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Introductory Chapter

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1. Introduction

Oxidative stress, which will be defined and described in details below, is inevitable attribute of most strong stresses. In this book, the induction of oxidative stress by environmental challenges like physical, chemical as well as biological factors is described. These factors can induce oxidative stress in direct and non-direct ways, which will be covered by several chapters. Substantial bulk of chapters will describe the defensive mechanisms against deleterious effects of reactive species in different organisms. The book gives a broad description of the processes related to production of reactive species and their elimination. Particular attention will be given to natural and chemically synthesised antioxidants.

2. Introduction in oxidative stress theory

Free radicals are relatively unstable particles with one or more unpaired electrons on outer atomic or molecular orbitals. Many of them have as short life time and they can exist for only microseconds or even less. That is why most scientists for long time believed that free radicals were too unstable to exist in biological systems. The presence of free radicals in biological systems was discovered about 60 years ago and was virtually immediately implicated by Rebecca Gerschman and colleagues (1954) in human diseases. Two years later Denham Harman (1956) suggested that free radicals could be involved in pathologies as well as animal and human aging, and he first proposed free radical hypothesis of aging. Since 1950th critically important discoveries on roles of free radicals in living organisms promoted deep understanding that they are involved in many pathologies of animal and human organisms. D. Harman also specified later mitochondria as a place in the cell principally determining lifespan and proposed that mitochondria could be the "biological clock" and in this manner govern longevity, and further the hypothesis proposed was developed in mitochondrial theory of aging with key role of free radicals (Harman, 1972). Investigations on ROS roles in living organisms, particularly, in organisms' aging culminated by the formulation of free radical theory of aging (Harman, 1983), which in different formulations has been applied to all organisms – bacteria, fungi, plants and animals (Lushchak, 2011a). In 1995, D. Harman was nominated for the Nobel Prize in medicine for his works on the role of free radicals in diseases and aging. It seems that among all theories of aging, the Harman's one has the most consistent experimental support to date. The development of the theory extended it to age-related pathologies and also disturbances not directly related to aging.

It should be noted that now the term "reactive oxygen species" (ROS), which include oxygen free radicals along with some other activated oxygen forms like peroxides (e.g. H_2O_2), is more commonly used than "oxygen free radicals" to underline the existence of activated oxygen forms with non-radical nature. The investigation with many organisms resulted in disclosing of molecular mechanisms leading to increased ROS production, corruption of defense systems and different combinations of these routs. The interest to free radical processes was stimulated by the discovery of enzymatic mechanism of ROS elimination by the enzyme superoxide dismutase in 1969 by Irvin Fridovich and Joe McCord (1969). Several years later, nitric oxide as one more reactive form was found to play important regulatory roles in muscle relaxation and many other processes (Gruetter et al., 1979). This led to discovery of nitric oxide synthase (NOS). Reactive species were also found to be involved in defense mechanisms of immune system for attack of invaders (Klebanoff, 1967). Identification of enzymatic finely controlled systems of ROS production like NADPH-oxidases producing $O_2^{\bullet -}$ and H_2O_2, and NOS producing $^{\bullet}NO$, filled up the gape to view free radical processes as controlled ones. Helmut Sies (1985) was the first who defined "oxidative stress" as "Oxidative stress" came to denote a disturbance in the prooxidant-antioxidant balance in favor of the former". Extensive investigations in the field of free radical processes and their role in living organisms as well as ROS dynamics, regulation and consequences of imbalance between production and elimination let me propose the next definition of oxidative stress: "Oxidative stress is a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents" (Lushchak, 2011b). In this definition, the dynamic character of ROS-involving processes and their effects on core and regulatory processes in living organisms are underlined.

To date, development of oxidative stress was described in all phyla of organisms – bacteria, fungi, plants and animals. Although ROS are mainly supposed to play negative roles in living organisms, more and more data accumulated demonstrate their involvement in regulation of many physiologically important processes such as development, metamorphosis, morphogenesis, aging, etc. Reactive species do that either directly affecting certain systems or influencing specific regulatory pathways. The question on the specificity of ROS-involving processes is very important and to now it is responded in complicated way as the concerting type, spatio-temporal production, available direct targets and sensors. In many cases, these issues have been described in details, although the chemical instability of reactive species dictates specific rules in the "game" with them.

