Chapter 2

Oxidative Stress and Lipid Peroxidation – A Lipid Metabolism Dysfunction

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1. Introduction

Free radicals are chemical compounds with unpaired electron(s), therefore being considered very active molecules. The cells had developed their own antioxidant defence systems in order to prevent the free radicals synthesis and to limit their toxic effects. These systems consist of enzymes which breakdown the peroxides, enzymes which bind transitional metals or compounds which are considered scavengers of the free radicals. Reactive species oxidize the biomolecules that will further elicit tissue injury and cell death. Evaluation of free radicals involvement in pathology is rather difficult due to their short life time.

2. Biochemistry of reactive oxygen species (ROS)

Free radicals can be formed by three mechanisms:

- Homolytic cleavage of a covalent bond of a molecule, each fragment retaining one electron

$$X{:}\ Y \rightarrow X^{\cdot} + Y^{\cdot}$$

- Loss by a molecule of a single electron

$$A \rightarrow A^{\cdot} + e^{-}$$

- Addition by a molecule of a single electron

$$A\ e^{-} \rightarrow A^{-\cdot}$$

- Heterolytic cleavage – covalent bond electrons are held up by only one of the molecule's fragments. Basically, charged ions occur.

$$X\,Y \rightarrow X{:}^{-} + Y$$

Oxygen activation is the main factor that induces enhanced formation of ROS. Due to its presence in the atmosphere, but also in the body, free radicals reaction with oxygen is inevitable. A second characteristic of oxygen refers to its electronic structure. Thus, O_2 has on the outer layer two unpaired electrons, each located on one orbital. Therefore, oxygen can be considered a free di-radical, but with a lower reactivity. Oxidation of this electron donor is achieved by spin inversion from the O_2 reaction with transition metals or by univalent reduction in two phases of one electron [5]. These two mechanisms underlie oxidation reactions that occur in nature. Although this process represents only 5%, following the univalent reduction of O_2, ROS occurs, with greater reactivity and toxicity, as is the hydroxyl radical OH.

In biological systems, the most important free radicals are oxygen derivate radicals formed by the following mechanisms:

O_2 reduction by the transfer of an electron will result in the synthesis of the superoxide anion ($O_2^{\cdot -}$). The formation of the superoxide anion is the first step of O_2 activation and occurs in the body during normal metabolic processes. In some cells, its production is continuous, which implies the existence of intracellular antioxidants [3].

$$O_2 + e^{-} \rightarrow O_{2-}$$

Tissue alteration by traumatic, chemical or infectious means causes cell lysis along with the release of iron from deposits or by the action of hydrolases on metalloproteinase.

Reduced transition metal autooxidation generates the superoxide anion. The reaction of transition metal ions with O_2 can be considered a reversible redox reaction, important in promoting ROS formation.

$$Fe^{2} + O_2 \rightarrow Fe^{3} + O_{2-}$$

$$Cu + O_2 \rightarrow Cu^{2} + O_{2-}$$

Degradation of H_2O_2 in the presence of transition metal ions leads to the formation of the most reactive and toxic ROS: the hydroxyl radical (OH.) (Fenton and Haber-Weiss reaction). To this radical, the body does not present antioxidant defense systems such as for the superoxide anion or hydrogen peroxide (H_2O_2). Although metallothioneins (natural antioxidants) are proteins that bind to metal ions, including Fe^{2+}, thus inhibiting the Haber-Weiss reaction, however they are found in too low concentrations in the body to be effective in the decomposition of the hydroxyl radical. But these reactions can be inhibited by specific scavengers for OH, such as mannitol and chelating agents: desferroxamine. However, chelators as EDTA stimulate this reaction.

