. Endothelial barrier function, activation of coagulation system, and receptor-mediated vasoreactivity are also compromised. These inflammation-mediated alterations can induce irreversible tubular injury and nephron failure. Oxidative stress and inflammation are inseparably linked, being major characteristics of CKD and drivers of CKD progression. The presence of systemic inflammation and its severity contribute to CKD-associated oxidative stress, which represents a condition in which the generation of ROS overrides the capacity of the antioxidant defense system. Activation of polymorphonuclear neutrophils is a well-recognized feature in CKD patients, with proven association between renal dysfunction and the different mediators and markers of inflammation, such as C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and fibrinogen, suggesting that CKD is a low-grade inflammatory process in itself. Myeloperoxidase, generated in response to the activation of polymorphonuclear neutrophils, triggers ROS activation and the inactivation of NO.

15.3.4 Adaptive Response to Oxidative Stress

When the body is subjected to increased oxidative stress, various defense mechanisms are mounted, which includes the upregulation of antioxidant status. An important role is played by a factor called nuclear factor erythroid 2-related factor 2 (Nrf2) [8].

15.3.4.1 Role Played by Nrf2

Normally, imbalance of oxidant–antioxidant status in favor of the former triggers an adaptive defense mechanism, resulting in an enhanced expression of antioxidant cytoprotective enzymes and other molecules. In mammals, an increased expression of these molecules is brought about by nuclear factor erythroid 2 p45-related factors 1 and 2 (Nrf2). Nuclear factor erythroid 2-related factor 2 (Nrf2) is present in the cytoplasm as an inactive complex bound to Kelch-like ECH-associated protein 1 (Keap1), a repressor molecule that facilitates Nrf2 ubiquitination. Keap1 contains several reactive cysteine residues that serve as sensors of the intracellular redox state. Oxidative or covalent modification of thiols in some of these cysteine residues leads to conformational changes in Keap1, which results in the disruption of its interaction with Nrf2. Inside the nucleus, Nrf2 binds to regulatory sequences (known as antioxidant response elements or electrophile response elements) in the promoter regions of genes responsible for expressing different antioxidants and molecules used in detoxification. Therefore, Nrf2 plays a central role in the defense against oxidative stress by its effect on the antioxidant and anti-inflammatory systems [11].

Glutathione is the principal and most abundant cellular endogenous antioxidant and plays a major role in the regulation of the cellular oxidative state. It is a tripeptide made up of glutamate, glycine, and cysteine (γ-glutamyl-cysteinyl-glycine). Glutathione directly helps in scavenging ROS and other oxidized substances by serving as a substrate for a number of antioxidant enzymes such as glutathione peroxidase. During the process, glutathione itself gets oxidized to G-S-S-G, or oxidized glutathione. It needs to be reconverted to its reduced form (GSH) with the help of an NADPH-requiring enzyme, glutathione reductase. Any impairment of the glutathione redox cycle increases the risk of ROS-mediated cell injury and consequences like atherosclerosis, diabetes mellitus, chronic liver disease, and cerebrovascular diseases. An association of these occurrences has been reported with elevated glutamate cysteine ligase enzyme, due to induced expression by Nrf2 and resulting in reduced GSH levels. Hence, impaired Nrf2 activation is associated with the severity of oxidative stress and inflammation and consequent disease progression in CKD [8].

15.4 Consequences of Increased Oxidative Stress in CKD

The principal consequences of increased oxidative stress in CKD pertain to the cardiovascular system. These mainly include dysfunction of the endothelium, hypertrophy of the left ventricle, and fibrosis of cardiac tissue [12].

15.4.1 Endothelial Dysfunction

Increased oxidative stress in the form of excessive ROS production is a major cause of endothelial dysfunction, the most important consequence. LDL molecules get easily modified in the presence of increased oxidative stress. These oxidized LDL

molecules are internalized by macrophages (via scavenger receptor class A), which then get transformed into foam cells. These foam cells are the principal components of fatty streak, one of the initial steps in the formation of atheromatous plaque. This initiates an antigenic reaction involving T lymphocytes and mounts an immunological response. The foam cells accumulating in the arterial intima produce an inflammatory response in the vessel wall. This involves inducing the expression of various chemotactic factors, including leukocyte adhesion, leading to the migration of a variety of circulatory inflammatory cells into the subendothelial space. Various cytokines and growth factors are also released at the site. The result of all these processes includes endothelial dysfunction, platelet aggregation, metalloproteinase expression, collagen deposition, fibrosis, and the consequent thrombogenesis. Ultimately, an occlusive thrombotic plaque develops in the vessel wall.

Endothelial dysfunction is an early indicator of atherosclerosis. It is an early predictor of cardiovascular events/disease, including unstable angina, myocardial infarction, heart failure, and death, and may also be associated with restenosis after a multitude of coronary interventions. Ischemia–reperfusion phenomenon is the main culprit in producing ROS in this event.

15.4.2 Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is considered to be the lesser appreciated adverse cardiovascular consequence of oxidative stress. Depending on the severity of renal dysfunction in CKD patients, the estimated LVH prevalence vary between 40% and 75%. Though the appearance of LVH in CKD is multifactorial, still factors like hypertension and volume overload (especially in patients undergoing dialysis) play an important role. The role of oxidative stress in the development of LVH in these patients cannot be overlooked. A variety of growth factors and hypertrophy signaling kinases (e.g., tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinase, and Jun-nuclear kinase), as well as transcription factors (like kB, Ets, and activator-protein-1), which stimulate matrix metalloproteinase expression, get induced by ROS. G-protein-coupled hypertrophic stimulation, as well as apoptosis induction, is also brought about by ROS. It has been reported that the activation of apoptosis signaling kinase-1 activates nuclear factor kB, which is an important mediator in the hypertrophy of cardiac tissue.

NO also pays a significant role in endothelial dysfunction. In conditions of increased oxidative stress, with oxidation of BH4, NOS starts to produce superoxide anion, further aggravating oxidative stress. This process is notably seen in endothelial dysfunction associated with hypertension, diabetes, and chronic smoking. Further, increased oxidative stress can inactivate endogenous NO, and NO deficiency has been reported to exacerbate LVH in experimental conditions.

15.4.3 Cardiac Fibrosis

The event mentioned above ultimately stimulates cardiac fibroblast proliferation and activates matrix metalloproteinases in the cardiac tissue, culminating in fibrosis.

15.4.3.1 Oxidative Stress and Pressure Overload: A Vicious Cycle

Increased ROS generation produces a hypertrophic stimulus and also acts as a mediator in bringing about tissue hypertrophy. A vicious cycle gets created as pressure overloading results in increased oxidative stress, which in turn leads to a hypertrophic response of the tissue, and the cycle goes on. Different causes of pressure overload in CKD include the expansion of extracellular volume, increased sympathetic activity, enhanced expression of endothelin protein, increased renin–angiotensin system activity, and exaggerated action of pump inhibitors like Na^+-K^+ ATPase and Ca^{2+}-ATPase inhibitors.

15.5 Important Enzymatic Markers of Oxidative Stress in CKD

Besides being produced in the mitochondrial respiratory chain, there are other metabolic reactions leading to the generation of ROS, e.g., nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase (XO), myeloperoxidase (MPO), and endothelial nitric oxide synthase (eNOS). These may be termed as markers of oxidative stress as their levels correlate with the degree of oxidant/antioxidant imbalance in the body [13].

15.5.1 NADPH Oxidases

NADPH oxidase is the major source of ROS production in different tissues, mainly endothelial cells, vascular smooth muscle cells, and renal parenchymal cells. This enzyme belongs to a big family, which consists of seven members: five different types of NADPH oxidases (NOX) and two dual oxidases (DUOX1–2). All the NOX enzymes consist of two heme containing oxidoreductases present as six transmembrane domains. The principal function of NOX is to catalyze the transfer of electrons intracellularly, i.e., within specialized compartments of the cell or from the cytosol to the extracellular space.

Cytosolic NADH or NADPH acts as the electron donor for the action of NOX. The different isoforms of NOX differ in their tissue distribution, intracellular localization, regulation, and binding proteins. The role played by NOX1 and NOX4 in a broad range of diseases has drawn the attention of researchers worldwide. NOX4 is the most important isoform present in renal tissue and has been isolated from renal tubules, glomerular mesangial cells, fibroblasts, and podocytes in the kidney, as well as from endothelial cells and fibroblasts in the vasculature. Under basal conditions, NOX have been found to show low activity but turn highly active in the presence of cytokines and growth factors and under the influence of conditions associated with

oxidative stress, like high glucose or cholesterol levels. Once active, these enzymes are responsible for producing ROS and setting in a chain reaction of initiation and propagation of oxidation and peroxidation.

15.5.2 Glucose-6-Phosphate Dehydrogenase (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway. The principal significance of the pentose phosphate pathway/hexose monophosphate (HMP) shunt pathway is the generation of ribose and NADPH. NADPH is required to keep glutathione in its reduced form, and because of this reason, altered activity of G6PD (enzymopathy) is associated with increased oxidative stress. Interestingly, high activity of G6PD is found in kidneys of rodents with experimental diabetic nephropathy, warranting its measurement in human models also.