3. Induction of redox disbalance

3.1 Stimulation of ROS production

High production of ROS is usually implicated as the main mechanisms for oxidative stress induction. Therefore, here I suppose to characterize briefly the main known to date sources of reactive species. They are electron transport chains (ETC) of mitochondria, endoplasmic reticulum (ER), plasmatic and nuclear membranes, photosynthetic apparatus in plants; certain oxidative enzymatic reactions catalysed by specific oxidases; and autooxidation of endogenous and exogenous (xenobiotics) compounds.

Reactive species may be generated due to "leakage" of electrons from electron transport chains. In mitochondria electrons can escape the electron transport chain in several places, but mainly at the level of coenzyme Q and complex III. In this case, electrons interact with molecular oxygen resulting in formation of superoxide anion radical, which further spontaneously or enzymatically at operation of superoxide dismutase can be converted to hydrogen peroxide. Similarly to mitochondria, in photosynthetic apparatus, leakage of electrons also leads to production of superoxide anion radical and hydrogen peroxide. However, here the light energy absorbed may result in formation of other ROS, for instance singlet oxygen (Hideg et al., 2011). In electron transport chain of endoplasmic reticulum, the electrons transported may also escape to oxygen with the production of corresponding ROS. Here, this process is catalyzed by the enzymes of cytochrome P450 family. It should be noted that ER may be a place of ROS production not only as the result of direct operation of cytochromes. Compounds transformed here not being initially ROS generators may become them after transformation followed by entrance in reversible autooxidation. The nuclear membrane, particularly nuclear pore complex, can also be ROS producer (Hahn et al., 2011). Xantine oxidase and glucose oxidase are the best known oxidases generating ROS during catalytic acts. Xantine oxidase can produce superoxide anion radical via NADH-oxidase activity and nitric oxide via nitrate and nitrite reductase activities (Berry and Hare, 2004), whereas glucose oxidase catalyses the oxidation of glucose to D-glucono-δ-lactone with co-production of hydrogen peroxide (Raba and Mottola, 1995). Reactive species may also be produced by certain oxidases of amino acids and polyamines.

NADPH oxidase of plasmatic membranes is a specific enzymatic system known to produce reactive species (Sirker et al., 2011). Using NADPH the enzyme adds electrons to molecular oxygen that was first found in phagocytic cells and implicated to be responsible for killing of microorganisms either intra- or extracellularly. The enzymes of this class were found in most animals and plants. Now it is known that they are not only responsible for attack of invaders, but also generate ROS for signaling purposes (Sirker et al., 2011). The system is under strict control, because ROS overproduction is harmful for the cell. The second group of enzymes, NOS produce $^{\bullet}NO$ in very well controlled manner similarly to NADPH oxidase. Nitric oxide is used not only for signaling purposes, but also to kill microorganisms (Vazquez-Torres et al., 2008). Moreover, in phagocytic cells two abovementioned enzymes cooperate to enhance the antimicrobial effects. The products of these enzymes namely, superoxide anion radical and nitric oxide, interact with the formation of very powerful oxidant peroxinitrite. Although the latter is not a free radical, it was found to be capable to enter nitrosylation reactions modifying in this manner proteins and nucleic acids. Moreover, it can spontaneously decompose with the formation of one of the most active oxidants – hydroxyl radical. These two enzymatic systems, in cooperation with myeloperoxidase, producing very strong oxidizing agent hypochlorite ion (ClO^{-}), also known as chlorate (I) anion, are responsible for antimicrobial activity of phagocytic cells (Arnhold and Flemmig, 2010).