The reduction of O_2 by two electrons leads to the formation of hydrogen peroxide, H_2O_2.

$$O_2 + 2e^- + 2H \rightarrow H_2O_2$$

H_2O_2 is often formed in biological systems via peroxide anion production.

$$2O_{2-} + 2H \rightarrow H_2O_2 + O_2$$

H_2O_2 is not a free radical, but falls within the category of reactive oxygen species that include not only free radicals but also its non-radical derivatives involved in producing these ROS. Of all free radicals, H_2O_2 is the most stable and the easiest to quantify. Intracellular formation of hydrogen peroxide, depending on the content of catalase, is the way by which the bactericidal mechanism is achieved in phagocytosis.

The formation of singlet oxygen ($^1O_2$). It represents an excited form of molecular oxygen, resulting from the absorption of an energy quantum. It is equated to a ROS due to its strong reactivity. Singlet oxygen has an electrophilic character, reacting with many organic compounds: polyunsaturated fatty acids, cholesterol, hydroperoxides or organic compounds containing S or N atoms, producing oxides. In plasma it is neutralized by the presence of antioxidants, especially albumin.

Singlet oxygen is formed in the following reactions:

- Reaction of hydrogen peroxide or hydroxyl radical with the superoxide anion
- Different enzymatic catalyzed reactions
- Decomposition of endoperoxides
- Degradation of hydroperoxides in liver microsomes

2.1. Free radicals resulting in lipid peroxidation propagation phase

Lipid peroxidation is a complex process consisting of three major phases: initiation, propagation and end of the reaction. The initiation phase is slow due to the need of accumulation of a sufficient quantity of ROS, followed by the activation process of oxygen which is the amplifier factor. The process' latency period is that which determines the continuation of reactions by altering the oxidative balance in favor of pro-oxidant factors. The evolution of these reactions is unpredictable due to the formation of own catalysts determining the complexity of the process [1].

Free radicals are very unstable, their lifetime being very short. Their reactivity results from their coupling at the end of the reaction, only for an unpaired electron to reappear, thus stimulate the propagation of the reaction by forming a new radical.

The end of the reaction occurs by:

- Free radical recombination among them or,
- Intervention of antioxidant systems with membrane or intracellular action: superoxide dismutase (SOD), catalase.

Peroxides and their decomposition products (aldehydes, lipofuscin) are the most stable and represent the final link of O_2 activation. They are produced directly by the hydroxyl or singlet oxygen radical. During these reactions, own catalysts are formed, represented by free radicals or degradation products that diversify and increase the oxidation reactions; the structures involved are diverse, and are represented by polyunsaturated fatty acids, hemoproteins, nucleic acids, carbohydrates or steroids [4].

3. The production of reactive oxygen species

3.1. Endogenous production

The electron transfer in the respiratory chain involves an incomplete reduction of molecular O_2 at a rate of 1-2% with the formation of superoxide anion and of singlet oxygen.

If the anion is released in a low in protons environment, it will initiate peroxidation, the substrate being formed by polyunsaturated fatty acids from cell membranes.

If the anion will reach a proton rich environment, dismutation will take place; this following auditioning an electron from another anion and by proton reaction will form hydrogen peroxide. Dismutation can occur spontaneously, but in this case it takes place very slowly or catalyzed by SOD, which increases 10^{10} the reaction rate to the body's pH. There is an inversely proportional relationship between reaction rate and pH value. The efficiency of this enzyme is proven by its presence in all aerobic cells, and cells exposed to oxygen action, as hepatocytes and erythrocytes, contain large amounts of SOD [6].

$$O_{2\text{-}} + e^- \rightarrow O_2^{--} + 2H \rightarrow H_2O_2$$

Superoxide anion production during mitochondrial respiration has a self-regulation mechanism. Superoxide anion formed in part by autoxidation of NADH dehydrogenase, can then induce this enzyme's inactivation, so the presence of SOD in the membrane matrix to achieve dismutation is absolutely necessary. It results that the two enzymes SOD and NADPH dehydrogenase are a metabolic control and energy preservation couple in the presence of oxygen [7].