15.5.3 Endothelial Nitric Oxide Synthase

The isoform of nitric oxide synthase synthesized in the endothelium is known as endothelial nitric oxide synthase (eNOS). There are three different isoforms of NO synthases (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and eNOS. L-arginine is metabolized by NOS to form L-citrulline and NO requiring NADPH and oxygen as cosubstrates and BH4 as a cofactor. NO performs a number of important functions like neurotransmission and the regulation of vascular tone. In vasculature, eNOS is the most abundant of the NOS isoforms, and NO synthesized in the endothelium acts as the major vasodilator or endothelium-derived relaxing factor (EDRF). Under certain pathological conditions, because of "eNOS uncoupling," i.e., electron transfer within the active site is uncoupled from L-arginine oxidation and oxygen gets reduced to $O_2^{\bullet -}$. Thus, eNOS can produce ROS in the form of superoxide radicals under these circumstances. This superoxide anion combines rapidly with NO to produce peroxynitrite (ONOO−), a highly reactive radical with great oxidative potential.

The uncoupling of eNOS may be brought about by a variety of mechanisms like deficiency of L-arginine or BH4 and by the accumulation of asymmetric dimethylarginine (ADMA), a naturally occurring L-arginine analog and endogenous NOS inhibitor. ROS, once generated, produce oxidation of either BH4 and protein arginine *N*-methyltransferase (PRMT type 1) or dimethylarginine dimethylaminohydrolase (DDAH), generating increased levels of ADMA. Thus, this may also become a vicious cycle.

15.5.4 Myeloperoxidase

Myeloperoxidase (MPO) is a heme-containing peroxidase that is synthesized during myeloid differentiation and gets stored in the azurophilic granules of different leucocytes. Under normal circumstances, MPO catalyzes the formation of hypochlorous acid (HClO) from the H_2O_2-mediated oxidation of halide ions. It has been reported that under various pathological situations, degranulation of cells leads to a release of MPO into the extracellular space, where it can oxidize other substrates also, besides halide ions. By the same mechanism, MPO is responsible for tissue damage in atherosclerosis as it causes oxidative modification of LDL particles. Oxidized LDL molecules are the main culprit molecules in the formation of atheromatous plaque, thus suggesting a link between MPO and coronary artery disease.

15.5.5 Xanthine Oxidase

The enzyme xanthine oxidoreductase is present as two interconvertible forms, i.e., as xanthine dehydrogenase (XDH) and xanthine oxidase (XO), though expressed by a single gene. Both XDH and XO catalyze the terminal two steps in purine degradation in humans, i.e., conversion of hypoxanthine to xanthine and further conversion to uric acid. For these reactions, XDH uses hypoxanthine or xanthine as a substrate and NAD^+ as a cofactor to produce uric acid and NADH. But under some pathological circumstances, especially involving inflammatory conditions as in CKD, XDH gets converted to XO by posttranslational modification involving oxidation of the cysteine residues in the protein molecule. XO has a greater affinity for oxygen as a cofactor, leading to the formation of uric acid, along with $O_2^{\bullet -}$ or H_2O_2, thus adding up to the existing oxidative stress.

15.5.6 Paraoxonase-1

The enzyme paraoxonase-1 (PON1) belongs to the paraoxonase family, which helps in protecting the body against oxidation of molecules, including lipoproteins; thus, it may be used as a marker of antioxidant status. The activity of PON1 has been found to be decreased in patients with CKD. The importance of this marker may be understood from the fact that the R allele of the Q192R variant of the PON1 gene is directly related to the severity of LVH and cardiovascular dysfunction in patients with CKD. It has also been observed that patients of CKD who were homozygous for the R allele of this gene showed significantly increased plasma levels of the lipid peroxidation marker, 8-isoprostane. Thus, PON-1 has the potential to be used as a marker of antioxidant status in patients with CKD.

15.6 Treatments to Target Oxidative Stress in CKD Patients

As oxidative stress has an important pathophysiological role in patients with chronic renal disease, different treatments to counter this have been suggested [14].

15.6.1 Antioxidant Vitamins (Vitamins C and E)

Patients of CKD are known to suffer from anemia and are commonly administered intravenous iron for treatment. Parenteral iron is a potential source for generating oxidants in the body. Supplementation of vitamin E by oral route in these patients has been observed to be associated with a decreased oxidative stress status and lesser oxidative susceptibility of LDL particles, conferring upon it a cardioprotective role. In this regard, administration of both vitamin E and vitamin C, both by oral and parenteral routes, has been found helpful, as assessed by an improvement in hematocrit and a lesser requirement of erythropoietin in these patients. Hemodialysis is an additional source for increasing oxidative stress in patients of CKD. Use of dialysis membranes that are antioxidant based has been found effective in decreasing endothelial dysfunction and the oxidation of LDL particles. The introduction of hemolipodialysis using vitamin-E-containing liposomes and vitamin C in the dialysate to reduce dialysis-induced oxidative stress also appears promising.

15.6.2 Angiotensin-Converting Enzyme (ACE) Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers have been observed to be effective in preventing or slowing the process of development of nephropathy in patients with diabetes. These act by interfering with the renin–angiotensin system and also inhibiting angiotensin-II-dependent NADPH oxidase activation quite effectively and have potential to be successful in limiting oxidative stress in patients with CKD/ESRD.

15.6.3 Statins

These are the drugs of choice to treat dyslipidemia, which may be associated with CKD. Besides inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase competitively, these produce an anti-inflammatory effect also. Reports from nonrenal patients put forward their potential as antioxidants also as these inhibit the activation of small GTPases such as Rac1-inhibiting NADPH oxidase, independent of its lipid-lowering effect. This effect of statins might be linked to their anti-inflammatory action.

15.6.4 Allopurinol

Allopurinol is the structural analog of purines and is known to inhibit the enzyme XO competitively. It has also been suggested to be a free radical scavenger, an antioxidant, and a "scavenger" of hypochlorous acid. It has been found to be cardioprotective too because of its capacity to improve endothelial dysfunction and oxidative stress in patients of CKD, and this action is much more prominent than that of exogenous antioxidant vitamins. This may possibly be because allopurinol prevents the formation of superoxide anions by inhibiting their significant source, i.e., the enzyme xanthine oxidase, while antioxidant vitamins try to scavenge the excess free radicals that have already been generated. Large doses of antioxidant vitamins are needed to produce the desired effect, giving allopurinol an upper hand in this regard.

15.6.5 Other Measures

Recent measures mainly target the uncoupling of eNOS to prevent the generation of excessive oxidative stress. Therefore, BH4 and its precursor, sepiapterin, a potential recoupler of eNOS, could prove to be beneficial in CKD.

The rationale behind the use of a glutathione peroxidase mimetic may be its ability to reconvert BH4 to a nonoxidized state, which helps in the normalization or recoupling of eNOS.

In an attempt to increase the production of NO, administering high doses of L-arginine has also been suggested. But this poses a challenge as L-arginine is, generally, given as a chloride salt, which, being an acidifying agent, is not advisable in renal patients, especially with ESRD.

15.7 Hemodialysis and Oxidative Stress

Patients with CKD on hemodialysis (HD) are subjected to increased oxidative stress. In these patients, altered dietary pattern may cause the depletion of antioxidants like vitamins C and E, mainly because of lesser intake of vegetables and fruits because of the risk of hyperkalemia. Besides this, malnutrition, loss of vitamins during HD procedure, reduced selenium levels, and reduced function of antioxidant enzymes are some of the other reasons. Further, additional factors responsible for increasing oxidative stress in these patients include chronic inflammatory state associated with CKD, uremia, comorbidities (e.g., hypertension, diabetes, obesity, dyslipidemia, advanced age, and vascular calcification), and other factors related to the HD procedure per se.

Chen et al. [4] suggested that HD procedure promotes the formation of $O2^{\bullet -}$, a powerful prooxidant reactive oxygen molecule, and that there is a direct increase in ROS levels in plasma after each HD session. It has been observed that within minutes after the initiation of an HD session, exposure to dialyzer membranes and

dialysate triggers the activation of complement factors, platelets, and PMNLs, followed by ROS production. PMNL stimulation is a significant biomarker for oxidant stress, which gets progressively enhanced with stages of CKD and is more pronounced in patients undergoing HD. Then within 30 min of HD initiation, lipid peroxidation products start increasing. The activation of complement or the production of free fatty acids induced by heparin might be the pathophysiologic mechanisms leading to these effects. The duration of HD treatment is also a significant independent factor of oxidative stress as prolonged dialysis sessions are characterized by increased inflammation and lipid peroxidation effects.

15.7.1 Antioxidants and Hemodialysis

The HD procedure *per se* is characterized by a significant depletion of antioxidants, and patients undergoing chronic hemodiafiltration have been observed to possess significantly lower plasma levels of vitamins C and E and lower activity of antioxidant enzymes, along with increased levels of several oxidants, as compared to their healthy counterparts. Further, it has been speculated that the administration of antioxidants such as vitamins E and C might be of benefit in HD patients. *In vitro*, vitamin E is the most powerful lipid-soluble antioxidant molecule in cell membranes. It not only preserves the stability of biological membranes and protects them from injury induced by ROS and lipid peroxides, but it also modifies cell reaction to oxidants via the regulation of signal transmission molecular pathways. It was observed in a study on salt-sensitive hypertensive rodents that supplementation of vitamins E and C, in combination, ameliorated the accumulation of oxidative products, improved kidney hemodynamics, delayed disease progression, and subsequently protected the kidney from further damage. However, there is accumulating data suggesting that supplementation of various antioxidants such as vitamins C, E, and N acetyl cysteine (NAC) might reduce oxidative stress in HD; the studies available in this regard are not consistent. This may be due to several factors [4]: oxidative status assessed by numerous different biomarkers in different time lines and in heterogeneous and cohorts of patients [13], dosage and route of administration of antioxidants differing between the trials [6], small sample size and nonclarity of pathophysiologic mechanism, and [15] the degree of OS abrogation by antioxidants. Therefore, antioxidant intake has not yet been adopted in guidelines or everyday clinical practice [16].

