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Peroxide Radicals Implications in the Inflammatory Rheumatic Disease

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The complex role of peroxide radicals in the organism is deemed to be essentially the result of three processes, represented by: the inclusion in important activities of the organism - phagocytosis and the fight against infections; the role in the pathogenicity of certain serious acute, but especially chronic, degenerative diseases; genesis of peroxide radicals within the eicosanoid synthesis process, usually having as starting point the arachidonic acid. The more and more frequent involvement of peroxide radicals in the pathology supports the study of the clinical significance of malonyl- dialdehyde and the use of this determination as clinical test.

Keywords: peroxide radicals, inflammatory rheumatic disease, arachidonic acid

The inflammatory condition continue to represent a major public health problem World Health Organization estimates 1% of global population affected by progressive inflammatory diseases, with various disabilities, limited working capacity or social dependence [1, 2].

Rheumatic diseases are defined as chronic inflammations of bones, joints, muscles, tendons and ligaments, with clinical correspondence of swollen, redness, stiffness, warmth and pain [3]. Over 100 entities rheumatic diseases are identified, however their etiopathology is complex and still incompletely clarified [4].

Ankylosing spondylitis is a chronic inflammatory disease with predominant injury of the spine and joints, usually beginning in the sacroiliac level, with upward extension, progressive fibrosis and spine ankylosis [5]. The genetic predisposition of this disorder was determined in human leukocyte antigen B27 (HLA-B27) the is found in 90-95% of the patients. However, considering the variations of HLA-B27 that gene and other additional genes with critical roles in the immune system, like ERAP1, IL1A, and IL23R, it is not clear how genetic factors increase the risk of developing ankylosing spondylitis [6, 7].

Rheumatoid arthritis (RA) is a symmetric, inflammatory, peripheral polyarthritis and frequent extraarticular manifestations. In the advanced stage, the inflammation of the joints leads to deformity, through the stretching of tendons and ligaments and the erosion of cartilage and bone. The etiology of rheumatoid arthritis is unknown, although immunological disorders following infection, traumatic, metabolic and endocrine factors are incriminated [8, 9].

Systemic lupus erythematosus is a chronic multisystem inflammatory disorder, with relapsing and remitting course, characterized by protean manifestations, including musculoskeletal manifestations [10]. This is an autoimmune disease, occurred more often in women than in men, although genetic, epigenetic, ethnic, hormonal, and environmental factors [8,9].

The present trends in rheumatology are the early diagnostic and treatment, according to the nosologic

criteria of each and every patient. The interest for non steroidal anti-inflammatory drugs in the management of rheumatic disorders are based on their anti-inflammatory, analgesic and antipyretic pharmacological action [7, 9, 11].

Theoretical hypothesis, theoretical results and applications

The biologically active lipids in the acute or chronic inflammation

The inflammation is a type of response developed by the body to various injuries. A large number of research works are focused on inflammation field, however recent reports have provided important data for understanding the pathogenesis of this process [5, 8, 11].

We consider that the active lipid system is the key stone in the pathogenesis of acute inflammation, as in the chronicity, the rehabilitation or reorganization of the tissues [11,12]. The active lipid system defines the eicosanoids (C20 fatty acids and their metabolites) as biologically mediators, embracing prostaglandins, thromboxanes and leukotrienes. High synthesis of eicosanoids corresponds to one of the biological cascade of inflammation. Their biosynthesis are enzymatic reactions regulated by cyclooxygenase, lipoxygenase and cytochrome P450 mono-oxygenases, that incorporate one molecule of oxygen into the polyenoic acids [12]. Also called essential fatty acids, the polyenoic acids are polyunsaturated monocarboxylic acid-long chain, such as the arachidonic, linoleic and linolenic. Since there are no endogenous precursors or cellular reservoirs, essential fatty acids are discharge from the phospholipid of the membrane, in a *turnover* process with various triggers. Phospholipid precursors are released by the enzymatic action of the phospholipase A2, either the damage of the cell membrane or of the internal organelles membranes induces, enzymatic activation [13,14].

Prostaglandins are derivatives of arachidonic acid and has been the most studied. We regard as the prostaglandins play the role at membrane level of

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continuous adaptation of the cellular response to exogenous factors [13]. They provide fluidity and flexibility to the cell membrane, regulate transmembrane active transport, modulate the binding of pharmacological agents to the receptors and act as cellular metabolic regulators [14,15].