The release of hydrogen peroxide is proportional to the partial pressure of O_2. In case of a cerebral or cardiac ischemia, extramitochondrial concentration decreases, disrupting oxidative phosphorylation and ATP levels. An inversely proportional relationship between mitochondrial H_2O_2 formation rate and lifetime exists. Thus, it was observed experimentally that old animals present an increase in lipid peroxide formation in the mitochondria as a result of increased production of superoxide anion compared with young animals.

Antimicrobial defense. Phagocytosis of bacterial germs is accompanied by a massive production of superoxide anions and other derivatives (OH^-, HOCl, H_2O_2, $1O_2$) from the leukocyte metabolism. A NADPH-dependent oxidase, activated by protein kinase C and arachidonic acid released under the action of phospholipase A allow anion synthesis with an increased consumption of O_2.

The sequence of reactions initiated in the membrane continues into the cytoplasm where a substantial amount of superoxide anion is formed which then is diffuses also extracellularly. Increased use of glucose occurs for energetic purposes and for restoring NADPH and oxygen consumption necessary for the production of ROS [8].

Hydrogen peroxide is toxic on the neutrophil, which is inhibited by the presence at this level of the three enzymes that degrade the excess of peroxide: GSH-peroxidase, catalase and myeloperoxidase.

The enzyme present in phagosome, myeloperoxidase, will catalyze in the presence of H_2O_2 and chloride ions, forming toxic halogenated derivatives.

$$H_2O_2 + Cl^- \rightarrow ClO^- + H_2O$$

In turn, hypochlorous acid can react with aminic groups or with the ammonium ion (NH_4) forming chloramines. In the presence of hydrogen peroxide, HOCl forms singlet oxygen. These products of activated leukocytes have bactericidal properties.

Based on the properties of leukocytes to emit chemiluminescence during phagocytosis, this method has a clinic utility. Chemiluminescence emission is due to formation of free radicals, lipid peroxides and prostaglandin synthesis, a process associated with phagocytosis. This property is suppressed by anesthesia, cytostatic agents and anti-inflammatory preparations. Drugs with anti-inflammatory effect inhibit the activity of cyclooxygenase, the enzyme involved in prostaglandin synthesis.

A deficiency in the leukocyte production of free radicals (septic granulomatosis) or decrease of myeloperoxidase activity (following corticotherapy) is characterized by particularly sensitivity to infections.

During phagocytosis, three cytotoxic and antimicrobial effect mechanisms take place:

- oxygen dependent mechanism involves activation of myeloperoxidase and other peroxidases
- Nitrogen compounds dependent mechanism involving participation of NO, NO_2, other nitrogen oxides and nitrites. In this mechanism both types of cytotoxic inorganic oxidants interact: oxygen and nitrogen reactive radicals.
- The third mechanism is independent of oxygen and nitrogen by changing phagolysosome pH that favors the action of antimicrobial substances present in the lysosomal or nuclear level.

The constitutive form of NO synthase is found in endothelial cells, neutrophils, neurons. The existence of the inducible form has been shown in macrophages, hepatocytes, endothelial cells, neutrophils and platelets. Glucocorticoids inhibit the expression of inducible NO synthase but not of the constitutive enzyme.

Nitrogen reactive radicals have a cytotoxic effect by inhibiting mitochondrial respiration, DNA synthesis, and mediate oxidation of protein and non-protein sulfhydryl groups.

Although NO has a protective role at the vascular level by a relaxing effect (EDRF), under certain conditions it may exert a cytotoxic effect, causing pathological vasodilatation, tissue destructions, inhibits platelet aggregation, modulates lymphocyte and immune response function.

Synthesis of prostaglandins. Phospholipase A_2 catalyzes the degradation of membrane phospholipids with arachidonic acid formation. Stimuli such as phagocytosis, antibody production, and immune complex formation, the action of bacterial endotoxins or cytokines stimulate the activation of this enzyme. There are two enzymatic forms: type I PLA_2, membrane bound, which is stimulated by Ca^{2+} at physiological pH, and the type II one, cytoplasmic, which is inhibited by Ca^{2+} and is active at acidic pH. Two enzymes, lipoxygenase and cyclooxygenase, bound to plasmic and microsomal membranes, convert arachidonic acid in derivatives such as: thromboxane, prostaglandins, leukotrienes [18].

