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Chapter 1

Pathological Aspects with Global Impact Induced by Toxicants at Cellular Level

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Additional information is available at the end of the chapter

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1. Introduction

In toxicology, the term "toxicant" defines a noxious substance that induces a series of deleterious effects on organs, tissues and biological functions and processes in living organisms. Another term used to describe a toxicant is "poison" [1]. The list of toxicants regardless their origin, of natural sources or ensued from human activities, is quite long.

Some of the main detrimental responses that a toxicant is able to generate at cellular level include: production of reactive oxygen species (ROS) and free radicals. These "basic" processes could be associated with carcinogenesis, immunotoxicity, teratogenesis and genotoxicity. The toxic mechanism of action in such cases is initiated by a terminal toxicant and a target molecule and might involve different types of reactions, including: covalent or non-covalent bonds, hydrogen subtraction, electron transfer, and enzymatic reactions.

The cascade of processes that occur at cellular level and involve ROS is initiated by events like ischemia and lipid peroxidation, which are noticed after the first exposure to reactive metabolites and are considered primary events. As secondary events were described important processes as follows: changes in structure and permeability of membranes, mitochondrial dysfunctions, cytoskeletal and DNA changes, lysosomal destabilization, intervention in apoptosis/necrosis and endoplasmic reticulum destruction. The final step of the cascade is associated with severe pathological destruction on organs level.

In the past decade, the toxicity of heavy metals and their risks on human health has been a subject of high interest, an argument in this regard being the impressive number of publications available (over 2000 articles according to PubMed database). Heavy metals are inorganic elements, natural components of earth's crust, and are labeled as the oldest toxins known by humans [2]. It has been demonstrated that heavy metals induce toxicity at different levels in

human body, including: gastrointestinal system, central and peripheral nervous systems, cardiovascular, renal and hematopoietic systems [2]. As regards the toxic mechanism of action of heavy metals, it has been stated that generation of reactive oxygen species represents one of the main mechanisms involved in heavy metals induced-toxicity. It is believed that generation of reactive oxygen species is responsible for the hepatotoxicity, neurotoxicity and nephrotoxicity associated to heavy metals [2, 3].

Free radicals and reactive oxygen species generated by toxicants were described to hold key roles in lipid peroxidation, DNA damage, oxidation of sulfhydryl groups of proteins, depletion of protein, and alteration of calcium homeostasis [2, 4].

Reactive oxygen species (ROS) are oxygen-free radicals that contain one or more unpaired electrons, formed during oxidative metabolism and were characterized as exceedingly active compounds which act by inducing oxidative changes of cellular proteins, lipids and polynucleotides [5-7].

Under normal conditions, ROS play essential functions in cellular homeostasis, as signal molecules in several signaling pathways involved in cell differentiation, organogenesis, stress response and wound healing, and as redox regulators [6]. Oxidative stress represents a status characterized by excessive cellular levels of ROS as a result of an imbalance in the redox homeostasis explained by increased production of ROS or declined antioxidant capacity [2, 6, 7]. A considerable number of studies endorse the fact that oxidative stress is linked to a plethora of pathologies including cardiovascular diseases, atherosclerosis, diabetes, chronic inflammatory processes, neurodegenerative disorders, and mostly to cancer [6-8].

This chapter summarizes an update of available data regarding ROS in physiological and pathophysiological conditions, the roles of ROS in cancer and heavy metals induced toxicity via ROS generation.

2. Redox homeostasis and ROS generation

The term “redox” refers to the oxidation-reduction status and is considered a key regulator of several metabolic cellular functions [9] and a fundamental keeper of cellular homeostasis [10].

During redox processes that occur in the cells are generated a variety of reactive oxygen species with functional roles in physiological and pathological conditions dependent on cell’s capacity to maintain the ratio between ROS production and ROS disposal in balance. Commonly, the term “redox signaling” is used to express the changes of protein’s oxidation status resulted in ROS-mediated events at cellular level [11].

The oxidative stress is characterized by a globally enhancement of intracellular ROS levels appeared from a dysfunction of the mechanisms involved in maintaining redox homeostasis: increased ROS generation or declined capacity of ROS elimination [10]. Mounting evidence suggest that oxidative stress is implicated in various pathologies such as aging, neurodegenerative disorders, development of brain damage, pathogenesis of multiple sclerosis lesions and cancer [10, 12, 13].

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ROS are highly reactive molecules, derived from oxygen, ceaselessly generated during oxidative metabolism and exuded into biological systems; outcomes of one or multielectron reductions of oxygen [7, 14]. The balance between ROS production and ROS disposal is ensured by the cell's keepers, antioxidant enzymes (superoxide dismutase – SOD, glutathione peroxidase, catalase and thioredoxin reductase) and non-enzymatic scavengers (ascorbate, tocopherols, tocotrienols, carotenoids, natural flavonoids, melatonin, gluthatione, thioredoxin) [7, 14].