Prostaglandins E (PGE) are the main factors involved in the pathway of the acute inflammatory process, inducing heavy vasodilatation, permeabilizing of the microvessel walls and increasing the phagocytosis process [11,15,16]. Moreover, prostaglandins are able to act like local hormones with brief duration, named autacoids. Higher amounts of autacoids are released by the activated macrophage phagocytes that are focused inside the site of inflammation than outside, proving that autacoids are part of the inflammatory mediators network [8, 15].

Deeping understanding of the inflammation mechanism evidence the involvement of lipid autacoids. Certainly, we notice that inhibitors of each metabolic pathway of a the linoleic, linolenic and arachidonic fatty acids achieve the anti-inflammatory effect [6, 16]. Thus, the theoretical knowledge of prostaglandins are applying as the base of new pharmacologically perspectives, particularly in the bone and joints therapy [6, 11].

The Arachidonic Acid and the Inflammation

Activated inflammatory cells from the inflammation site generate chemically reactive oxygen species, that can initiate lipid peroxidation [Fig.1]. The phospholipids in the cellular membranes are primary targets of chemical and enzymatic oxidation, inducing serious damages of the cellular membranes and necrosis. Oxidized lipids as oxygenated arachidonic acid products, the prostaglandins, leukotrienes, and thromboxane A₂, have potent effects throughout the inflammatory and reparative responses [17]. The various lipid peroxidation products and their metabolites could be quantified as biomarkers and applied in the diagnosis and of inflammation. Furthermore, we remark that supply of the arachidonic acid amplify the inflammatory process, while the non-steroidal anti-inflammatory drugs as indomethacin are inhibitors [18].

The peroxides are the result of cysteine reactions, that are catalyzed by metallic ions. Competing with prostaglandines production, the enzymes glutathion-peroxidase and catalase decompose the excess of

peroxides. Catalase is not directly involved in the decomposition reaction of the lipid peroxides. Actually, this enzyme antagonize the effects of the arachidonic acid, such the aggregation of the thrombocytes and the contraction of the small vessels [18].

Referring to the advanced rheumatic diseases, there are observed increase in the lipid peroxidation and low plasmatic level of sulfhydryl (-SH) groups [19]. The applications of these results are focused to detect and to quantify the free radical species, mainly the phagocytic products of macrophages [18].

Protection systems against the action of the free radicals

Free Radicals (FR) are molecules that derive from incompletely oxidized compounds that have undergone partial burning. Their oxygen groups are able to act in the surface of the cellular membranes or organelles, to initiate aggressive oxidation reactions and to damage the cells [20].

The most active free radicals are ions: superoxide ($O_2^{\cdot-}$), peroxide ($O_2^{\cdot}$), hydroxide (OH $\cdot$), nitric oxide (NO $\cdot$). The sources of aggressive oxidants as peroxides and superoxides are the hydrogen peroxide (H_2O_2) and the metabolites of fatty food, as lipid peroxides. Physiological mechanisms of cellular defence are acting *in vivo* and balance the aggression of free radicals [16, 20].

The protection of antioxidant system involve the enzymatic and non-enzymatic pathways. Because the antagonist protective system is promptly initiated, the lipid peroxidation is difficult to be proved *in vivo*, either on the individual cells, or on the global organism. However, the presence of antioxidant system himself confirms the theory of lipid peroxidation [21].

Superoxide - dismutases (SOD) are metal-containing enzymes depending on metal as manganese, copper or zinc, that are produced in the eukaryotes aerobic cells, but also in the facultative aerobic bacteria. Recognized as the main enzymatic factor that is able to acquire the tolerance to oxidative stress, SOD has been described since 1969 in [22, 23]. Nowadays, there are described three types of SOD, based on the type of metal from the catalytic center: the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in cytoplasm. The role of SOD is to catalyze the conversion of two superoxides into oxygen and