Under the action of lipoxygenase, arachidonic acid is converted into a hydroperoxide: hydroperoxyeicosatetraenoic acid (HPETE) which will release the hydroxyl radical during its transformation into hydroxyeicosatetraenoic acid (HETE). Hill et al. have emphasized the role of glutathione peroxidase (GSH-Px) and of glutathione in this reaction: blocking the activity of this enzyme, they have noticed a significant decrease (of 66%) of HPETE conversion in HETE [14, 16].

Under the action of cyclooxygenase, arachidonic acid incorporates two oxygen molecules to form an endoperoxide, PGG; it loses the OH group to form PGH. This transformation, which is accompanied by the release of hydroxyl radical, exerts a negative retro-control to prostaglandin synthesis, inactivating the cyclooxygenase. Some of the products developed have a complex effect on the inflammatory process: thus, in the first phase, PGE_2 acts on cells from the vascular wall with a procoagulant effect, and in the late phase it has an inflammatory effect by inhibiting leukocyte activation and oxidative metabolism of these cells during phagocytosis. The byproducts resulting from this process will be the ones to modulate the intensity of the next phase [15, 22].

The two endoperoxides formed, PGG_2 and PGH_2, have an inducible role on the production of PCI_2 or TxA, being involved in the mechanism that ensures homeostasis of the vascular and platelet phase of hemostasis.

The other enzyme has a dual effect, and promotes the initiation of lipid peroxidation and the decomposition of resulting products of these reactions.

3.2. Exogenous production

The human body is subjected to aggression from various agents capable of producing free radicals. Thus, UVs induce the synthesis of ROS and free radicals generating molecules via photosensitizing agents.

Ingestion of alcohol causes ROS synthesis by different mechanisms: xanthine oxidase and aldehyde oxidase can oxidase the main metabolite of ethanol (acetaldehyde) resulting in superoxide anion.

Ethanol also stimulates the production of superoxide anion and, by inducing NADPH-oxidase synthesis, NADPH cytochrome reductase and P450 cytochrome.

The alcohol ingestion decreases the activity of protective enzymes (SOD, glutathione peroxidase). Also low serum concentrations of selenium and vitamin E have been found in alcoholics.

Toxic substances as nitrogen oxide and nitrogen dioxide in the environment are responsible for autoxidation of polyunsaturated fatty acids in lung alveoli. The reaction may be reversible or irreversible. $NO^{\cdot}$ and NO_2 may react with H_2O_2 produced by alveolar macrophages and can generate the hydroxyl radical.

The reduction of carbon tetrachloride (CCl_4) in $CCl_3^{\cdot}$ performed under the action of cytochrome P_{450} or in the presence of Fe_2 is another factor that induces autoxidation of polyunsaturated fatty acids, increasing lipid hydroperoxides concentration.

Anticancer drugs are able to synthesize free radicals, this process depending on the mode of action and their toxicity.

These drugs under the action of cytochrome P450-dependent enzymes produce the activation of O_2 with the formation of ROS which will attack GSH and other thiols (hemoglobin), causing the formation of lipid peroxides and activation of Ca^{2+}-dependent endonucleases.

These mechanisms can induce disturbances of the coagulation system (increased hemolysis), severe forms of cardiomyopathies, because of the low level of cardiac antioxidants (AO) [25].

4. Reactive oxygen species and oxidative stress

In physiological conditions a delicate balance exists between ROS production and the antioxidant capacity. A higher ROS production and/or a decreased antioxidant capacity is responsible for the harmful effects of free radicals or the oxidative stress (OS). Oxidative stress represents an important pathogenic mechanism involved in inflammation, cancerogenesis or aging [24].