ROS can be generated by multiple endogenous and exogenous sources. The main endogenous source of ROS is mitochondria, which produces reactive species as by-products of normal cell metabolism during the electron leakage that passes in the conversion of molecular oxygen process. Hereupon it might be added the activity of some enzymes, like: membrane-associated NADPH oxidases, cytochrome p450s, P-450-dependent monooxygenases, lipoxygenase, cyclooxygenase and xanthine oxidase [6, 13]. At mitochondrial level responsible for the formation of ROS are complexes I (NADH dehydrogenase (ubiquinone)) and II (succinate dehydrogenase), which produce ROS on the inner side of mitochondrial matrix, and complex III (ubiquinol-cytochrome c reductase) that delivers the generated species (superoxide radical) into the intermembrane space or mitochondrial matrix [14].

Other endogenous sources of ROS are the microsomes and peroxisomes (generate especially H_2O_2) and it was, also, demonstrated that immune cells' (neutrophils and macrophages) mechanism of action against invading microorganisms involves ROS [10]. The biosynthesis of prostaglandins, prostacyclins and thromboxane A2 from arachidonic acid, process catalyzed by cyclooxygenases, it is also a source of ROS [13, 15].

As exogenous sources of ROS, there were indicated the following agents: atmospheric pollutants, tobacco smoke, irradiation (UV irradiation, x-ray, gamma-ray), chemicals, iron salts, heavy metals and chemicals [2, 10, 14].

The group of reactive oxygen species comprises two different kinds of species:

a. free radicals – possess unpaired electrons in their outer orbitals: superoxide anion radical ($O_2^{\cdot -}$), hydroxyl radical (OH•), hydroperoxyl radical (HOO•), alkoxy (RO•) and peroxy radicals (ROO•), and

b. non-radical oxygen species – possess unpaired electrons, too, are very reactive and are able to form ROS radicals: hydrogen peroxide (H_2O_2), organic hydroperoxide (ROOH), ozone (O_3) and trioxidan (HOOOH) [2, 6, 14].

Free radicals (also known as pro-oxidants) can be recognized by some specific features, like: high instability and reactivity, the presence of unpaired electrons in the outmost orbital of their atoms, the need to acquire equilibrium by bonding with electrons of neighboring atoms, what leads to chain reactions and the ability to react with different cellular molecules [16, 17].

Some of the main ROS species will be presented in Table 1, outlining the generation process and their specific characteristics.

ROS name	Generation	Characteristics
$O2^{-}$, superoxide anion	- *at mitochondrial level:* as a side-product of mitochondria formed during aerobic metabolism; there are two sites of mitochondrial ROS production, complex I (NADH – ubiquinone oxidoreductase) and complex III (ubiquinol - cytochrome c oxidoreductase) [10, 18, 19] $O_2 + e^- \rightarrow O_{2\cdot}$ [20] - *enzymatically:* it is produced is by NADPH oxidase (Nox) expressed in the phagocytes' cell membrane, by cytochrome P450-dependent oxygenases in the endoplasmic reticulum of the liver, lung and small intestine and by the xanthine oxidase (XO) located in the cytosol [14, 16, 18, 19, 21, 22].. - *non-enzymatically*: the generation process consists in the transfer of a single electron to oxygen by coenzymes in reduced form, flavins or iron sulfur clusters or xenobiotics that suffered a reduction reaction [19, 23].	- is described as the first free radical obtained during mitochondrial electron transfer chain process and it is easily converted to hydrogen peroxide via a dismutation reaction catalyzed by superoxide dismutase (SOD) [6] - it's a short-lived molecule and presents only one reduction equivalent - it cannot be considered a candidate molecule for signal transduction in the cell since the ability of this radical to cross the mitochondrial outer membrane is rather low [14] - at mitochondrial level it is involved in the generation of peroxynitrite ($ONOO_{2}^{\cdot-}$), a noxious oxidant that induces DNA damage, disruption of mitochondrial integrity, and irreversible modification of proteins [14] - releases Fe^{2+} from iron-sulfur proteins and ferritin [2] is the precursor of most ROS and a mediator in oxidative chain reactions [19]
H_2O_2, hydrogen peroxide	- by direct reduction of O_2 [2] -by dismutation from superoxide radical under the action of superoxide dismutases (SOD1 – in mitochondrial intermembrane space and SOD2 – in mitochondrial matrix) [11, 16, 24]. $2\ O_2^{\cdot-} \rightarrow H_2O_2 + O_2$ -up-to 80% of H2O2 is formed by peroxisomal (eg. D-amino acid oxidase, D-aspartate oxidase or polyamine oxidase) and microsomal (microsomal CYP-mediated ω-oxidation of fatty acids) enzymes [4, 18, 25, 26].	- presents a two-electron reduction state [2] - is a mitochondrial ROS, a electrophobic molecule able to pass through membranes H2O2 [2, 11]. - mitochondrial concentrations of hydrogen peroxide are 100 times greater than that of superoxide anion [11]. - it can be regarded as a fundamental ROS in carcinogenesis [18]. - its conversion into oxygen and water is mediated by catalases and gluthatione peroxidases [18]. - is a strong oxidant, precursor of hydroxy radical (·OH) via Haber Weiss reaction [18]. - due to its lipophilic character lightly crosses mitochondrial and plasmatic membranes reaching into the cytosol and extracellular environment where asserts its effects [14]. - has the capacity to generate highly reactive hydroxyl radicals via reactions with metals (iron and copper) [20, 27].

