OXIDATIVE STRESS

ENVIRONMENTAL INDUCTION AND DIETARY ANTIOXIDANTS

Edited by **Volodymyr I. Lushchak**

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Preface

Free radicals discovered in biological systems in 1950es were immediately suggested to be involved in diseases and aging (Harman, 1956; 1985). The term "free radicals" was later extended to denote a wider group of activated oxygen forms whose activity is higher than molecular oxygen, and were collectively named reactive oxygen species (ROS), which include singlet oxygen, superoxide anion radical, hydrogen peroxide, hydroxyl radical, and many of their derivatives. In 1969, J. McCord and I. Fridovich described the catalytic function for erythrocuprein (hemocuprein) as superoxide dismutase responsible for elimination of the superoxide anion. The information on free radical processes in biological systems allowed Helmut Sies (1985) to systematize "Oxidative stress" and came to denote a disturbance in the prooxidant-antioxidant balance in favor of the former. Recently, we modified this definition as "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). The last definition included accumulated the up-to-date knowledge on the effects of ROS on core and regulatory processes, and underlined the idea on their steady-state level in biological systems. Our understanding of the ROS roles in biological systems has gone through three phases: their appreciation as damaging ones, protection against infections and, finally, signaling and regulatory molecules in diverse biological processes. We can now state that all listed components operate in organisms in concert and are absolutely necessary for realization of biological functions.

Intensive research was invested into discovering whether the environmental factors can affect intracellular ROS steady-state levels. That resulted in understanding that this level may be modified by many external physical, chemical and biological factors. Since it is difficult to register ROS levels *in situ*, these data were mainly gained through indirect methods with the evaluation of levels of ROS-modified molecules of both external and internal origin. Therefore, this book mainly contains the information on oxidative stress induced by physical and chemical factors and a portion of the book includes the information on antioxidants capable to modify ROS levels.

On January 2, 2012, a Google Scholar search for "oxidative stress environment" yielded about 589,000 publication hits, whereas in Scopus and Pubmed databases it yielded 4,428 and 6,302 hits, respectively. We have presented 17 chapters in this book,

covering several important aspects of environmentally induced oxidative stress and its prevention by antioxidants. Since oxidative stress seems to be an inevitable component of virtually all stresses that are strong enough, the book provides the interested readers with information needed to recognize this.

The Introduction section (V. I. Lushchak) covers general aspects of oxidative stress theory and briefly analyses potential ways of oxidative stress induction by environmental factors – stimulation of ROS production and depletion of antioxidants. The role of antioxidants is also highlighted.

The book is divided into four parts. The first section, entitled "Physical Factors" demonstrates the induction of oxidative stress by exercise, light and temperature fluctuations. The chapter written by V. Lj. Jakovljevic and colleagues extensively introduces the biology of reactive oxygen and nitrogen species, measurement of redox status, levels of superoxide anion radical, hydrogen peroxide, glutathione, lipid peroxides, activities of superoxide dismutase and catalase, and then demonstrates that exercise may increase the production of ROS and modify redox status. Interestingly, it has been demonstrated that perturbations of free radical processes depend on the intensity and type of exercise, as well as specialization of athletes and their physical state. Different light types possessing high energy can also induce damage to cellular components, even in specialized organs. K. Engelmann et al. described the operation of human retina, ROS-related processes, protective role of specific parts of the light spectrum and retina protection by tinted intraocular lenses in detail. The next two experimental chapters deal with oxidative stress induced by temperature changes – in fungi and plants. Using two Antarctic fungi, *Penicillium sp.* and *Aspergillus glaucus*, N. Kostadinova et al. demonstrated a relationship between cold shock and oxidative stress evidenced by an increased level of oxidized proteins and activation of antioxidant enzymes. Since abscisic acid may increase freezing tolerance of plants, M. E. Mora-Herrera et al. were able to demonstrate that ther decrease in temperature affected the level of hydrogen peroxide and catalase isoforms in potato microplants, which was related to tolerance to low temperatures.

The induction of oxidative stress by chemical factors is presented in the second section of the book. Ions of metals may induce oxidative stress in at least two ways – entering Fenton reaction and replacing other metal ions in their binding centers (Valko et al., 2007). The detailed description of toxicokinetics of lead and cadmium, induction and role of oxidative stress in neurochemical changes in the hypothalamus and pituitary of F1 generation PND 56 male and female rats are presented by P. Pillai et al. Herbicides are well known inducers of oxidative stress and many mechanisms were described in this case. 2,4-Dichlorophenoxyacetic herbicide is one of the broadly used ones, and W. Tayeb et al. describe the general phenomenology and potential mechanisms of induction of oxidative stress in different organisms. The chapter by O. B. Stoliar and V. I. Lushchak is devoted to analysis of oxidative stress induced in fish by different environmental pollutants.