End products of free radicals action, aldehydes, inhibit the activity of membrane enzymes (glucose-6-phosphate, adenylate cyclase). These aldehydes react selectively with proteins or enzymes containing SH groups and cause tissue destructions.

The emergence of OS is one of the most important pathogenic mechanisms involved in inflammation, carcinogenesis, radiation disease and aging.

The objective of various experimental models was to study erythrocyte response to oxidant substances action. Erythrocyte characteristics and test substance dosing allowed the evaluation of OS; these experiments can be extrapolated to explain various physiological or pathological processes in the body.

Oxidative stress is an ongoing process in the body, and under physiologic conditions there are effective mechanisms that negate its effects, thus high concentration of erythrocyte GSH and related enzyme equipment provide a defense against ROS.

Erythrocyte congenital enzyme deficiencies confer erythrocytes an increased sensitivity to OS.

A section of the body intensely studied to assess OS is the liver due to its role in the metabolism of a wide range of endogenous and exogenous products. Thus, by the metabolism of aromatic compounds, drugs or carcinogenic hydrocarbons in the live, a large amount of FR occurs, which will initiate in the next phase OS from this level.

Liver antioxidant systems are represented by SOD, GSH and dependent enzymes (transferase and peroxidase). Using ESR and spin trapping, FR resulting from chemical pollutants metabolism were identified, and a strong correlation between the functional impairment of the hepatic parenchyma, free radicals formation and decrease in GSH was noted. Under these conditions, free radicals of that substance occur which can cause tissue destructions also without O_2 activation.

The experimental poisoning of rats with alcohol (1.5 mmol/kg) showed significant decrease at one hour of ingestion of GSH, vitamin E and C along with hepatic necrosis and formation of lipid peroxides [2, 11].

GSH is an important protective factor against OS. Its level is interrelated with other antioxidants (vitamin C and E) that stimulate its preservation in reduced form.

Stress proteins. Structurally altered intracellular protein group, whose synthesis is induced by oxidative stress has been named stress proteins group. Of particular interest is the 32 kDa protein whose synthesis is induced by the action of ionizing radiation, hydrogen peroxide. This protein is a marker of generalized response to oxidative stress. Free radicals affect cytokines (endogenous pyrogens: IL-1, IL-2, TNF-a, IFN) that play a role in regulating signal transmission in response to stress which will cause the synthesis of these proteins. It is considered that SOD itself is a stress protein.

The tissue repair process is enzymatically catalyzed (repair enzymes) that break down damaged cellular particles, take intact aminoacids to synthesize new defense proteins.

5. The effects of reactive oxygen species

5.1. Oxygen free radicals – Intracellular messengers

Before discussing the negative effect of oxygen activation on the body, we should also take into consideration their involvement in certain physiological processes, when these ROS are

produced in quantities which do not exceed the antioxidant capacity. Thus, the superoxide anion is produced by leukocytes during phagocytosis and in smooth muscle cells, epithelial cells, skin fibroblasts and endothelial cells [27].

The anion produced by macrophages and endothelial cells induces conformational changes of receptors on the LDL lipoprotein surface, allowing their recognition and involvement in atherogenesis [10, 12].

It is also involved in cascade-type metabolic reactions of arachidonic acid and in achieving platelet adhesion and aggregation function.

5.2. Lipid peroxidation

Formation of peroxides, especially lipid ones, is a consequence of the activation of O_2, the interconversion of reactive species and natural systems protection overcoming. In biological environments, the most favorable substrate for peroxidation is represented by polyunsaturated fatty acids (PUFA), components of cell and subcellular membranes.

Peroxidation is a complex process that includes three phases: initiation, propagation, end-decomposition, which interpose, so that only end products can be determined chemically: aldehydes (malondialdehyde), polymerized carbonyl compounds (lipofuscin) [9].