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ROS name	Generation	Characteristics
HO•, hydroxyl radical	- via Fenton reaction from hydrogen peroxide [10, 16, 19]. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + OH^-$ - by Haber-Weiss reaction - oxidation of superoxide anion radical [19]: $O_2^{\cdot -} + H_2O_2 \rightarrow HO\cdot + OH^- + O_2$ -by decomposition of peroxynitrite [2]	- presents a three-electron reduction state [2] - is a extremely reactive specie with a short half-life [2, 18]. - induces DNA damage by generating 8-hydroxy 2'-deoxyguanosine (8-OHdG), molecule responsible for DNA mutations, a key factor for the carcinogenic risk [18]. - induces changes to other cellular molecules, changes defined by enzymes' denaturation, proteins' structure modifications or peroxidation of polyunsaturated fatty acids [18].
$HO_2^{-\bullet}$, hydroperoxyl radical	- is formed when an oxygen molecule binds to a proton [16] - it can be obtained during the second step of oxygen complete reduction reaction [20]: $O_2^{\cdot -} + H_2O \rightarrow HO_2^{\cdot -} + OH^-$ -via Fenton reaction [20] $Fe^{2+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\cdot -} + H^+$	- this oxygen-centered reactive specie is capable to abstract H from a lipid molecule (polyunsaturated fatty acid), particularly in the presence of metals like copper or iron, leading to an autocatalytic chain reaction. The result of this process is the formation of a lipid hydroperoxide (or peroxide) [20, 28].

Table 1. Description of the main ROS species.

3. ROS in physiological conditions

Reactive oxygen species are continuously produced in the cells, especially during mitochondrial electron transport chain and eliminated in biological systems, where in physiological conditions (low levels), they play crucial roles in the regulation of different cell's functions, including cell proliferation, apoptosis, transformation, and senescence [7].

The mechanism of action of ROS in activation of cell proliferation or different signal pathways implies the interaction between ROS and cysteine residues leading to the formation of disulfide bonds and activation of signal transducing pathways. The activation of these processes may occur via kinase activation or phosphatase inhibition, and via regulation of proteinases, including matrix metalloproteinases (see Figure 1) [14].

Besides their toxic effects, it was demonstrated that reactive oxygen species interfere in main cellular processes (differentiation, organogenesis, wound healing, cell fate regulation), in the activity of different enzymes (kinases and phosphatase), transcription factors, ionic channels and transporters [6]. Moreover, it appears that ROS acts as an essential cellular messenger alongside with the acknowledged second messengers (Ca^{2+}, arachidonic acid, cAMP and IP3) [6].

Pan and coworkers stated that ROS are involved in the microbicidal activity of phagocytes, regulation of signal transduction and gene expression, and act as inducers of oxidative damage to macromolecules (nucleic acids, proteins, and lipids) (see Figure 1) [29].

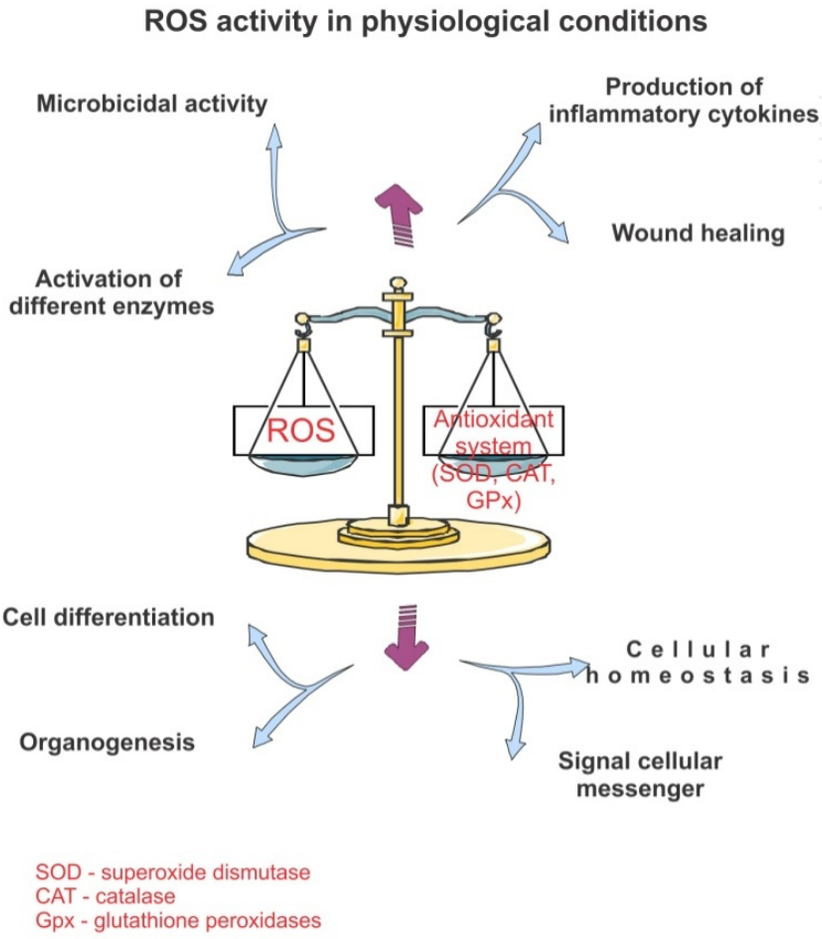

Figure 1. ROS cellular functions in physiological conditions (the picture was obtained by using Servier Medical Art templates).