15.8 Conclusion

Thus, it may be concluded that oxidative stress plays a major role in CKD and its consequent complications. Furthermore, patients on hemodialysis for treatment are subjected to an augmented oxidative stress. Therapeutic strategies incorporating the measures to tackle oxidative stress at different levels may prove to be promising in this regard.

References

1. Preuss HG (1993) Basics of renal anatomy and physiology. Clin Lab Med 13(1):1–11
2. Mihai S, Codrici E, Popescu ID et al (2018) Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. J Immunol Res 2018:2180373, 16p. https://doi.org/10.1155/2018/2180373
3. Tonelli M, Wiebe N, Culleton B et al (2006) Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol 17(7):2034–2047. https://doi.org/10.1681/ASN.2005101085
4. Chen TK, Knicely DH, Grams ME (2019) Chronic kidney disease diagnosis and management: a review. JAMA 322(13):1294–1304. https://doi.org/10.1001/jama.2019.14745
5. Locatelli F, Marcelli D, Conte F, D'Amico M, Del Vecchio L, Limido A et al (2001) Survival and development of cardiovascular disease by modality of treatment in patients with end-stage renal disease. J Am Soc Nephrol 12(11):2411–2417
6. Forbes JM, Coughlan MT, Cooper ME (2008) Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 57(6):1446–1454. https://doi.org/10.2337/db08-0057
7. Scholze A, Jankowski J, Pedraza-Chaverri J, Evenepoel P (2016) Oxidative stress in chronic kidney disease. Oxidative Med Cellular Longevity 2016:8375186, 2 p. https://doi.org/10.1155/2016/8375186
8. Yonova D, Trendafilov I, Georgieva I, Dimitrova V, Arabadjieva D, Velkova N (2018) Oxidative stress (OS) in chronic kidney disease (CKD): a mini review. Nephrol Renal Dis 3 (3):1–3. https://doi.org/10.15761/NRD.1000147
9. Jacek R, Anna G, Danilo F, Timo S, Andrzej W (2014) Chronic kidney disease-different role for HDL? Curr Med Chem 21(25):2910–2916
10. Nakanishi T, Kuragano T, Nanami M, Nagasawa Y, Hasuike Y (2019) Misdistribution of iron and oxidative stress in chronic kidney disease. Free Radic Biol Med 133:248–253. https://doi.org/10.1016/j.freeradbiomed.2018.06.025
11. Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320
12. Ling XC, Kuo K (2018) Oxidative stress in chronic kidney disease. Ren Replace Ther 4:53. https://doi.org/10.1186/s41100-018-0195-2
13. Daenen K, Andries A, Mekahli D, Schepdael AV, Jouret F, Bammens B (2019) Oxidative stress in chronic kidney disease. Pediatr Nephrol 34:975. https://doi.org/10.1007/s00467-018-4005-4
14. Kao MPC, Ang DSC, Pall A, Struthers AD (2010) Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J Hum Hypertens 24(1):18. https://doi.org/10.1038/jhh.2009.70
15. González Rico M, Puchades MJ, García Ramón R, Saez G, Tormos MC, Miguel A (2006) Effect of oxidative stress in patients with chronic renal failure. Nefrologia 26(2):218–225
16. Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR (2017) Oxidative stress in hemodialysis patients: a review of the literature. Oxid Med Cell Longev 2017:3081856, 22p. https://doi.org/10.1155/2017/3081856

Role of Oxidative Stress in the Pathophysiology of Type 2 Diabetes and Cardiovascular Diseases

16

Olorunfemi R. Molehin, Stephen A. Adefegha, and Anne A. Adeyanju

Abstract

Many of the cell components like mitochondria, as well as other cytosolic components, are central to and has pivotal significance in reactive oxygen species (ROS) production. A state of imbalance between ROS production and antioxidant defense mechanisms, in which ROS are favored, is termed oxidative stress. Oxidative stress is a common denominator in the progression of several deteriorating ailments, for instance, diabetes mellitus (DM) and cardiovascular diseases (CVDs). DM is a worldwide health burden, affecting people globally, and it is one of the main causative factors for death and a main risk factor for CVDs. The most prevalent type of DM is type 2 diabetes (T2D), which is a derangement of metabolism with a peculiar feature of insulin resistance and hyperglycemia. Oxidative stress promotes "overload of glucose, oxidative phosphorylation influx of polyol pathway, elevated advanced glycation products (AGEs), formation and expression at receptors site, activation of protein kinase C isoforms, and hexosamine pathway initiation." CVDs are complex in nature with multifaceted mechanisms regarding its pathophysiology. CVDs refers to conditions that involve blocked heart and/or blood vessels resulting to diseases such as coronary heart diseases, ischemic heart diseases, atherosclerosis, stroke,

O. R. Molehin (✉)
Department of Biochemistry, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria
e-mail: olorunfemi.molehin@eksu.edu.ng

S. A. Adefegha
Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria
e-mail: saadefegha@futa.edu.ng

A. A. Adeyanju
Department of Biological Sciences, Faculty of Applied Sciences, KolaDaisi University, Ibadan, Oyo State, Nigeria
e-mail: anne.adeyanju@koladaisiuniversity.edu.ng

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_16

with hypertension, hypercholesterolemia, and/or dyslipidemia as major risk factors. We examine in this chapter the association of oxidative stress with hyperglycemia, insulin resistance, hyperinsulinemia, hypertension, hypercholesterolemia, and/or dyslipidemia, to mention a few factors in the pathogenesis of T2D and CVDs. These metabolic processes or enzymes implicated in type 2 diabetes and cardiovascular diseases are current therapeutic targets and could be explored for future/novel drug design.

Keywords

Type 2 diabetes · Oxidative stress · Antioxidant · Hyperglycemia · Cardiovascular diseases

16.1 Introduction

Diabetes mellitus (DM) is a growing health burden globally and a leading causative factor for global morbidity and mortality and has been documented to be a risk agent for cardiovascular diseases. DM, notably T2DM, is a very dreadful pathological condition worldwide [1]. Despite comprehensive research outputs, the actual course of the pathogenesis of T2DM has not been fully understood. Therefore, many investigations are actively ongoing to vividly explain the pathogenesis of T2DM, notably the progression of T2DM, including oxidative stress and inflammation pathway [2]. This metabolic disorder gives birth to oxidative stress, which alters "the activity of insulin by diver's means of interacting pathways, producing the reactive oxygen species (ROS) such as H_2O_2 and superoxide anions. These species has damaging potential on the islets β-cells of the pancreas leading to a shortening in the release of insulin [3, 4]."

DM exists in two major types: types 1 and 2 diabetes. "Type 1 diabetes (T1D) is an autoimmune condition that causes β-cells of pancreas destruction and accounts for 5–10% of diagnosed cases of DM. Meanwhile, T2DM comprises of reduced insulin secretion by β-cells or increase in insulin resistance, and an estimation of about 95% of all cases of DM. The occurrence of T2D in a high number of chronic comorbidities has been reported and it has been verified that this disease affects the manner of patient's life which leads to the progression of CVDs. Many early deaths is as a result of DM, caused majorly by coronary artery disease or renal dysfunction [1, 4]." Several types of CVD have been documented to be linked to overgeneration of ROS. Biologically, "free radicals are highly unstable molecules, which possess free electrons, which can react with a variety of organic substrates like proteins and DNA [5]." The major player in intracellular oxidant production in most types of cells is the mitochondria. Other sources of intrinsic cellular ROS generation includes enzymes such as "xanthine oxidase, nitric oxide synthase, cyclooxygenases, cytochrome P450 enzymes, and lipoxygenases [6]. These biomolecules are the first line cellular structures that are affected by ROS and RNS."

16.2 Reactive Oxygen Species

ROS are free radical species and non-radical species called oxidants. ROS are composed of "free radicals containing oxygen, e.g. hydroxyl radicals, as well as non-radical components, such as ozone and hydrogen peroxide." Free radicals are well known for playing a dual role: it has a damaging effect and is involved in signaling mechanisms. "Hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), and peroxynitrite ($ONOO^-$), among others are oxygen-free radicals called ROS. Other non-radical derivatives of oxygen such as hydrogen peroxide (H_2O_2), are also considered ROS, due to its ability to produce free radicals [5]."

16.2.1 Generation and Sources of Free Radicals and Non-radicals

ROS are produced via the metabolism of normal cell and plays crucial functions biologically. Although ROS are vital to life, they are harmful to macromolecules, owing to their high chemical reactivity. Therefore, defense systems are initiated in cells to checkmate the production of ROS to avert any cellular damage. The defense armory against ROS are mostly enzymatic in nature and they scavenge overproduced ROS. "Examples are superoxide dismutases (SODs), catalase, peroxiredoxins, thioredoxins, and glutathione peroxidases" [6].

Mitochondria as an organelle play significant functions in the production of energy in metabolism, known as adenosine triphosphate (ATP), which occurs during oxidative phosphorylation. During this process, in the electron transport chain, ATP, ROS, and mainly O_2 are generated via electron transport chain (ETC) (Fig. 16.1).