A radical character initiator (which may have different structures and origins, including peroxy $ROO^{\cdot}$ radicals) removes a hydrogen atom from polyunsaturated fatty acid diallyl carbon, forming a favorable reactive center for oxygen action. The peroxy $ROO^{\cdot}$ radicals which become hydroperoxides result. In fact, due to side reactions, other locations of the peroxide group per PUFA molecule occur [28].

5.3. Cell structural alterations

Since the formation of peroxides and their decomposition products, the sequence of reactions passes from a molecular level to a cellular one due to structural changes that occur in membranes: structural disorganization of the membrane and deterioration of pores crossing the double phospholipid layers. Peroxidation leads to changes in fatty acid qualitative composition of phospholipids composition with changing the ratio between PUFA and other acids. The first two effects induce the third, which consists in a decrease in membrane fluidity and altered active ion transport; these effects finally lead to changes in ion and other intracellular compounds concentration [26].

Numerous experimental studies have shown that tissue injury caused by free radicals determined at one point an imbalance of Ca^{2+} (i.e., increases in intracellular Ca^{2+} concentration). Under physiological conditions, there are effective homeostatic mechanisms (enzyme systems, protein transporters) to keep an optimum ratio between intracellular (0.1-0.4 microM) and extracellular of the mM order concentration. Overcoming these mechanisms (in this case by producing free radicals) determines the accumulation of

calcium in the cell which will lead to structural membrane alterations, production of unsaturated lipids, efflux of GSH, its transition to an oxidized form and the creation of an intracellular oxidative potential [21, 23].

Experimental studies on isolated hepatocytes have shown the correlation between the cellular toxicity of calcium and the decrease of tocopherols levels, substances with strong antioxidant character.

Maintaining the cell functional state ultimately depends on the level of proteins containing SH groups. Thus, the role of GSH in protection against oxidative stress is precisely regeneration of protein SH groups which in turn will ensure intracellular calcium homeostasis. Vitamin E stabilizes ATPase activity dependent of calcium in the endoplasmic reticulum by maintaining SH groups in the structure of the enzyme in reduced state. Also, vitamin E is protective against the compounds resulting from lipid peroxidation: a molecule of alpha-tocopherol protects against 500 molecules of polyunsaturated fatty acids.

5.4. DNA destruction

The results of chromatographic technique used to determine the urinary excretion products resulting from scission of DNA in humans showed a normal excretion in average of 100 nmol products. This total represents 10^3 thymine molecules oxidized per day for each of the $6X10^{13}$ cells in the body.

Between eliminating these products and the specific metabolic rate (SMR) there is a linear correlation.

The specific metabolic rate of an organism is dependent on the O_2 use rate by its tissues and it is proportional to the free radicals production rate. In this case, the ratio between the total concentration of antioxidants (enzymatic and non-enzymatic systems) and the metabolic rate represents the protection degree of a tissue or body to free radicals. It seems that there is a genetic programming of the metabolic rate for each species and individual.

Looking at the hypothesis on free radicals involvement in aging, it has been shown that there is an inversely proportional relationship between the metabolic rate, free radicals production, respectively, and he maximum lifespan potential (MLP). Thus, on the evolutionary scale, metabolic rate decreased and lifespan increased, in mammals their product being constant.

One can calculate the lifespan energy potential (LEP), expressed in kcal-kg as follows: LEP 2.70 X MLP X SMR. This potential is directly proportional to the total concentration of antioxidants.

During aging, the formation of free radicals amplifies by exposure to prooxidant factors from the environment, and by the decreased antioxidant defense capacity.

At an intracellular level (especially in muscles and neurons), deposits of lipofuscin pigments, lipid peroxides and their breakdown products are formed.

These deposits are mainly localized in the myocardium, brain, and, by the age of 80, they represent 70% of cytoplasmic volume of neurons and 6% of that of myocardiocytes.