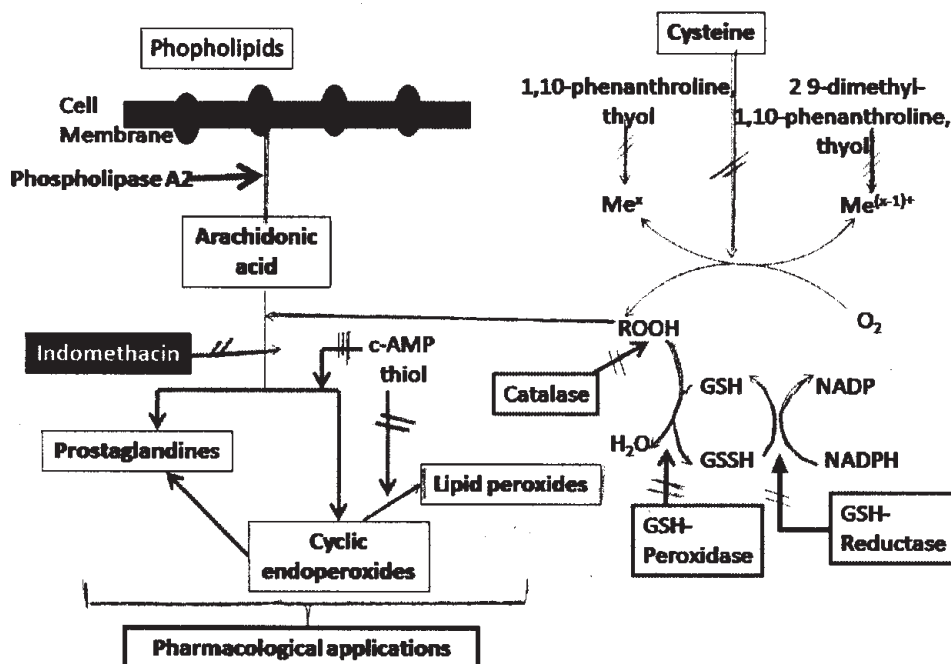
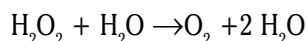


Fig. 1. The chart of the presumptive interactions between the arachidonic acid and the natural mediators or drugs, considering the role of the arachidonic acid in the inflammations and the interaction with certain anti-inflammatory agents

Legend: $\square$: inhibitor action; GSH: glutathion; GSSH: glutathion oxidized disulfide form; Me: metal ions catalyst; NADP: nicotinamide adenine dinucleotide phosphate; NADPH: NADP reduced form

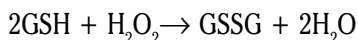
hydrogen peroxide, that is substantially less toxic than superoxide. Actually, the catalytic action of SOD is to accelerate over 1000 folds the dismutation of the superoxide in the hydrogen peroxide [18,22].

Catalase is an enzyme widely disseminated in the tissues, localised in the peroxisomes of mammalian cells. The activity level is varying depending on the tissue, but also within the same cell [16]. *Catalase* acts in the reactions of hydrogen peroxide conversion and uses hydrogen peroxide to oxidize toxins, including phenols, formic acid, formaldehyde and alcohols. Its major antioxidant role is the catalysis of the chemical decomposition of hydrogen peroxide into two molecules of water and one molecule of oxygen:

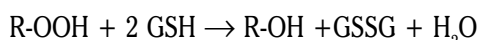


Glutathion peroxidase (GSH-Px) is one of the catalyzing enzymes involved in the degradation of organic hydroperoxide provided during the normal metabolic course. Also called the "life protein" glutathione is a bioactive polypeptide found in the most cells of the human body, acting as a coenzyme. Glutathione synthesis occurs in the liver, based on cysteine, glutamine and glycine amino acids [22, 23]. The activity of GSH-Px surrounded by the physiological conditions is difficult to be quantified, since for the same substrate there are multiple competitions with other enzymes. Owing to the high specificity of its substrate, the antioxidant potential of GSH-Px is superior than SOD and catalase. The importance of GSH-Px in detoxification process is based on the ability to decompose the peroxide radicals, the hydroperoxides (mainly the lipid peroxides) and even H_2O_2 . The role of *GSH-Px* is to protect the proteins, lipids and nucleic acids, regarding the free radicals [16, 20, 21].

This reaction consists of an electron donation, that is dislocated from the molecules of glutathione, thioredoxin or glutaredoxin [24].



or



Hydroperoxides (ROOH) are sources of peroxides, as H_2O_2 or any peroxide derived from the nucleic acids, polyunsaturated or steroid fatty acids. Glutathione (GSH) is the reduced monomeric compound, and GS-SG represents glutathione disulfide [25].

The reduction and the oxidation of glutathione are associated reactions and together are coupled with the reduction sequence of NADP^+ in $\text{NADPH} \cdot \text{H}^+$. Glutathione is a selenium depending enzyme. Consequently, we realize that the selenium deficiency increases the lipid peroxidation. Excepting hydrogen peroxide, similar activity on hydroperoxides it was found as well as other enzymes, called glutathione S – transferases, although they are not selenium depending [25].