16.2.2 Antioxidants

Antioxidants are substances that have the ability to prevent the oxidation of an oxidizable substrate to a significant extent when found in minimum concentrations. The term "oxidizable substrate" comprises of almost all things present in living cells. Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and can thus prevent or repair the damage done to the body's cells by oxygen. Antioxidants protect by contributing an electron of their own. In so doing, they neutralize free radicals and help prevent cumulative damage to body cells and tissues [5].

Effective antioxidant molecules should contain hydrogen or electron-donating substituents with requisite reduction potentials with respect to those of the redox couple of the radicals to be scavenged and should be able to perform delocalization of the resultant radical. In addition, it must possess transition metal-chelating potential, which is a function of the nature of the functional groups and their framework within the molecule.

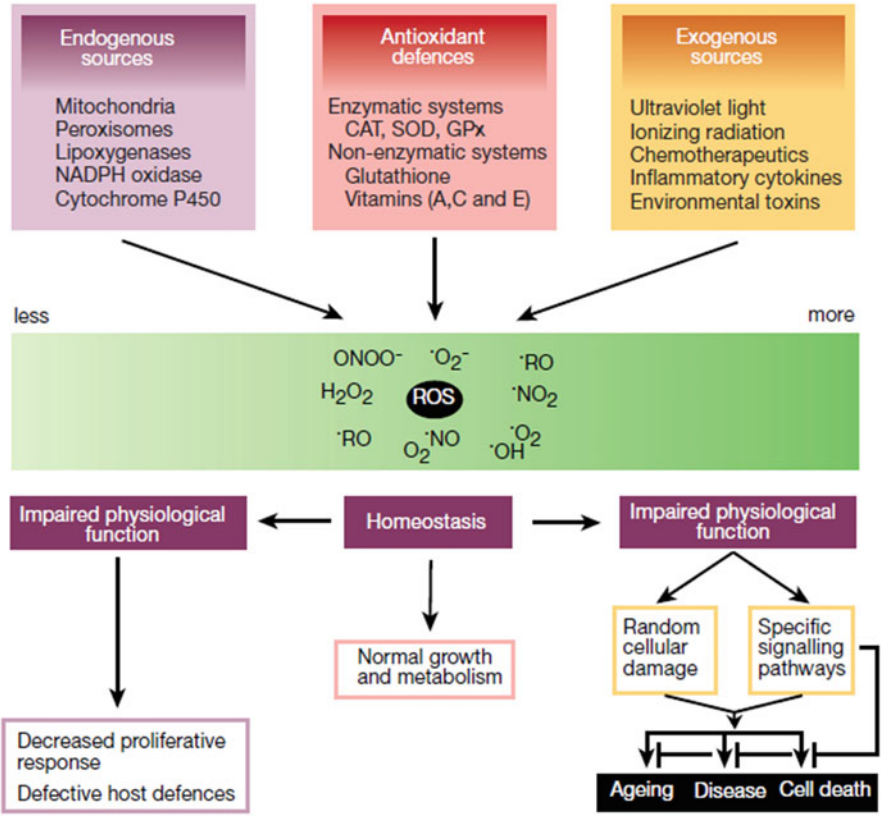


Fig. 16.1 Sources and cellular responses to reactive oxygen species [7]

Oxidative stress is usually counteracted by generating antioxidants from natural sources, "*in situ*" (endogenous antioxidants), or from diets related sources (exogenous antioxidants). Antioxidants act as scavengers of free radicals, reducing agents, chelating factors for transition metals, singlet oxygen molecules quenchers and activators of anti-oxidative defense enzyme mechanisms, to suppress biological systems radical injury [5].

16.2.2.1 Endogenous Antioxidants

Endogenous antioxidants are divided into enzymatic and nonenzymatic antioxidants, which are products of the body's metabolism. They are involved in the neutralization of ROS. Examples includes: glutathione reductase (GRx), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Endogenous Antioxidants

Superoxide Dismutase (SOD)

SOD dismutates superoxide anion into H_2O_2 and O_2, thus protecting oxygen-metabolizing cells against the harmful effects of superoxide free radicals:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Superoxide dismutase is widespread in nature and is domiciled in the mitochondria and cytosol of all oxygen-metabolizing cells. It is noteworthy that SOD is mainly an intracellular enzyme in human tissue. Only trace quantity is found in extracellular fluids such as lymph, cerebrospinal fluids, plasma, and synovial fluid. In mammals, there are several types of SODs, which are different from each other based on their location in cells and the metallic ions required for activity. The activation of SOD by H_2O_2 is likely protected by catalase, with which it is usually combined together [6].

Catalase

This is an enzyme containing iron with a single substrate, H_2O_2. It is found mainly in the small membrane-encased cell compositions known as peroxisomes. It plays significant role in the detoxification of H_2O_2 and other various molecules. Catalase eliminates H_2O_2 by catalyzing a reaction between two H_2O_2 molecules resulting in the formation of water and O_2. It is ubiquitously distributed in all tissues of all species:

$$2H_2O_2 \rightarrow O_2 + H_2O$$

In addition, "catalase can support H_2O_2 interactions with compounds that acts as hydrogen donors so that H_2O_2 can be converted to one molecule of H_2O, and the reduced donor becomes oxidized."

Glutathione Peroxidase

Glutathione peroxidase components comprise many compartments like the enzymes glutathione peroxidase, glutathione reductase, etc. Glutathione is an essential cofactor for antioxidant enzymes, namely GSH peroxidases, which exist in two forms: Se dependent and non-Se dependent. Currently, it is called phospholipid hydroperoxide GSH peroxidase. GSH peroxidases play a major role in detoxifying peroxides in the aqueous phase by conjugating them to GSH [6].

Non-enzymatic Endogenous Antioxidants

Examples of these class of antioxidants are vitamins C, E, and A, glutathione, α-lipoic acid, and carotenoids. Some trace elements such as selenium, copper and

cofactors like folic acid, uric acid, and albumin. Are also part of the nonezymatic endogenous antioxidants.

Vitamin C

Vitamin C as a water-soluble vitamin is significant for the biosynthesis of neurotransmitters, collagens, and carnitines. It has been documented to exhibit antioxidant, immunomodulatory, anticarcinogenic activities, etc. Vitamin C acts, in a synergistic role, with its E counterpart to quash free radicals and to reproduce vitamin E to its reduced version. Acid fruits, green vegetables, and tomatoes are natural origins of vitamin C. It is a labile molecule.

Vitamin E

Vitamin E is soluble in fats and has a strong antioxidant ability. It exists as a chiral-centered compound, having in its stereochemistry eight isomers. The most bioactive form in humans is α-tocopherol. Studies have shown that naturally D-α-tocopherol is nearly twice in action as its synthetic version of L-α-tocopherol. Vitamin E confers protection on cell membranes from free radical damages. The action of α-tocopherol antioxidants involves protection against lipid peroxidation. Vitamin E is also involved in the prevention of some CVDs, ischemia arthritis, and certain neurological disorders.

β-Carotene

β-carotene is also part of the carotenoid family. It is fat soluble, and it can be changed to active form vitamin A, hence the term provitamins. It is changed to retinol, a process fundamental to vision. It has a potent antioxidative property and is very good in quenching singlet oxygen. β-carotene is found in many fruits, vegetables, grains, and oil.

Glutathione (GSH)

"Glutathione (γ-glutamylcysteinylglycine, GSH) is an antioxidant that contains a sulphurhydryl (–SH) group". It is also an antitoxin and acts as a cofactor for enzyme activity. Glutathione is predominantly found in plants, animals, and microorganisms. Its water soluble nature, makes it to be easily dispersed mainly by the cell cytosolic cell components and hydrophilic phases of the living system.

Glutathione exists in two forms: the reduced glutathione known as GSH, which is also a tripeptide, and the oxidized form, glutathione disulfide GSSG, which is also a sulfur–sulfur linked compound. The GSSG/GSH ratio is a likely diagnostic index of oxidative stress. GSH has a strong redox potential (GSH/GSSG, $E'o = 0.33$ v), thus giving it its high-potent electron-donor capacity [6].

16.2.2.2 Dietary Antioxidants

These are antioxidants that are derived from plant food. They are of beneficial functions due to their protective function against oxidative stress, a known pivotal state in the progression of multiple diseases state like a process of carcinogenesis, DM. The use of antioxidants from synthetic sources such as *tert*-butyl hydroxyanisole (BHA) in food industry is well recognized. However, the synthetic antioxidants have some particular disadvantages, such as in instability at increased temperature, toxic properties, its volatility and some legal restrictions been placed on them. Consumers are increasingly avoiding food preparation from these synthetic antioxidants and natural alternatives are thus sought after to achieve better prolonged shelf life of foods and a high sense of food safety [8].

Natural antioxidants abrogate free radicals and help to counteract deleterious effects to body cells and tissues. Hence, the consumption of foods like spices, fruits and vegetables with vast richness in antioxidant is suggested to be a practical intervention towards promoting antioxidant status in humans, by boosting health benefits and preventing ailments. Most of the antioxidative properties of plant food is a result of the presence of phenolic compounds and vitamins in them [8]. "Several methods exist to test the antioxidant properties in foods such as: 2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging method, 1,1-diphenyl-2-picrylhydrazine (DPPH) radical assay, lipid peroxidation (LPO) method nitro blue tetrazolium (NBT) reduction assay or superoxide anion scavenging activity, hydroxyl radical scavenging activity and ammonium thiocyanate (ATC) assay method [8]."