Most of the free radicals generated by various cellular metabolic systems originate from oxygen. The percent of molecular oxygen that is converted into superoxide and hydroxyl radicals at mitochondrial level is 5%. Mounting evidence indicates that the free radicals resulted have major function in the normal metabolism of cells: they are used in the synthesis of prostaglandins, cholesterol and steroidal hormones. Furthermore, the biosynthesis of collagen demands the participation of hydroxyl free radicals [16].

The effects and functions of ROS are distinct and dependent of their concentration, for example: low concentrations of mitochondrial ROS are associated with metabolic adaptation in hypoxic conditions; moderate concentrations are involved in the regulation of inflammatory response and high levels stimulate apoptosis/autophagy pathways responsible of inducing cell death [11].

Other beneficial effects of ROS refer to their involvement in the intracellular killing of bacteria by neutrophil granulocytes, detoxification of the liver and certain cell signaling processes [20, 30].

There is also considerable information regarding the roles of mitochondrial ROS (superoxide anion and hydrogen peroxide) in inflammatory cytokine production and innate immune responses by activation of newly characterized RIG-I-like receptors (RLRs), inflammasomes, and mitogen activated protein kinases (MAPK) [11, 31, 32].

4. ROS in pathophysiological conditions

The phenomenon characterized by an impaired balance between ROS generation and ROS elimination is known as oxidative stress. The oxidative damage was frequently associated with different pathologies including: diabetes type II, neurodegenerative disease, atherosclerosis, multiple sclerosis, chronic inflammatory disease, aging and carcinogenesis (see Figure 2). The ability of free radicals to chemically react with most of cell components it was considered a risk, especially for large molecules (nucleic acids, proteins, polymerized carbohydrates - polysaccharides), and lipids, since these components are targets for the oxygenated free radicals [16,20].

The free radical induced-toxicity at cellular level is expressed as lipid and protein peroxidation and damaged nucleic acids (see Figure 2) [20]. The hydroxyl radical is the most reactive free radical molecule capable to cause severe cell damage and to other intracellular structures due to its ability to induce covalent cross-linking of a variety of biological molecules [20].

Superoxide anion is a reactive oxygen molecule that plays important roles in the body because is considered the precursor of the other free radicals that determine cell injury. The toxic mechanism of action of this free radical involves the disassembly of iron-sulphur ([Fe–S]) clusters in proteins via the inactivation of iron regulatory protein-1 (IRP-1), leading to clearing of iron and damage of –SH residues [20,34].

The noxious effects of ROS might be exerted at: DNA level, also known as DNA oxidation what leads to mutations and possibly cancer [20,33]; at protein level causing enzyme inhibition, denaturation and protein degradation, and at lipid level leading to lipid peroxidation [20]. ROS-induced DNA peroxidation inhibits gene transcription and determines gene mutations. The main toxic products resulted from ROS-induced DNA damage are 8-hydroxyadenine (8-OH-Ade), 8-hydroxyguanine (8-OH-Gua), 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, Tg) [19].

The lipid peroxidation process is a chemical chain reaction that consists of three stages: initiation, propagation and termination [10,20]. During the initiation stage occur the following processes: a ROS (usually a hydroxyl radical due to its high reactivity) reacts with a peroxide-free lipid system in order to remove a hydrogen (H) atom from a methylene group ($-CH_2-$), the result being the generation of free radicals such as conjugated dienes and peroxyl radical [10,20].