The next section is devoted to induction of oxidative stress by biological factors. Diverse pathogens invading the host organism are attacked by the immune system equipped by machinery to produce reactive species. R. C. Ebel and N. Kumar investigated the involvement of reactive oxygen species in combating *Xanthomonas citri pv citri* (Xcc), causing citrus canker in *Citrus sp.* and found that pathogen-induced oxidative stress was differently expressed in different representatives of the genera studied. K. Okabayashi et al. were able to demonstrate that ethacrynic acid, a thiol-modulating reagent, inhibited amylase release induced by β-adrenergic agonist in rat parotid acinar cells and the effect was independent of depletion of glutathione in the cells. The authors concluded that the inhibitory effect of ethacrynic acid on amylase release induced by β-adrenergic agonist was caused by the thiol-modulation of β-adrenergic receptors.

It is very attractive to use antioxidants to prevent ROS-induced modification of organisms' functions. Intuitively developed at the beginning of ROS investigation in living organisms, it looked promising to use them for prophylactics and treatment of ROS-modulated damages. However, the promises were not realized and it became clear that there are no absolutely direct links between ROS-induced changes and pathologies. The last section of the book presents a broad discussion of positive effects of diverse antioxidants. The Estonian team led by T. Kullisaar provides an interesting topic – after short surveys on probiotics and oxidative stress they share extensive information on the potential use of different probiotics in functional foods and capsules that may be helpful to combat oxidative stress related to many pathologies, like cardiovascular diseases, metabolic syndrome, allergy, atopic dermatitis, radiation-induced problems in the intestinal tract. Diabetes is a very common human disease, which, in addition to health problems caused, is accompanied by many complications related with oxidative stress and the system character of the pathology therefore clearly needs specific approaches. It is very attractive to use a food stuff instead drugs and B. Alipoor et al. describe the potential of one of the most common drinks, tea, with health benefits particularly for diabetes and related complications. Sulphur mustard as a bifunctional alkylating agent readily reacts with a variety of macromolecules including nucleic acids, proteins and lipids, as well as small molecular mass metabolites such as glutathione, which is in the focus of chapter written by R. Vijayaraghavan and A. Gautam. Since sulphur mustard also induces oxidative stress, antioxidants can be useful and the authors analyze available data on the use of flavonoids, particularly from *Hippophae rhamnoides*. Bee products accompanied people since ancient times and only now do we start to understand the molecular mechanisms of many processes modulated by these products. Therefore, P. Tatli Seven provide an extensive analysis of beneficial properties of propolis with the focus on its antioxidant, antimicrobial, anti-inflammatory and antitumor effects. The antioxidant potential of 152 samples of Thai fruits, vegetables and herbs, and 33 brands of tea was measured by W. Sangkitikomol and this study shows that the products are a good source of compounds with health benefits. Since the toxicity of cyanide is associated with the induction of oxidative stress, F. G. Elsaid suggests and proves that it can be

reduced by the application of aqueous extracts of *Allium kurrat* and *Ricinus communis* which possess antioxidant properties. Due to high sugar and fat diets and sedentary lifestyles, modern people are frequently subjected to atherosclerosis and obesity, which are important risk factors for metabolic syndrome and greatly predispose individuals to liver diseases, cardiovascular disease, type 2 diabetes, dyslipidemia, hypertension and numerous cancers, and is associated with markedly diminished life expectancy. The French team (S. Gaillet, D. Lacan, J.-M. Rouanet) presents results of titanic systematic work to identify the beneficial diets and find a broad set of diary foods and beverages possessing antioxidant properties and helping to combat the mentioned pathologies. These products are fresh and possessed fruits grapes, and berries, preparations from them as well as selenium-enriched microalgae, algal and fungal polysaccharides. Recently, while screening more than 250 cyanine dyes for their neurotrophin-like activity, the compound called NK-4 and some related compounds were found to be potent neurotrophic agents for the promotion of growth and differentiation of neuronal rat adrenal pheochromocytoma cell line PC12. NK-4 is a divalent cationic pentamethine trinuclear cyanine dye that contains three quinolinium rings, N-alkyl side chains, and two iodine anions. In the last chapter of the book, the Japanese team (H. Ohta, K. Akita & T. Ohta) summarized the data on the biological effects in different models and found that NK-4 possesses free radical-scavenging activity, neuroprotective against various cytotoxic stresses, neuroprotective effects against β-amyloid (Aβ) toxicity, and intracellular signaling. Therefore, the authors suggest that this dye can be used to protect animal organisms against neurodegeneration.

This book is expected to be interesting to experts in the field of basic investigations of reactive oxygen species and oxidative stress, as well as to practical users in the diverse fields like environmental sciences, medicine, and toxicology.

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Section 1

Introduction

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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 •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