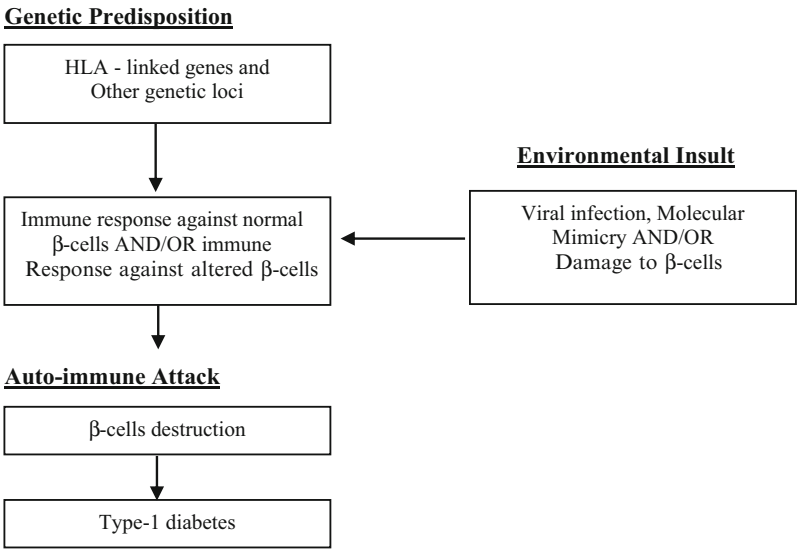


Fig. 16.2 Type 1 diabetes mellitus and its pathogenesis [9]

16.3 Diabetes: Types of Diabetes

16.3.1 Insulin-Dependent Diabetes Mellitus (Type 1 Diabetes)

This type of DM usually results in insulin deficiency, and it occurs when there is inhibition in the generation of insulin from the β-cells of the pancreas. Affected individuals are usually treated by administration of insulin. It is commonly seen in infants and young ones; however, the onset of any disease can occur at any age.

Certain risks factors have been associated with IDDM; these may be autoimmune, genetic, or environmental factors. Several clinical trials have been put into consideration for the prevention and management of IDDM [2] (Fig. 16.2).

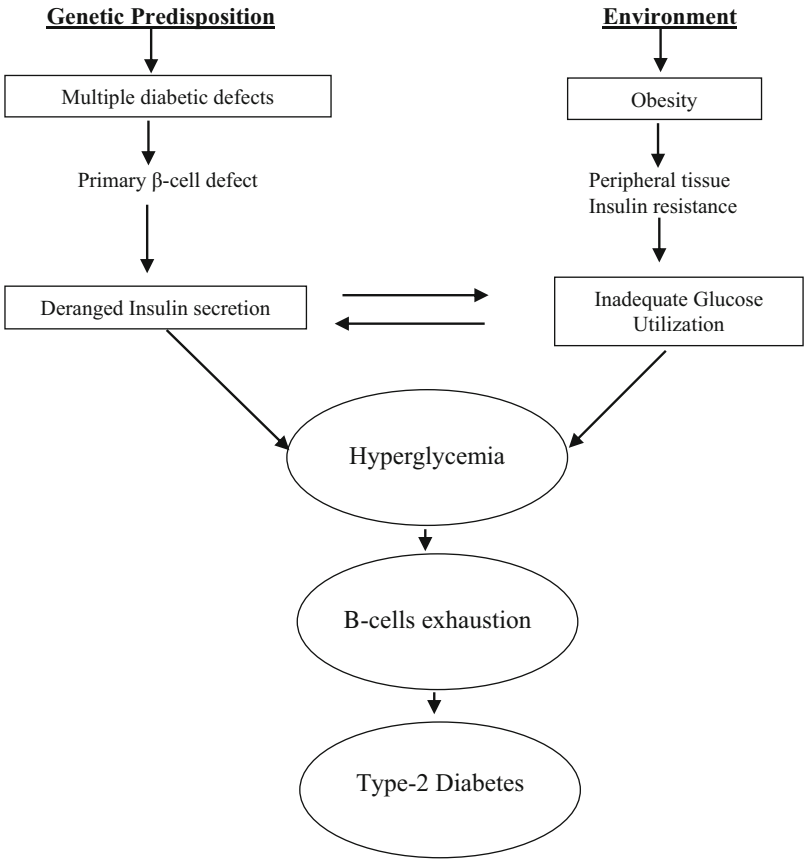


Fig. 16.3 An overview of type 2 diabetes mellitus pathogenesis [9]

16.3.2 Non-insulin-Dependent Diabetes Mellitus

16.3.2.1 Type 2 Diabetes

This is also called non-insulin-dependent diabetes mellitus (NIDDM). NIDDM usually occurs in adults when there is no proper utilization of insulin by the cells. As the need for insulin increases, there is also a decrease in the ability of the pancreas to generate insulin. NIDDM is usually characterized by two metabolic defects, namely dysfunction in the β-cell secretion of insulin and decreased response of peripheral tissues to insulin (insulin resistance).

At the start of NIDDM, the predominant derangement is reduction in insulin awareness with a peculiar feature of increased blood levels of insulin, thus leading to hyperglycemia. This can be prevented by arresting measures that improve insulin awareness or a reduction in glucose production by the liver [9]. At the advance stage of NIDDM, deterioration of insulin secretion disintegrates and replacement of insulin is often a needed therapeutic approach.

NIDDM is associated with the following risk factors: old age, unhealthy lifestyle, obesity, deranged glucose metabolism, and physical inactivity (Fig. 16.3).

16.3.3 Symptoms of Diabetes

The prevalent diabetic symptoms are excessive thirst and hunger, blurred vision, weight loss or gain, fatigue, frequent urination, and slow-healing wounds. Persistent high blood pressure can lead to severe life-threatening complications.

16.3.4 Hyperglycemia-Induced Oxidative Damage

Hyperglycemia has been known to increase free radical concentrations in the plasma, giving birth to oxidative damage. The enolization of glucose usually leads to the production of reduced molecular oxygen, producing oxidized intermediates that promptly result in cellular damage. This process is known as autoxidative glycosylation. The reduced oxygen products formed during autoxidative reactions are $O_2^{\bullet -}$, $OH^{\bullet}$ and H_2O_2. However, these reactive molecules can be deleterious to proteins in the case of cross-linking, fragmentation, and lipid peroxidation.

It has been reported that continuous exposure to elevated level of glucose would lead to an increase in sorbitol and fructose in intracellular cells as a result of increased enzyme activities of aldose reductase (AR) and sorbitol dehydrogenase in non-dependent insulin for the uptake of glucose.

16.3.4.1 Hyperinsulinemia and Oxidative Stress

Reduction in fitness, increased body fat, and fat distribution along the upper body fat are frequently linked to hyperinsulinemia and insulin resistance. Substantive data seem to associate hyperinsulinemia with free radical generation. Exposure of intact

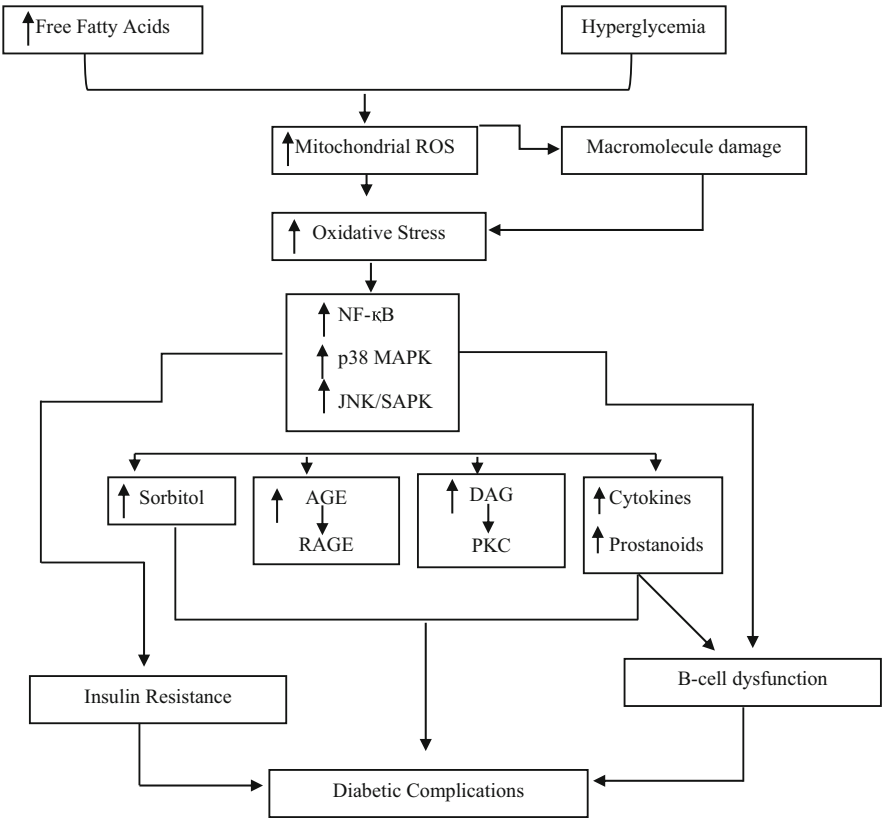


Fig. 16.4 Hyperglycemia-induced diabetic complications [10]

human fat cells to insulin leads to a buildup of hydrogen peroxidase in a time and concentration dependent fashion [9] (Fig. 16.4).