Experimental studies demonstrated that in 50 years a person accumulates 13.4 mg/lipofuscin/gram of myocardium, pigment formation taking place once with exceeding the absorption of 0.6 free radicals micromoles/gram of tissue.

There is an inversely proportional relationship between the formation of these products and the concentration of vitamin E in the body.

To control the effects of aging, ones requires a moderate diet, which reduces metabolic rate and O_2 consumption with an optimal concentration of lipids and a quantitatively and qualitatively balanced intake of antioxidants and other factors that enhance assimilation and their metabolism. It is also necessary to achieve a balanced interaction of endogenous antioxidants.

The antioxidants level varies greatly depending on the age of the body, that organ and subcellular components; thus an increase of GSH-Px activity was noted in mitochondria of cardiac cells and erythrocytes in the elderly, and a decrease of activity in liver and kidneys. The decrease of SOD activity in the liver of the elderly was highlighted and no significant changes in the concentration of intramitochondrial SOD in the heart were noted.

Also, there is a correlation between the intensity of DNA destructions caused by FR and xanthine oxidase concentration. This enzyme, present in low concentrations, in tissue or plasma, increases under tissue injury.

5.5. Effects on molecules

Free radicals are responsible for the inactivation of enzymes especially of serine proteases, the fragmentation of macromolecules (collagen, proteoglycans, hyaluronic acid), the formation of dimers, the protein aggregates in the cytoplasmic membranes. The most susceptible amino acids to their action are tryptophan, tyrosine, phenylalanine, methionine and cysteine.

Transition metal ions (Fe, Cu, Ni, Co, Cd) have a pro-oxidant action by intensifying reactions in which FR are formed and those in which the decomposition of lipid peroxides takes place. At the molecular level, Fe^{2+} ion contributes to the induction of oxidative stress by increasing non-enzymatic oxidation of catecholamines and GSH, promoting lipid peroxide decomposition and the formation of the most toxic free radicals, the hydroxyl radical. Fe^{2+}, under complexed form as transferrin, is inactive against peroxides. Fe^{2+} release from transferrin takes place under pH decrease as it does

in hypoxia, leukocyte activation or in muscle tissue during strenuous physical exercise. Another source of free Fe^{2+} is represented by hemoglobin, which at low concentrations acts as a pro-oxidant favoring PUFA peroxidation. Proteins that bind Fe^{2+} have a different action: thus, ferritin has a pro-oxidant capacity, while hemosiderin and lactoferrin are antioxidants.

Bilirubin, resulting from the metabolism of hemoglobin, as transition metal ions, causes alterations in the membrane structure by initiating PUFA peroxidation. Bilirubin crosses the blood-brain barrier, inhibits oxidative phosphorylation and decreases AMPc and GSH concentration. Thus, the encephalopathy caused by intense hemolytic jaundice in neonates is correlated with elevated levels of bilirubin, blood lipid peroxides and GSH decrease.

The same changes were observed in hepatitis of various etiologies (viral, ethanolic) and were correlated with graded morphological changes of the steatosis type, up to the irreversible ones, cirrhosis, caused by exceeding the protective antioxidant systems.

The bilirubin has an antioxidant effect, enhanced by binding to albumin, its plasma transport form. This different behavior of bilirubin depends on the concentration and the environment, like ascorbic acid, which features a pro- and antioxidant character, widely accepted today.