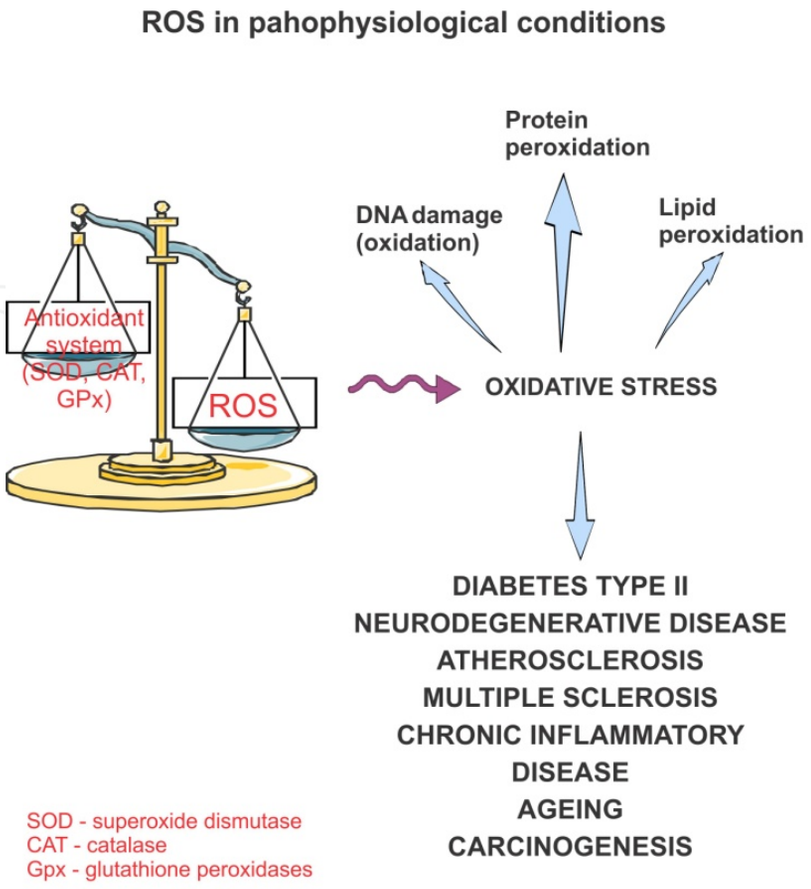

Figure 2. ROS cellular functions in pathophysiological conditions (the picture was obtained by using Servier Medical Art templates).

The propagation stage is characterized by the attack of the peroxyl radical resulted during the initiation stage to other lipid molecules (fatty acids) via an autocatalytic chain reaction catalyzed by metals such as iron or copper and it results a lipid hydroperoxide (or peroxide) [10,20]. Polyunsaturated fatty acids are considered easy targets for lipid peroxidation due to the unsaturated chemical structure. The ROS-induced peroxidation end products (α, β unsaturated reactive aldehydes, such as malondialdehyde - MDA, 4-hydroxy-2-nonenal - HNE, acrolein and isoprostanes) have also deleterious effects on tissues (see Figure 3) [10,20,35].

The injury caused by ROS to membrane phospholipids can affect in a cascade manner the membrane integrity, followed by altered membrane integrity, suppression of enzymes and membrane receptors, an increased tissue permeability, an impaired cellular function and cell death [10,20,29].

The end-products of lipid peroxidation possess a high reactivity and can easily interact with proteins, phospholipids and nucleic acids via different reactions resulting stable products (adduct between proteins and lipid peroxides or glycation products) with potential roles in the pathogenesis of several maladies [20, 36]. The stable products mediate cell death by

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aggregation of bulky protein complexes which inhibit the activity of 26S and 20S proteosome and determine accumulation of injured proteins (see Figure 3) [10].

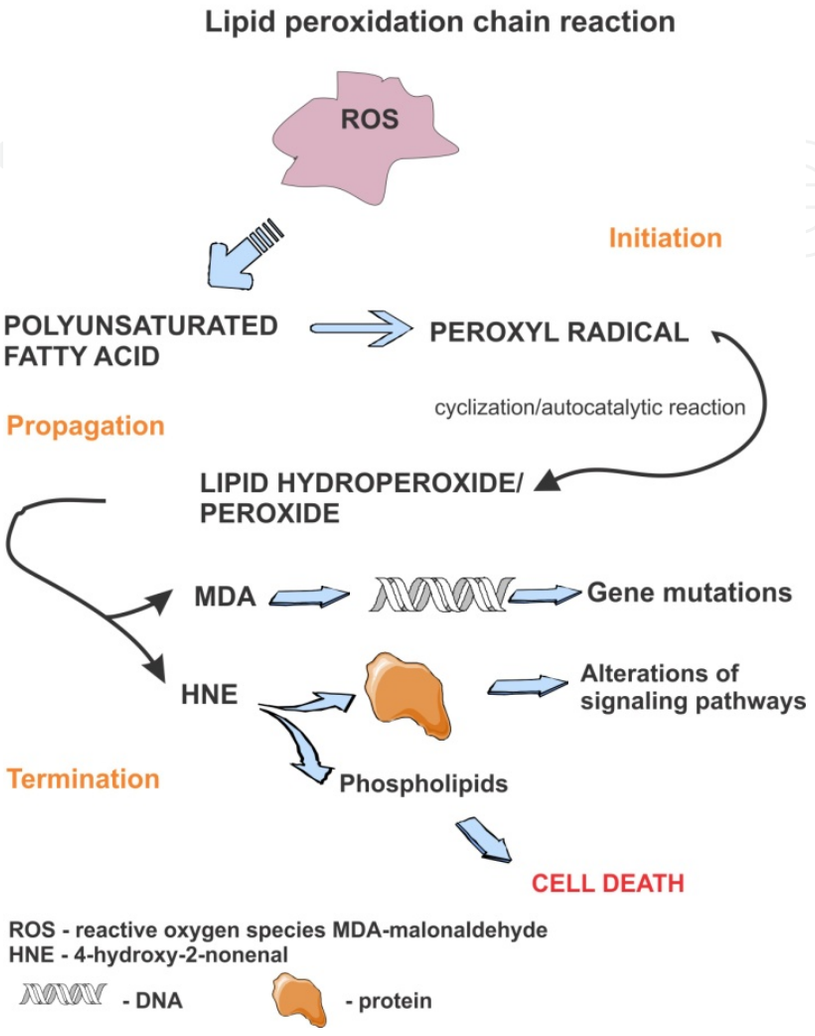

Figure 3. ROS-induced lipid peroxidation (the picture was obtained by using Servier Medical Art templates).