Since a trademark of insulin resistance is fasting hyperinsulinemia, affiliation between insulin resistance and free radical concentration in the plasma cannot be excluded [10]. However, the origin of free radical concentration in insulin unyielding conditions might be as a result of: (1) an insulin-intermediate overdrive of sympathetic nervous system activity; (2) an increase in free fatty acid (FFA) plasma concentration.

16.3.4.2 Polyol Pathway

The polyol pathway of glucose metabolism is initiated under high blood glucose conditions and an estimate of about 30% of internal cell glucose is metabolized in this pathway. However, about 3% of aldohexose is changed into sorbitol under normal glycemic states. The second stage of the pathway is a reversible reaction, which involves the oxidation of sorbitol to fructose catalyzed by the enzyme sorbitol dehydrogenase. The polyol pathway's contribution to oxidative stress caused by

hyperglycemia occurs via three mechanisms. First, the use of nicotinamide adenine dinucleotide phosphate (NADPH) by AR can lead to less availability of a cofactor for glutathione reductase, which is pivotal for the intracellular pool of GSH maintenance. Second, a much improved activity of succinate dehydrogenase (SDH) elevates the concentration of NADPH, which can likely be a substrate for NADPH-dependent oxidase, giving birth to the production of superoxide anion. The third mechanism involves the conversion of fructose to fructose-3-phosphate and 3-deoxyglucosone. "These compounds can form AGEs and consequently fund ROS production [10]."

16.3.4.3 Advanced Glycation End-Product (AGE) Formation

The free amino group of proteins reacts spontaneously with glucose to form Schiff bases. By means of complex reactions, these Schiff bases are converted to AGEs. It is well recognized that AGEs can cause damage to tissues by forming cross-links, which causes alteration of the protein architectural framework. "The interaction of AGE with AGE-cell surface receptors on endothelial cells and macrophages results in cell signaling activation of expression of gene that initiates oxidative stress and inflammation."

16.3.4.4 NADPH Oxidase

Biochemically, the principal origin of ROS is NADPH oxidase, especially the superoxide in the vasculature and kidney. Under pathological conditions, ROS obtained from NADPH oxidase activity have played a crucial role in kidney dysfunctions and damage to the vascular cells. This enzyme is involved in the catalysis of molecular oxygen reduction by NADPH, which acts as an electron donor, hence producing superoxide. The humoral and mechanical signaling causes an upregulation of NADPH oxidase in hypertensive state. The most researched stimulus of NADPH oxidase is AT-II, whereas ET-1 and urotensin II may also likely take part in NADPH oxidase, consequently producing an elevated ROS. It is suggested that the most recognized role of superoxide obtained from NADPH oxidase is nitric oxide (NO) inactivation in the reaction that forms peroxynitrite. This reaction gives birth to endothelial NO synthase (eNOS), which has a strong link to hypertension [8].

16.3.5 Complications of Diabetes

16.3.5.1 Hypertension

Hypertension is a major risk agent for the occurrence of CVDs, and oxidative stress is documented to be a major initiator in the development of CVDs. In experimental studies, a rise in ROS generation, a reduction in nitric oxide (NO) levels, and a decline in antioxidant bioavailability have been observed in hypertensive cases. Superoxide production in the vascular wall is fundamentally obtained by NADPH oxidase when triggered by hormones like endothelin-1 (ET-1), urotensin and angiotensin II (AT-II). Moreover, the mechanical stimuli on the vascular wall also contribute to elevated ROS, which portends hypertension. The production of ROS

causes vasoconstriction, thus leading to increased calcium concentration at the cellular level, thereby partaking in the progression of hypertension [11].

16.3.5.2 Neuropathy

High blood glucose level is involved in a critical function in the advancement of diabetic neuropathy. The mechanism of action through which hyperglycemia induces kidney cell deterioration is by means of elevated oxidative stress occasioned by DM. Metabolic and oxidative insults are known to cause swift changes in glial cells. A diagnostic parameters useful for this scenarios are increased "glial fibrillary acidic protein (GFAP) synthesis and S100B, both astrocytic markers [11].

16.3.5.3 Retinopathy

Retinopathy is a diabetic complication affecting the eye. This is caused by damage to the blood vessels of the light-sensitive retina tissue, hence resulting in vision problems. In the progression of diabetic retinopathy, endothelial and pericytes cells are selectively lost before other histopathological lesions is visible. The capillary walls of the retinal undergoes cell death via apoptosis prior to the progression of other lesions, peculiar of retinopathy in diabetes. This scenario can be a predictive tool for histopathological lesions of retinopathy.

Nuclear transcriptional factor NF-κB, is a key regulator of antioxidant enzymes which is redox sensitive. The activation of NF-κB is regarded as an essential signaling pathway through which hyperglycemia causes programmed cell death in endothelial cells. In the retina, NF-κB is localized in sub-retinal membranes and in microvessels. It is activated very early in the course of development of retinopathy in diabetes. Activated NF-κB binds to nuclear DNA and modulates the expression of several genes. Its amplification cascade in turn results in increased free radical production which can eventually lead to cell death. Recent studies have shown that NF-κB activation in retinal pericytes is responsible for the hyperglycemia-induced accelerated loss of pericytes observed in diabetic retinopathy [10].

16.3.5.4 Nephropathy

Diabetic nephropathy is a kidney damage that arises from having diabetes, wherein leakages of proteins are brought into the urine. It occurs in about one-third of patients with insulin-dependent diabetes and it is the single largest cause of end-stage renal disease requiring chronic dialysis or transplantation. The pathophysiology of diabetic nephropathy is not well defined. Recent studies have indicated that ROS play a key intermediate role in the development of diabetic nephropathy. High glucose directly increases hydrogen peroxide production by mesangial cells and lipid peroxidation of glomerular mesangial cells. Hyperglycemia-induced secondary mediator's activation such as protein kinase C (PKC), mitogen-activated protein (MAP) kinases and cytokine production is also responsible for oxidative stress-induced renal injury in the diabetic condition [2].

16.3.5.5 Erectile Dysfunction

Erectile dysfunction is frequently linked with DM, and it occurs at an earlier age in such patients than in the general population of DM cases. "The pathogenesis of erectile dysfunction as a diabetic complication remains incompletely understood. Diabetes has a known pathologic effect on peripheral tissue innervation and vascularization, both of which are critical for erectile function. Oxidative stress to cavernous tissue may be an important contributory factor to erectile dysfunction in diabetics [8]."

16.4 Inflammation and Inflammatory Processes in Type 2 Diabetes

Inflammation is an essential physiological response of the body to various pathological processes such as pathogen invasion, tissue injury, and irritants. This response involves infiltration and subsequent activation of the innate cells and adaptive immune system to the site of injury and the production cytokines as an example of inflammatory mediators. It is believed that inflammatory mediators are released based on promptings of high glucose concentration and are mediated by oxidative stress. Chronic inflammation and oxidative stress have been implicated in the pathophysiology of diabetes mellitus, and these two are inseparable when it comes to their links to physiological and disease states. Inflammation is the first-line reaction of the immune system to remove pathogens in order to restore normalcy to cells or replace damaged tissue. Following activation, innate immune system cells secrete pro-inflammatory cytokines and chemokines that stimulate the production of ROS and/or reactive nitrogen species (RNS). Pro-inflammatory cytokines can indirectly induce oxidative stress by activating macrophages which is recognized to act in removing the pathogen via the generation of reactive oxygen species. It is important to note that chronic inflammation is a prolonged pathological condition characterized by tissue destruction and fibrosis, culminating in cell damage due to the overproduction of ROS from the inflammatory cells. Consequently, oxidative stress may in turn stimulate the production of inflammatory cytokines and chemokines.

16.4.1 Role of Antioxidants in Type 2 Diabetes

Hyperglycemic conditions in DM promotes "auto-oxidation of glucose to form free radicals, which on excess quantity, beyond the scavenging prowess of endogenous antioxidant armory of defense results in macro- and micro vascular dysfunction." Any substance capable of inhibiting the oxidation of other molecules is termed antioxidant. In recent times, antioxidant therapy is documented to be effective in the management of numerous ailments, especially diabetes. Several studies and research have suggested the efficacy of antioxidants in managing and preventing diabetes complications. The mechanism of action of this antioxidant therapy is the

protection of beta cells against oxidative-stress-induced apoptosis, thus preserving the function of the beta cells.

The therapeutic use of antioxidants can be divided into antioxidant enzyme, combined drugs, synthetic carotenoids, antioxidants, and dietary antioxidants. The most common antioxidants from diets are vitamins C, E, and A [10, 11]."

16.4.1.1 Alpha Amylase

Alpha-amylase inhibitors are also known as Carbo-blockers and these compounds are not directly involved in the weight loss process. However, they are indirectly helpful in weight loss due to inhibition of sugar assimilation, through inhibition of starch breakdown. With reduced amount of α-amylase available for break down, the complex carbohydrate has a better chance of traveling through the body without being assimilated, and is eventually excreted from the body instead of being converted into storage fat.

16.4.1.2 Alpha Glucosidase

Alpha-glucosidase inhibitors are oral antidiabetic drugs used for "DM type-2. It works by preventing the digestion of carbohydrates (such as starch and table sugar). Carbohydrates are normally converted into simple sugars (monosaccharides), which can be absorbed through the intestine. Hence, α-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar. Examples include acarbose, miglitol, and voglibose.