Finally, different small molecules may enter autooxidation reactions and being capable of revesible oxidation can donate electrons to molecular oxygen and other compounds. Catecholamines, polyamines, polyphenols and some other endogenous compounds are known to enter autooxidation. However, most attention in this direction is paid to exogenous compounds (xenobiotics) capable to generate ROS in the organisms via

autooxidation process. Xenobiotics affecting living organisms via generation of reactive species include number of pesticides, ions of metals with changeable valence, some industrial chemicals, pollutants, drugs, etc. (Lushchak, 2011b). It is important to note, that many xenobiotics may initially not be capable to enter autooxidation, but after certain reactions carried out by enzymatic systems may become ROS generators. For example, some chlorinated phenolic compounds, which are not ROS generators, after hydroxylation in ER by cytochrome P450 become potential ROS sources (Dreiem et al., 2009).

As we could see, there are number routs of ROS generation in living organisms. So, there are also many potential possibilities to increase ROS production. In electron transport chains, it may be reached by the inhibition of electron flow through the transport chains in different manners. For instance, mitochondrial ETC operation may be inhibited by the limitation of oxygen supply, or presence of cyanides and other respiratory toxins, which inhibit cytochrome oxidase. In the case of plastid ETC in plants, high intensity illumination can significantly increase production of singlet oxygen, $O_2^{\bullet-}$, and H_2O_2. The stimulation of general oxygen consumption due to increased energy needs at the change of physiological state of organisms may also enhance electron flux through the ETC resulting in extra ROS production. The increment of ROS production in ER may be related to the presence of substrates for oxidases like at ethanol oxidation in liver of animals (Yang et al., 2010), or methanol oxidation in certain yeasts (Ozimek et al., 2005), and after oxidation the formed products may enter autooxidation.

Some microorganisms, components of their bodies or excreted products can stimulate ROS production by animal immune system (Langermans et al., 1994). The process is tightly controlled by the immune system cells via reversible phosphorylation of NAPH oxidase and NOS, or by second messengers like calcium ions. Concerning the most chapters in this book, it is worthy to note that environmental factors can be very powerful inducers of ROS production in all living organisms. They may do this via different mechanisms. But according to materials of this subsection, we have to mention mainly the introduction of xenobiotics, which may enhance ROS generation. Of course, organisms possesses powerful and efficient antioxidant systems defending them against ROS.

3.2 Depletion of antioxidants

The second principal way to increase the steady-state ROS level is connected with depletion of antioxidant system, which consists of both enzymatic and non-enzymatic components. The first includes so-called antioxidant enzymes directly dealing with ROS and are represented by superoxide dismutases, catalases, peroxidases including glutathione-dependent ones, thioredioxine reductases, etc., and associated ones supplying reductive equivalents, building blocks for antioxidant synthesis, and energy sources (Hermes-Lima, 2004a,b).

The activity of antioxidant enzymes can be decreased in different ways. First of all, they can be inactivated in direct and non-direct ways. For example, certain pesticides may extract from enzyme molecules metal ions needed for catalytic activity. For example, copper ions may be removed from Cu,Zn-SOD by diethyldithiocarbamate (Lushchak et al., 2005). The activity of catalases can be decreased due to interaction of aminotriasole pesticides with iron ions in active centre of the enzymes (Bayliak et al., 2008). The second way leading to

decreased activities of antioxidant enzymes is connected with direct chemical modification, for example, by oxidation (Wedgwood et al., 2011) or interaction with diverse compounds like carbohydrates (Shin et al., 2006). Finally, the activity of antioxidant enzymes can be decreased due to suppressed expression of corresponding genes or stimulated degradation.

Depletion of reserves of low molecular mass antioxidants also can result in the development of oxidative stress. This group of antioxidants consists of tocopherols, carotenoids, antocyanes, ascorbic and uric acids, etc. Glutathione, a cysteine-containing tripeptide (γ-glutamyl-cysteinyl-glycine) is important endogenous antioxidant, level of which is tightly controlled by the organisms at stages of biosynthesis, transport and consumption (Lushchak, 2011c). In any case, depletion of reserves of low molecular mass antioxidants may decrease the efficiency of elimination of reactive species that can result in increased steady-state ROS levels and lead to development of oxidative stress. Once oxidized by reactive species, cellular components usually became not effective components of living organisms. Therefore, there are two principal routs to deal with them: reparation or elimination.