6. Reactive oxygen species – Implications in cardiovascular pathology

Atherosclerosis (ATS) and its notable complication, coronary heart disease, still represent the major cause of premature death worldwide. Several lines of evidence suggest that the major risk factors (hypertension, diabetes mellitus, hyperlipemia, smoking) elicit oxidative stress at the luminal surface of vascular wall that will be further responsible for the oxidative damage of lipoproteins, formation of lipid peroxides, platelet aggregation and activation of macrophages [10]. LDL lipoproteins are the easiest to be oxidized because of their high PUFA content; at variance from native LDL, oxidatively modified LDLs are more avidly taken up by macrophages via the scavenger receptor thus generating the well-known “foam cells” of the atherosclerotic plaques. Experimental studies demonstrated that LDL can be oxidized by all of the major cells of the arterial wall (macrophages, endothelial cells, smooth muscle cells). Besides its rapid uptake by macrophages, oxidized LDL elicit a chemoatractive effect facilitating monocyte adhesion to the endothelium and a toxic affect at the level of endothelial cells by inhibiting the release of nitric oxide. *In vivo* identification of oxidized LDL in atherosclerotic plaques clearly established in the late 80s the oxidative-modification theory of ATS. Much effort was further directed towards identification of factors that influence the susceptibility of LDL particles to oxidation. Among these, the presence of small dense LDS particles, of preformed lipid peroxides, as well as glycation or binding of LDLs to proteoglycans were proven to facilitate oxidation [12].

Highly reactive aldehydes are one of the major causative factors in oxidative related cardiovascular pathology and ageing. Specific aldehydes (e.g., 4-hydroxynonenal acetaldehyde, acrolein) were reported to be transiently increased in the settings of heart failure and ischemia-reperfusion injury [13] and to interfere with transcriptional regulation of endogenous anti-oxidant networks in mitochondria [1]. Recently, accumulation of reactive aldehydes was studied from the point of view of the subsequent protein carbonylation and its implication in cardiovascular pathophysiology [4].

On the other hand, decreased antioxidant defense further contributes to the oxidative damage. Low concentration of GSH-peroxidase in the vascular wall creates conditions favorable to the actions of hydrogen peroxide and other FR on lipids and lipoproteins [28]. In physiological conditions, nitric oxide acts as an antioxidant, inhibiting LDL peroxidation and their destructive effect on interstitial proteoglycans. With the increased production of FR, NO may become a prooxidant factor, stimulating LDL peroxidation by a mechanism involving myoglobin. Deficiency of other protective factors will favor oxidative injury. Lipid-soluble antioxidants such as tocopherols and ubiquinol are present in the hydrophobic environment of the lipoproteins in order to protect PUFA from FR attack. *In vitro* experimental data showed that: i) exposure of LDL to oxidative stress will trigger lipid peroxidation only after the loss of its above mentioned antioxidants and ii) enrichment of LDL with vitamin E will make LDL oxidation more difficult [6].

Accordingly, the beneficial role of antioxidant supplementation has been extensively investigated in the past decades in a variety of animal models. Most investigators reported beneficial effects, i.e., prevention of atherosclerotic lesions with vitamin E supplementation, yet an early study by Keaney et al. mentioned a deleterious effect of high doses of tocopherol on endothelial-dependent relaxation in cholesterol fed rabbits [11]. Unfortunately, despite the promising observational experimental data, several prospective, double-blind, placebo-controlled trials did not support a causal relationship between vitamin C and E supplementation and a lower risk of coronary heart disease [21]. Similarly, lack of beneficial effect with long term vitamin E supplementation was recently reported in large clinical trial (the Women's Health Study) that addressed the role of antioxidant therapy in the primary prevention of heart failure [2].

These negative results may be related to the fact that antioxidant supplements could abolish the physiological role of ROS as signaling molecules [18], especially when considering that most cardiovascular patients are treated with "pleiotropic" drugs such as statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, that besides their major effects are reported to reduce ROS formation [23]. Indeed, a large body of evidence demonstrated unequivocally that reduced amounts of reactive oxgen species, most probably of mitochondrial origin [17] but not exclusively, are essential in regulating cardiovascular homeostasis [19] as well as the powerful mechanisms of endogenous cardioprotection at postischemic reperfusion, namely pre- and postconditioning [20].

In conclusion, increasing the level of endogenous antioxidants, as recently suggested via the supplimentation of weak "pro-oxidants" [8], and not chronic supplementation with large dose of exogenous antioxidants could become in the future a more appropriate approach to treat diseases that share oxidative stress as a common denominator.

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