"Acarbose also blocks pancreatic α-amylase in addition to inhibiting membrane-bound α-glucosidases. Pancreaticα-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules."

16.4.1.3 Dipeptidyl Peptidase-4 (DPP-4)

DPP-4 is documented to be linked to T2DM patients suffering from incretin deficiency. DPP-4 is also known as adenosine deaminase binding protein or cluster of differentiation 26 (CD-26). It is a serine exopeptidase capable of inactivating various oligopeptides through the removal of N-terminal dipeptides. Incretins are hormones, which regulate the secretion of insulin after meals. There are two main endogenous incretins secreted by the gastrointestinal tract: (1) glucose-dependent insulinotropic polypeptide (GIP), and (2) glucagon-like peptide 1 (GLP-1). Both proteins enhance the secretion of insulin whilst suppressing glucagon secretion. DPP-4 functions specifically by removing incretin from the body, which is the normal metabolic process in individuals without diabetes. Thus, molecular platforms, which serve as inhibitors of DPP-4, have been recognized as a workable therapy for T2DM management.

Recent drug use involves combined treatments with metformin such as Janumet (sitagliptin and metformin), Jentadueto (linagliptin and metformin), Kazano (alogliptin and metformin), Komboglyze (saxagliptin and metformin) and Oseni (alogliptin and pioglitazone) [12]. Juvisync is a combination of sitagliptin and

simvastatin, which is used as a treatment in individuals with hypercholesterolemia. DPP-4 inhibitors have been implicated in improving blood glucose control and reducing both fasting and postprandial blood glucose levels without causing weight gain. The inhibition of DPP-4 by a chemotherapeutic mediator may enhance the levels of moving endogenous GLP-1 by extending its half-life, which will subsequently increase the advantageous effects of GLP-1 in glucose-dependent insulin secretion and β-cell renovation. DPP-4 is a class of the serine protease family. Other members of this family include fibroblast activation protein-a (FAP), DPP-8, and DPP-9. Selectivity towards the inhibition of DPP-4 over DPP-8 and DPP-9 is mostly attained through empirical iterative optimization. It is essential that DPP-4 inhibitors do not inhibit DPP-8 or DPP-9, as inhibition of these enzymes has been implicated in multiorgan toxicity in rats and dogs, as well as *in vitro* reduction of human T-cell activation. The prevalence of using DPP-4 inhibitors leads to a risk of hypoglycemia being lowered, no weight gain with possible restoration and separation of pancreatic β-cells.

16.5 Cardiovascular Diseases

Cardiovascular diseases are group of diseases that usually affect the heart and blood vessels. Cardiovascular disease is linked with various risk factors including hypercholesterolemia, hypertension, smoking, diabetes, and physical inactivity just to mention a few. It has been established that diabetics are prone to cardiovascular diseases as diabetes itself has been considered an independent risk factor for premature atherosclerosis [2, 8]. The incidence of CVDs in people with DM is three to four times of that of normal individuals. Thus, the diabetic state itself is an independent risk factor for premature atherosclerosis. One of the potential mechanisms that could mediate the premature atherosclerosis in diabetes is oxidative stress.

Increased concentrations of autoantibodies to both oxidized and glycated LDL have been documented in diabetes suggesting that in NIDDM, enhanced oxidative stress occurs *in vivo* and that LDL glycation may represent a predisposing event that facilitates subsequent oxidative modification. Several lines of evidence support a proatherogenic role for oxidized LDL (Ox-LDL) and its *in vivo* existence. Ox-LDL is not recognized by the LDL receptor but by the scavenger receptor pathway on macrophages, which results in unregulated cholesterol accumulation, leading to foam cell formation.

16.5.1 Hypertension

16.5.1.1 Oxidative Stress and Hypertension

Hypertension is due to persistent increase in blood pressure. Oxidative stress constitutes a united basis for the occurrence of disease scenarios that are occasioned by excessive production of ROS. The ROS family constitutes several molecules that

have a broad spectrum of effect on cellular function. Notably, many of these ROS activities are associated with morphological observations seen in CVDs. The effects of ROS are triggered via a redox-sensitive control of multiple-signaling molecules and second messengers [7]. In addition, nitric oxide (NO) has been found to play a significant role in maintaining normal physiological conditions within the cardiovascular system. However, reduced NO generation has been attributed to increased production of ROS. There is also an increased production of ROS when the level of angiotensin-II (a vasoconstrictor) increases above normal level, thus elevating the level of blood pressure [7].

16.5.1.2 Inflammation and Hypertension

Inflammation is a strategy developed by higher organisms to protect themselves during cellular invasions like microbial infection, injury to tissues, and other toxic states. An important response by the immune system is the host enabling the removal of the harmful stimuli and the healing of damaged tissue. The molecular mechanism behind the inflammatory process is very complex, which is triggered by the recognition of specific molecular signs associated with either infection or tissue injury. The whole state of inflammatory response is controlled by a variety of key regulators included in the selective expression of pro-inflammatory molecules. Prolonged inflammations are often related to deleterious side effects on the health. Alterations in inflammatory responses due to persistent inducers or genetic variations are on the increase in the past years, causing a variety of inflammatory diseases and pathophysiological conditions.

Several studies have shown that numerous clinical trials conducted on the management of hypertension commonly involve increased plasma CRP (C-reactive protein) levels. However, CRP has been considered to be one of the strongest inflammatory markers that have been greatly associated with hypertension. More so, CRP plays a significant role in stimulating monocytes to release pro-inflammatory cytokines.

Inflammation is a protective response to injury or infection. It is a complex process that involves inflammatory cells first identifying the affected tissue, leukocyte recruitment into tissue, elimination of the offending agent and repair of the site of injury. Inflammation requires interactions between cell surfaces, extracellular matrix and proinflammatory mediators. Excessive inflammation can have detrimental effects and contribute to the progression of chronic and/or prolonged diseases such as atherosclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

"CRP can stimulate monocytes to release proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), tumour necrosis factor alpha (TNF-α) and also endothelial cells to express intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, effects which will further promote inflammation. CRP is considered the inflammatory marker with the strongest association with hypertension." It has been demonstrated in numerous clinical trials that hypertensive patients commonly have increased plasma CRP levels.

16.5.1.3 Endothelial Dysfunction

Traditional cardiovascular risk factors such as diabetes, hypertension, dyslipidemia, and tobacco toxins are associated with endothelial dysfunction. Endothelial dysfunction is one of the detrimental effects of hypertension. "Healthy endothelial vessel walls become stiffer, and vasorelaxation is diminished. As such, measures of endothelial dysfunction can be used as a marker for hypertension. One of the main causes for endothelial dysfunction is the reduced availability of nitric oxide (NO), resulting in increased oxidative stress in the endothelium. Endothelial dysfunction can also precede hypertension, and less compliant vessels will establish higher blood pressures." Eventually, endothelial dysfunction leads to a pro-inflammatory, prothrombotic, vasoconstrictive state with increased cell adhesion and oxidative stress.

16.5.1.4 Inflammation: Myeloperoxidase Activity

Myeloperoxidase (MPO) is a member of the superfamily of heme peroxidases that is mainly expressed in neutrophils and monocytes. "MPO-derived reactive species play a key role in neutrophil antimicrobial activity and human defense against various pathogens primarily by participating in phagocytosis. Elevated MPO levels in circulation are associated with inflammation and increased oxidative stress. Multiple lines of evidence suggest an association between MPO and cardiovascular disease (CVD) including coronary artery disease, congestive heart failure, arterial hypertension, pulmonary arterial hypertension, peripheral arterial disease, myocardial ischemia/reperfusion-related injury, stroke, cardiac arrhythmia and venous thrombosis. Elevated MPO levels are associated with a poor prognosis including increased risk for overall and CVD-related mortality. Elevated MPO may signify an increased risk for CVD for at least two reasons. Firstly, low-grade inflammation and increased oxidative stress coexist with many metabolic abnormalities and comorbidities. Consequently, an elevated MPO level may represent an increased cardiometabolic risk in general. Secondly, MPO produces a large number of highly reactive species which can attack, destroy or modify the function of every known cellular component. The most common MPO actions relevant to CVD are generation of dysfunctional lipoproteins with an increased atherogenicity potential, reduced NO availability, endothelial dysfunction, impaired vasoreactivity and atherosclerotic plaque instability. These actions strongly suggest that MPO is directly involved in the pathophysiology of CVD. As a consequence, MPO may be seen as a mediator by which inflammation promotes CVD at molecular and cellular levels [13].

16.5.1.5 Vascular Damage: Nitric Oxide Production

NO has an essential role as a key paracrine controller of vascular tone. At physiological level, NO inhibits leukocyte–endothelial cell adhesion, VSMC proliferation and migration, and platelet aggregation to maintain the health of the vascular endothelium. Therefore, it has many beneficial effects. The decrease in bioavailability of NO in the vasculature reduces vasodilatory capacity and contributes to

hypertension. The enzyme that catalyzes the formation of NO from oxygen and arginine is nitric oxide synthase (NOS).

Receptor-mediated agonist stimulation leads to rapid enzyme activation. Furthermore, shear stress and allosteric modulators are important modulators of eNOS activity. In addition to its vasorelaxing and antiproliferative roles, NO has an important role in antagonizing the effects of AT-II, endothelins and ROS. NO diffuses as gas to the adjacent smooth muscle wherein it interacts with different receptor molecules, such as the soluble guanylyl cyclase.