Non-enzymatic systems comprise the liposoluble vitamins E and A. Vitamin E is the generic name of four compounds of tocopherol, characterized by the common tocol structure and different position of methyl groups: α , β , γ , δ . α -tocopherol contains a chromanol ring with 2-methyl-6-hydroxy substitution and a saturated radical containing 16 atoms of carbon. The antioxidant effect of vitamin E is more powerful *in vivo* than *in vitro*. Strategically placed α -tocopherol in the membranes presents the ability to block the lipid peroxidation [26].

The "radical vitamin E" generated by the hydroxy group fixed on benzenic cycle, is working up as an aggressive reducer. Although free radicals following a radical chain reaction are weakly reactive, they interrupt the membrane [18, 24].

Vitamin A is a less potent antioxidant, mainly acting by "blocking" the singlet oxygen. Since long time, carotenoids have been considered antioxidants, owing to their competence to scavenge the free radicals, mainly singlet oxygen, and therefore to block the lipids peroxidation. Significant antioxidant activity of β -carotene is induced by the xanthine oxidase system and act by inhibition of the lipoperoxidase [16].

The preference of lipid peroxidation for destructive action on the biological membranes is related to the high amount of polyunsaturated fatty acids specific to the phospholipid structure [18, 24].

Iron is one of the potent catalyzers of the peroxidation and as well as the usual structural metal of cytochromes. Cytochromes are hemoproteins capable of performing oxidation and reduction, held to be bound to unsaturated lipids of mitochondrial membranes. Therefore, free radicals could be produced by the lipid peroxidation of the fatty acids released from phospholipids of the membrane. Cytochrome P450 is an enzymatic system present in every cell of the organism, excepting the erythrocytes and the skeletal muscle cells. Particularly, free radicals disturb the electronic transfer of microsomal P450 cytochrome system, concerning the metabolism of certain substances in the organism, such as steroids, liposoluble vitamins, fatty acids, prostaglandins and alkaloids. The P450 cytochrome system detoxifies drugs and a large number of the environmental polluting agents [20, 27].

Moreover, the free radicals are involved in the inflammation mechanisms, acting as cytotoxic and chemotactic factors for the phagocyte leukocytes, respectively polymorphonuclears and macrophages [6, 28].

Phagocytosis is a complex process used to remove pathogens, cell debris and other foreign particles (fig. 2). The polymorphonuclears have multi-lobed nucleus and a very short life, while the mononuclear phagocytes have a large nucleus and a long life. Appointed by the chemotactic factors, the phagocytes are activated and recruited, moving towards the inflammation area, but first arrive the polymorphonuclears and next the macrophages [16]. Phagocytes adhere to the bacteria and ingest them inside the phagosome, using oxygen-dependent bactericidal mechanisms that activate the NADPH^+ oxidase and produce peroxide derivatives [23, 24].

The release of these reactive species requires to couple the phagocytosis with the glycolysis, as energy resource (fig. 3). Intracellular respiration increases, because augmented oxygen consumption is required for its activation and producing the free radicals [11,18].

Additionally, lysosomes migrate towards the phagosomes and discharge hydrolytic enzymes. The intracellular pH decreases considerably and generate optimal setting for hydrolytic enzymes activity. The combined action of the free radicals produced by the antimicrobial systems and the host cells hydrolytic enzymes, destroys the bacterial wall by halogenation, proteolysis and decarboxylation [18, 22].

Secondary metabolites of these processes, such as amino aldehydes or lipid peroxides and their decomposition products, are also toxic for some bacterial species. Various proteins such as lactoferrin, lysozyme and phospholipase

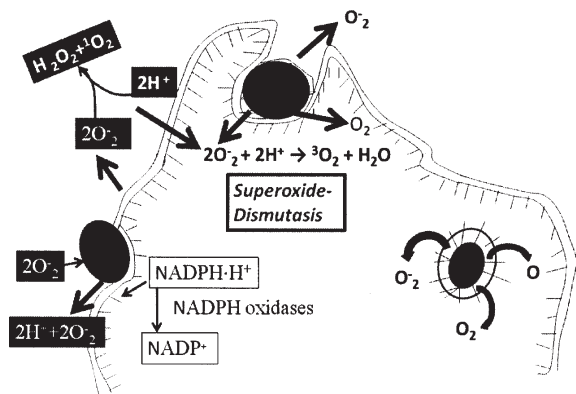


Fig. 2. Phagocytosis process: micro organisms adherence to the phagocytes → NADPH-oxidase activation → invagination through the pseudopods.