Recent studies demonstrated that ROS is involved in the mitochondrial energetically dysfunction associated to ethanol-induced gastric mucosa damage [29,37]. It was stated that impairment of mitochondria and/or up-regulation of NADPH-oxidase complex are linked to various cancers and play major function in establishing the anticancer therapeutic strategies [14].

In the last years, the subject regarding the roles of ROS in cancer gained a lot of interest. The cellular injury initiated by ROS is considered an important step in cancer development. It seems like the signal transduction messenger function of ROS is associated with cancer initiation by activation of different signaling pathways like MAPK, PI3K and NF-κB [7].

The mechanism of action of ROS in ageing is still a matter of debate. There are studies that sustain the fact that elevated ROS levels along with mitochondrial dysfunction and metabolic

alterations are features of cellular senescence. Other studies have proposed the hypothesis that ROS is cause and consequence of NF-κB pathway activation during senescence [38].

5. ROS and cancer

The imbalance between cellular generation of ROS and its removal leads to oxidative stress which was associated, among other deleterious effects, with the initiation and progression of cancer.

Regarding the carcinogenesis, it is important to note that reactive oxygen species are considered to be regulators of the following major signaling mechanisms: extracellular signal-regulated kinases (ERKs), mitogen-activated protein kinases (MAPKs), phosphoinositide 3-kinases (PI3Ks) and transcription factors such as hypoxia-inducible factors (HIFs). All of these signaling pathways play key roles in cell proliferation, cell growth and cell survival. It was observed that high levels of ROS can induce irreversible oxidative damage in lipids, proteins and nucleic acids. Furthermore, ROS are active in multistage carcinogenesis from initiation to malignant conversion, by inducing oxidative DNA damage and mutations in protooncogenes and tumor suppressor genes, and subsequent activation of signal transduction pathways [14].

In the first stage of carcinogenesis, cancer cells usually express genetic instability and a significant increase in ROS concentration as a consequence of a "vicious circle": ROS induce genetic mutations (especially in mitochondrial DNA), which lead to metabolic dysfunction and additional ROS generation [14].

ROS cause almost all forms of DNA damage, such as changes of the nucleotide bases, strand breakage and DNA protein cross-links, but the end-products depend on the type of ROS. It was mentioned that the mutations induced by specific ROS are implicated in the genesis of cancer. Another mechanism of action of ROS in carcinogenesis was to induce and keep the oncogenic phenotypes of tumor cells. At present, oxidative stress is widely accepted as a key contributor to cancer development [14,29].

Previous studies have demonstrated that oxidative stress is associated with carcinogenesis and is also related to the incidence of cancer [39,40]. During the carcinogenesis process, the imbalance between ROS production and ROS elimination is represented by the increased concentrations of reactive oxygen species in cancer cells and a reduction of antioxidants levels. The increase of ROS in these cells occurs due to the influence of intrinsic or extrinsic factors, resulting in gene mutations and changes in transcriptional processes as well as changes in signaling pathways and, ultimately, the occurrence of cancer [40]. Other contributory factors for the enhanced production of ROS in cancer cells are: cancer-associated fibroblasts (CAFs), cancer-associated macrophages (CAMs), and hypoxia. Cancer-associated macrophages are able to generate ROS via NADPH oxidase in tumor cells [39].

It was also shown that ROS affects the expression of the p53 suppressor gene which is a key factor in apoptosis. In addition, oxidative injury induced by changes in gene expression, cell

proliferation, apoptosis, and angiogenesis plays a significant role in tumor initiation and progression [39].

There are recent studies that sustain the idea that ROS induced by oxidative stress might lead to apoptotic or necrotic cell death of skin cells. Especially, the accumulated ROS plays a critical role in the intrinsic aging and photo-aging of human skin in vivo, what leads to the hypothesis that ROS are responsible for different skin cancers and other cutaneous inflammatory maladies. Ultraviolet radiation type B (UVB) is considered a complete carcinogen and generates increased levels of ROS, leading to oxidative damage at skin level. According to several studies, exposure of mammalian skin cells to UVB radiation determines alterations of cellular function via oxidation of macromolecules, DNA damage, generation of ROS, and changes in signaling pathways. As major sources of H_2O_2 were described UVB-induced leukocyte infiltration in the skin, and inflammatory leukocytes and it was stated that H_2O_2 plays an important role in inflammatory skin diseases and skin cancer [5].

ROS exert key roles in a variety of processes associated with epithelial malignancy such as cell proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, apoptosis evasion and enhancement of metastatic potential [41].

Free radicals in carcinogenesis, ROS and RNS (reactive nitrogen species) contribute in different ways to carcinogenesis and the malignant progression of tumor cells, enhancing their metastatic potential. In fact, they are now considered a distinctive characteristic of cancer. These species lead to genomic damage and genetic instability, and they participate as intermediaries in mitogenic and survival signals via growth factor receptors and adhesion molecules, promoting cell mobility, inducing inflammation/repair and angiogenesis in the tumor microenvironment [16].