Cells actively fix ROS-caused damages to DNA (Lu et al., 2001) and some oxidized amino acid residues in proteins can be also repaired (Lushchak, 2007). That needs operation of very efficient specific reparation mechanisms. After oxidation carbohydrates, lipids, proteins, RNA and free nucleotides are further mainly degraded with very few exceptions described for proteins. The necessity to degrade nonfunctional constituents is not only dictated by their useless, but also potential hazard due to disruption of cellular structures like membranes and cytoskeletons. In addition, in many cases the products of ROS-induced modification of lipids, carbohydrates, proteins and nucleic acids can themselves generate reactive species. It is absolutely clear, that oxidatively modified cellular components should be degraded, and this work is mainly carried out by diverse hydrolases like lipases, proteases, nucleases, etc.

4. Induction of oxidative stress

The factors, which induce oxidative stress, can be grouped in external (physical and chemical) and internal. The physical factors include variation of temperature, light and irradiation. The chemical factors consist of diverse compounds of various natures, which entering organisms cause increase in levels of reactive species. Finally, internal factors may not be directly related to metabolism of reactive species, but induce oxidative stress in non-direct way like energy depletion.

The potential mechanisms of oxidative stress induction by physical factors include both activation of ROS production and corruption of ROS-eliminating routs. Increased temperature may disturb membrane structure enhancing electron leakage from electron-transport chains and their interaction with molecular oxygen. Illumination by visible light may transform some photosensibilizators entered organisms like quercetin via excitation to activated electron donors. Another mechanism of ROS generation by extensive illumination can be connected with light absorbtion by specific cellular compounds like chlorophylls of thylacoids or eye retina. Radiation dependently on the type and intensity may either corrupt defense mechanisms or at extensive irradiation promote homolytic fission of covalent bonds followed by ROS formation.

Due to many reasons, most attention in environmentally induced oxidative stress field is paid to chemicals. The compounds can enter organisms via different routs – with food and beverages, through lungs, skin, and gills. There are several groups of mechanisms of oxidative stress induction by exogenous compounds (xenobiotics): (*i*) compounds once entered the organism may be directly involved in redox processes yielding ROS; (*ii*) in organism some chemicals may be converted to redox active compounds due to metabolism; and (*iii*) the compounds entering organisms may non-directly stimulate ROS production or corrupt defense systems. Certain compounds may realize their effects via several mechanisms simultaneously.

This book provides the information on induction of oxidative stress in diverse living organisms by physical and chemical factors. Substantial part of the book is devoted to antioxidants, i.e. compounds protecting an organism against deleterious ROS effects.

5. Acknowledgments

The editor would like to thank all authors who participated in this project for their contributions and hard work to prepare interesting chapters on the induction of oxidative stress by physical and chemical factors as well as protection of organisms against deleterious effects of reactive species by antioxidants. I also thank to colleagues from Precarpathian National University, who helped to develop the ideology of this book during many years of collaboration, helpful, creative, and sometimes "hot" discussions, which stimulated to perfect my knowledge on the role of reactive species in diverse living processes. I am also grateful to the "In-Tech" Publisher personnel, especially to Ms. Sasa Leporic who excellently assisted me in the arrangement of the book and scheduling the activities.

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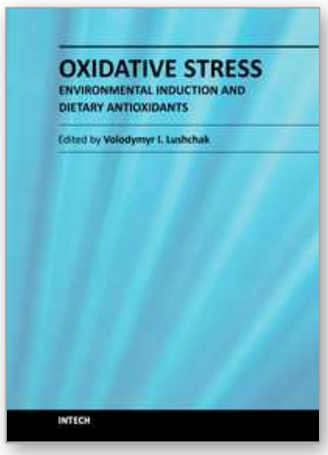

Oxidative Stress - Environmental Induction and Dietary Antioxidants

Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0553-4
Hard cover, 388 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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