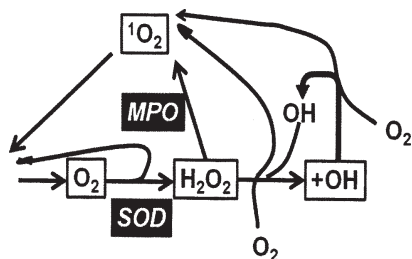


Fig. 3. The interactions between the activated forms of oxygen. We can notice the intervention of the superoxide-dismutase (SOD) and of the myeloperoxidase (MPO)

are involved in destructive actions, but their mechanism is still unclarified. The leukocytes involved in the phagocytosis carry inclusions of the microorganisms incompletely digested, mainly from Gram negative bacteria and circulate for a while [11,15].

In the chart of figure 4 we noticed that phagocytes are able to catalyze the decomposition of the excess of H_2O_2 by three enzymatic systems: peroxide-glutathione-peroxidase, catalase and myeloperoxidase [20, 29].

The antibacterial systems based on the hydrogen peroxide were detected in some cells. The main products of their activity are the free radicals of oxygen, although additional molecules, including the cyanides, are released. The cyanides are cytotoxic factors, explained by the large amount of energy from the radicals.

Conclusions

Normally, the largest part of the oxygen of human metabolism is combined with the hydrogen, resulting water. However, 4-5 % of the oxygen is transformed into superoxide anion and hydrogen peroxide, following an enzyme catalysis process. Since the high oxidative activity, they are very unstable.

The free radicals are active molecules produced during the oxygenation reactions of the basic activity of the human body. The chemical structure of free radicals is a superoxide-anion and hydrogen peroxide.

The functions of free radicals are to regularize the cells growth, to transmit the biological signals and to suppress the viruses or bacteria. The excess of free radicals in our body, attack the cellular and the mitochondrial membranes, reacting with the unsaturated fatty acids from the membranes and intensifying lipid oxidation.

Resulting of the lipid oxidation, free radicals are generating a chain reaction, follow-on by the alteration of the structural integrity of the membranes and cell injuries, related to inflammatory diseases and accelerated aging.

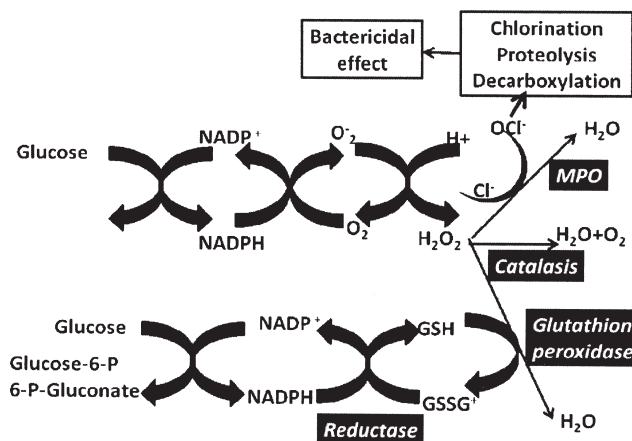


Fig. 4. The synthesis and decomposition of hydrogen peroxide (H_2O_2). The hydrogen peroxide is formed of the action of the superoxide-dismutase or through the pentose- phosphates shunt and is used for the catalyzed reaction of myeloperoxidase (MPO). The excess of H_2O_2 is decomposed through the action of the catalase and of the glutathione-peroxidase

Both useful and harmful effects on the human body are attributed to the free radicals. The benefits of free radicals are the antimicrobial protection by phagocytosis, stimulation of the cellular growth and proliferation, induction of the cellular apoptosis.

Oxidative stress is the consequence of unbalance between the production of the free radicals and the antioxidant competence. The main endogenous antioxidants are the enzymatic systems of superoxidismutase (SOD), catalase and glutathione peroxidase. Since endogenous antioxidants are not completely efficient, exogenous source are necessary in order to balance the free radicals actions.

Applications of free radicals are found in pharmaceutical area. Antioxidant pharmaceutical agents protect the organism against the oxidative stress by neutralizing mechanism, blocking the oxidation of structural lipids of the cellular membranes and of the circulating low density lipoproteins, rising the antioxidant enzymes or blocking the degenerative processes of premature ageing [30].

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