The normal production of NO has a crucial role in the maintenance of physiological conditions within the cardiovascular system. L-arginine, which is a substrate for eNOS, seems to be a promising molecule in the preservation of NO formation. However, L-arginine failed to prevent blood pressure increases and left ventricle remodeling due to chronic treatment with methyl ester of *N*-nitro-L-arginine, which is an inhibitor of eNOS. The ACE inhibitor captopril completely prevented NO-deficient hypertension without improving NOS activity. NO also has an ACE down-regulating effect. Thiols protect NO from oxidation by scavenging ROS and by forming nitrosothiols. Both effects prolong NO half-life and the duration of NO action.

16.5.2 Damage to Critical Metabolic Pathway

16.5.2.1 RAAS (Renin–Angiotensin–Aldosterone System)

The renin–angiotensin system plays a key role in the development of cardiovascular disease. AT-II is a potent vasoactive peptide that can be formed in vascular beds that are rich in ACE. When AT-II production increases above normal levels, it induces vascular remodeling and endothelial dysfunction in association with increases in the levels of blood pressure. As a potent activator of NADPH oxidase, AT-II contributes to the production of ROS. In rats and mice where hypertension is induced by AT-II infusion, the expression of NADPH oxidase subunits, oxidase activity and the generation of ROS are all increased. AT-II not only increases NADPH oxidase activity but also upregulates superoxide dismutase activity, possibly to compensate the increased levels of ROS. In situations where this compensatory effect is efficient, ROS levels may appear normal even under prooxidant conditions. However, when ROS production becomes overwhelming, compensatory mechanisms are inadequate and pathophysiological consequences occur.

16.5.2.2 Acetylcholine Release

Acetylcholine induces endothelium-dependent dilation via the production of endothelial factors, mainly NO, which then diffuses to the underlying VSMC, thereby inducing VSMC relaxation. The decrease in NO bioavailability will significantly lead to reduced acetylcholine-mediated vasodilation. The consequence of an overall increase in ROS is a reduction in the bioavailability of NO.

ET-1: endothelins is one of the vasoconstrictor isopeptides that are produced in different vascular tissues. “ET-1 is the main endothelin generated by the

endothelium and is the most important endothelin in the cardiovascular system. When ET-1 is administered in high concentrations, it behaves as a potent vasoconstrictor that is capable of exerting an array of physiological effects, including the potential to distort arterial pressure. ET-1 mediates its effects via two receptors, ETA and ETB. The ETA receptor mediates contractions via the activation of NADPH oxidase, xanthine oxidase, lipoxygenase, uncoupled NOS and mitochondrial respiratory chain enzymes. The ETB receptor induces relaxation in endothelial cells. Many factors that normally stimulate ET-1 synthesis, (for example, thrombin and AT-II) also cause the release of vasodilators, such as prostacyclin (PGI2) and/or NO, which oppose the vasoconstricting function of ET-1."

16.6 Complication of Hypertension

16.6.1 Kidney Damage

ROS have been confirmed to have a key role in the pathophysiological processes of several renal diseases. These renal diseases are known to be involved in the induction of hypertension. Regarding glomerular alterations, ROS act as an intermediary between lipoprotein glomerulopathy and other inflammatory glomerular lesions. A recent study showed that NADPH oxidase activation and the production of ROS through lipid raft clustering is an important molecular mechanism that triggers homocysteine-mediated oxidative injury of podocytes. This injury may represent an early event that initiates glomerulosclerosis during hyperhomocysteinemia. One of the underlying mechanisms of ROS-mediated tubulointerstitial injury is the exposure of tubular cells to LDL, which may result in tubulointerstitial damage due to NADPH oxidase-mediated ROS production. AT-II has a vital role in obstructive nephropathy as it activates NADPH oxidase, thereby generating superoxide that leads to hypertrophy of the renal tubular cells.

There are several oxidative-stress-mediated mechanisms involved in endothelial dysfunction in chronic kidney disease (CKD). "ROS are elevated in CKD and are related to endothelium-dependent vascular reactivity and systolic blood pressure. High ROS and increased levels of the endogenous asymmetric dimethylarginine were reported to be novel risk factors for endothelial dysfunction. Moreover, high levels of asymmetric dimethylarginine were reported in CKD and were associated with increased intima-media thickness and cardiovascular events." In renovascular hypertension, oxidative stress in the ischemic kidney plays a major role in the maintenance of hypertension in two-kidney, one-clip rats [13].

16.7 Conclusion

Diabetes mellitus and cardiovascular diseases rank among the paramount causes of death across the globe. They are global public health problems affecting the poor, the rich, the educated, and the uneducated people, as well as the urban and rural dwellers

in both developed and developing countries. Different factors have influence in the pathogenesis and progression of diabetes mellitus and cardiovascular diseases. This review focused on the role of oxidative stress and inflammation in the pathogenesis and progression of diabetes mellitus and cardiovascular diseases. It critically examined the interplay between oxidative stress, inflammation, diabetes mellitus, cardiovascular diseases, and their complications based on existing scientific evidence. The evidence from this review is very clear and shows that inflammation plays a role in the development and progression of diabetes mellitus. It shows that inflammatory mediators such as interleukin-1-beta 6 and tumor necrosis factor alpha are greatly altered in type 2 diabetes mellitus and cardiovascular diseases. These mediators contribute immensely to increased generation of reactive oxygen species and oxidative processes, together with hyperglycemia and dyslipidemia, leading to several complications. In the presence of defective antioxidant defense system, resulting from either endogenous antioxidant alteration or exogenous inadequacy, that tilts the oxidative balance in favour of reactive oxygen species, oxidative stress develops. Various experimental and clinical studies (*in vitro*, *in vivo*, *in silico*, *ex vivo* and clinical trials) support the direct link between oxidative stress and diabetes mellitus via the measurement of oxidative biomarkers in diabetic, non-diabetic and cardiovascular diseases conditions." Oxidative stress is strongly involved in chronic hyperglycemic-induced insulin resistance. To the best of our knowledge, the review has been able to bridge the gap between the release of inflammatory mediators, the production of reactive oxygen species, and oxidative stress in the incidence and pathogenesis of diabetes and cardiovascular diseases.

References

1. Mazidi M, Kengne AP, Mikhailidis DP, Toth PP, Ray KK, Banach M (2017) Dietary food patterns and glucose/insulin homeostasis: a cross-sectional study involving 24,182 adult Americans. Lipids Health Dis. 16:192–197. https://doi.org/10.1186/s12944-017-0571-x
2. Oguntibeju OO (2019) Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 11(3):45–63
3. Evans JL, Maddux BA, Goldfine ID (2005) The molecular basis for oxidative stress-induced insulin resistance. Antioxid. Redox Signal. 7:1040–1052. https://doi.org/10.1089/ars.2005.7.1040
4. Zheng Y, Ley SH, Hu FB (2018) Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 14:88–98
5. Liochev SI (2013) Reactive oxygen species and the free radical theory of aging. Free Radic. Biol. Med. 60:1–4
6. Holmström KM, Finkel T (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat. Rev. Mol. Cell Biol. 15:411–442
7. Kangralkar VA, Patil SD, Bandivadekar RM (2010) Oxidative stress and diabetes. Int J Pharm Appl 1(1):38–45
8. Adefegha SA (2018) Functional foods and nutraceuticals as dietary intervention in chronic diseases; novel perspectives for health promotion and disease prevention. J Diet Suppl 15 (6):997–1009
9. Cheng AYY, Funtus IG (2005) Oral antihyperglycemic therapy for Type-2 diabetes mellitus. Can. Med. Assoc. J. 172:213–226

10. Evans JL, Goldfine I, Maddux BA, Grodsky GM (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocrine Rev 23(5):599–622
11. Adefegha SA, Oboh G, Olasehinde TA, Boligon AA (2018) Dietary supplementation with Ethiopian pepper (*Xylopia aethiopica*) modulates Angiotensin-I converting enzyme activity, antioxidant status and extenuates hypercholesterolemia in high cholesterol fed Wistar rats. Pharma Nutr 6:9–16
12. Gallwitz B (2011) Small molecule dipeptidyl peptidase IV inhibitors under investigation for diabetes mellitus therapy. Expert Opin Investig Drugs 20:723–732
13. Einarson TR, Acs A, Ludwig C, Panton UH (2018) Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007–2017. Cardiovasc. Diabetol. 17:83

Correction to: Oxidative Stress and Immunological Complexities in Multidrug-Resistant Tuberculosis

Ridhima Wadhwa, Nikita Sehgal, Naresh G, Taru Aggarwal, Saurabh Satija, Meenu Mehta, Gaurav Gupta, Dinesh K. Chellappan, Murtaza M. Tambuwala, Brain Oliver, Trudi Collet, Pawan K. Maurya, Philip M. Hansbro, and Kamal Dua

Correction to:
Chapter 7 in: P. K. Maurya, K. Dua (eds.),
***Role of Oxidative Stress in Pathophysiology of Diseases*,**
https://doi.org/10.1007/978-981-15-1568-2_7

The online version of the book was inadvertently published with an error in the last name of one of the authors in Chapter 7 as Murtaza M. Chellappan. The name has been corrected as Murtaza M. Tambuwala.

The updated version of this chapter can be found at
https://doi.org/10.1007/978-981-15-1568-2_7

P. K. Maurya, K. Dua (eds.), *Role of Oxidative Stress in Pathophysiology of Diseases*, https://doi.org/10.1007/978-981-15-1568-2_17