6. Redox modulation of toxicants — Heavy metals-induced toxicity

The essential metals are very important for the maintenance of cell homeostasis. Among the 23 chemical elements with physiological functions in humans, half of them are metals, including heavy metals [42]. The heavy metals, generally defined as metallic elements with a relative density above 5 mg/ml, have the potential to cause human toxicity; the main examples are: Pb, Hg, Fe, Cd, Tl, Bi, Mn, and As. The intoxications produced by metals, characterized mainly by neurotoxicity, genotoxicity, or carcinogenicity, are widely known [43]. After their absorption into organism, the metals bind to proteins and lead to impaired enzymatic activity; the result being the damage of many organs.

Cellular redox processes are controlled by two systems (thioredoxin, Trx, and glutathione, GSH) [44]. Exposure to ions of heavy metals can amplify the production of reactive oxygen species (ROS), which can react with cellular components followed by the debut of many physiological processes [45]. ROS have a double character as both deleterious and useful compounds: on one hand they act within cells as 2nd messengers in intracellular signaling cascades, inducing and keeping the oncogenic phenotype of cancer cells, and on

the other hand, ROS induce the cellular senescence and apoptosis and can be considered anticancer species. The cumulative production of ROS (called oxidative stress) is common for many types of cancer cell which are linked with altered redox regulation of the cellular signaling pathways [46].

Metal-induced formation of ROS causes changes to DNA, increased lipid peroxidation, and altered Ca and -SH homeostasis. Lipid peroxides, formed by ROS attack on phospholipids, can react with redox metals finally producing carcinogenic products [27].

Redox active metals (Fe, Cu, Cr, Co) are part of redox cycling reactions and have the ability to produce ROS. Perturbation of metal ion homeostasis can lead to oxidative stress, a state where increased production of ROS overcome the body antioxidant protection and induces DNA damage, lipid peroxidation, and proteins changes. The action mechanism of these metals involves formation of ROS, finally producing mutagenic and carcinogenic products. Redox inactive metals (Cd, As and Pb) show their toxic effects via bonding to proteins -SH groups and depletion of GSH [47].

Lead (Pb) is a chemical element from group 14, and period 6 (p-block). Pb was removed from alimentary cans, paints, and petrol because it was the most common cause of heavy metal poisoning; an important problem remains the water pipes from older houses, some occupations, and traditional remedies. Pb causes toxicity to mitochondria by depletion of GSH, which results in excessive ROS production and mitochondrial damage. It was discovered that Pb toxicity leads to cellular damage via two pathways: (1) the production of ROS, and (2) the direct reduction of antioxidant reserves. Mitochondrial antioxidant enzymes play an important role in cellular defense mechanism against oxidative damage [48]. A possible molecular mechanism of Pb toxicity is represented by the oxidative stress, which appears when ROS production exceed the capacity of antioxidant defense mechanisms. Pb is capable of causing oxidative damage to heart, liver, brain, and erythrocytes [49].

Mercury (Hg) is a chemical element from group 12, and period 6 (d-block); it is poorly absorbed from bowels and the ingestion is usually harmless. Hg compounds have the ability to provoke cellular damage through an increase of ROS levels (the molecular mechanism involved in its genotoxicity). In response to Hg exposure, the amount of intracellular GSH increase to chelate Hg in order to protect the cells by its antioxidant role. Tchounwou and *colab*. already demonstrated that GSH levels are higher in human populations exposed to methylmercury intoxication by a fish-rich diet [50].

Iron (Fe) is a chemical element from group 8, and period 4 (d-block) and it is one of the most abundant elements in the crust of earth. ROS can play a role in Fe-induced cell toxicity because of its salts' powerful prooxidant activity. In the presence of cellular reductants, Fe from low molecular weight salts can be an initiator of free radical reactions. In Fe overload, hepatocellular Ca homeostasis may be spoiled through mitochondrial damage and microsomal Ca sequestration. DNA has also been reported to be a target of Fe-induced damage in the liver; this may lead to malignant transformation [51].

Due to its oxidation states (3+ and 2+), Fe is considered an intrinsic producer of ROS, leading to neuronal oxidative stress. Paradoxically, Fe redox properties determine its participation in

potentially cytotoxic reactions: bivalent form catalyze the formation of hydroxyl radical, considered the most reactive and damaging intermediate of cellular metabolism, while trivalent form can be reduced to Fe^{2+} after reacting with superoxide anion. Both forms are also involved in the propagation of lipid peroxidation, by a complex mechanism; however, it likely involves the direct interaction of Fe with ROS [52].

Cadmium (Cd) is a chemical element from group 12, and period 5 (d-block); it was discovered in the 19th century and the first studies upon its toxicological properties were initiated shortly after. The smoke and food are considered the main sources of Cd. Cd inhibits the activity of antioxidant enzymes; it displaces Zn and Cu leading to a decreased level of these two metals in the enzymes and an increased level in the cytoplasm. Thus appear conformational changes and inhibition of enzyme activity, and deregulation of Cu homeostasis which can lead to ROS production via the Fenton reaction [53].

Thallium (Tl) is a chemical element from group 13, and period 6 (p-block); Tl and its compounds must be manipulated with an increased attention due to their important toxicity. Different authors indicate that Tl induce ROS formation, GSH oxidation, and membrane lipid peroxidation; the liver mitochondria seems to be the main targets of its toxicity because liver is its storage site [54].

Bismuth (Bi) is a chemical element from group 15, and period 6 (p-block); it has a few industrial uses in pigments, ceramics and alloys with low melting points. Bi causes kidney damage and the promotion of a reversible encephalopathy; chelating agents may be used as treatment.

In a few studies of Woods and Fowler there were evaluated Bi effects on organelle structure and heme biosynthetic parameters in liver and cells; their study revealed that action of the metal on membrane enzymes only partially accounts for deterioration of the membrane enzymes' activity. They showed that Bi initial acute effects in liver and kidney cells include deformation of mitochondrial membranes and inhibition of specific heme pathway enzymes. Both effects contribute to deterioration of membrane-associated enzymatic functions [55].

D. Bagchi and *colab*. investigated the effects of acute and chronic stress on the enhanced production of ROS; the precautionary ability of bismuth subsalicylate (BSS) was evaluated against the gastrointestinal mucosal injury induced by oxidative stress. Their findings revealed that BSS decreased chronic stress-induced lipid peroxidation, DNA fragmentation, and membrane microviscosity by approx. 40-50% in gastric and in the intestinal mucosa. It was found that oxidative stress produce gastrointestinal mucosal injury through improved production of ROS, and that BSS protect against gastrointestinal mucosal injury [56].

Manganese (Mn) is a chemical element from group 7, and period 4 (d-block); it is an essential dietary nutrient, but its excess lead to an accumulation with toxic effects (the manganism is a disease associated with Mn accumulation and it is due to ROS production. The bivalent ion is a central component of some enzymes and an activator of many metal-enzyme complexes. On the other hand, the trivalent ion is found in the essential enzymes manganese catalase and Mn-superoxide dismutase (SOD), both of which break down oxidants using the Mn3+ in their reactive catalytic center. The bivalent ion (Mn2+) intends to bind to almost all Ca2+ and Mg2+

binding sites leading to substitutions of these ions in many biological processes; this is due to the similarities of their electron structure [57].

Arsenic (As) is a chemical element from group 15, and period 4 (p-block); it is contained in many minerals, but it also appear as a pure elemental crystal. As inhibits lipoic acid, which is a cofactor for pyruvate dehydrogenase into the citric acid cycle. Another important aspect is the fact that AsO_4^{3-} decouples the oxidative phosphorylation leading to the inhibition of energy-linked reduction of NAD^+, mitochondrial respiration, and ATP synthesis. The production of H_2O_2 is also increased, which lead to ROS production and oxidative stress. The frequency of human cancers is increased in the case of long term exposure at As probably due to the ROS production [58].

Zhang Z and *colab*. showed that As can activate p47(phox) and p67(phox), proteins which activate NADPH oxidase and it generate ROS in DLD1 cells. It was found that tumor volumes of group treated with As were much larger than those without As treatment. Many researchers found that ROS have a role in the initiation of cellular injury induced by As, which can lead to cancer development. ROS induce direct cellular injury, which may start a set of radical reactions leading to an increase of secondary ROS generation. More than that, the increased ROS production may stimulate the inflammatory processes involving secretion of chemotactic factors, growth factors, proteolytic enzymes, lipoxygenases, and cyclooxygenase, inactivation of anti-proteolytic enzymes, and the release of signaling proteins. NADPH oxidase complex is an important physiological system for ROS production; As is highly capable of activating NADPH oxidase and disrupting of mitochondrias' membrane, leading to the generation of different ROS. It has been generally accepted that ROS are critical regulators for a wide range of cellular responses, from kinase activation, gene expression, DNA damage, cell proliferation, to cell migration in the arsenic treated cells [59].

7. Conclusions

Reactive oxygen species (ROS) represented a matter of debate/concern in the last years due to the dual role played by these compounds: beneficial effects at low concentrations (signal molecules, mediators of cellular homeostasis, activators of different enzymes) and deleterious effects at high concentrations (DNA and protein damage, lipid peroxidation and oxidative stress).

The oxidative stress was described as an underlying mechanism in different pathologies, including: neurodegenerative diseases, multiple sclerosis, diabetes, atherosclerosis, ageing, chronic inflammatory diseases and cancer. In cancer development, a possible theory regards the activity of ROS as regulators of major signaling pathways: extracellular signal-regulated kinases (ERKs), mitogen-activated protein kinases (MAPKs), phosphoinositide 3-kinases (PI3Ks) and transcription factors such as hypoxia-inducible factors (HIFs). In addition, it was demonstrated that the toxicity associated to heavy metals (lead, mercury, arsen, cadmium, thallium, bismuth, manganese, iron) is mediated via ROS: ROS generation, mitochondrial injury or inhibition of the antioxidant cellular systems.

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