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ANTIOXIDANTS AS DEFENSE MECHANISMS AGAINST VIRAL PATHOGENS

Cristina Iulia MITRAN, Madalina Irina MITRAN and Mircea Ioan POPA

[1] Department of Microbiology, "Carol Davila" University of Medicine and Pharmacy, 050474 Bucharest, Romania
[2] "Cantacuzino" National Medico-Military Institute for Research and Development, 011233 Bucharest, Romania

Corresponding author: Cristina Iulia Mitran, cristina.iulia.mitran@gmail.com

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Antioxidants are molecules that interact with reactive oxygen species (ROS), leading to their neutralization and thus protecting cells from their toxic effects. When high levels of ROS are generated, they exert harmful effects on cellular components (lipids, proteins and nucleic acids), with the alteration of cellular functions. Many studies have shown that there is a link between oxidative stress and viral infections. ROS can modulate the viral replication and influence the spread of the infection. In turn, viruses are able to induce ROS-generating enzymes, such as xanthine oxidase and alter the oxidant-antioxidant balance. The increase in the production of ROS has been highlighted in numerous viral infections (human immunodeficiency virus, hepatitis B and C viruses, Epstein Barr virus, herpes simplex viruses, etc). Antioxidants may represent useful adjuvants in the therapy of viral infections.

Keywords: antioxidants, oxidative stress, viral infection

INTRODUCTION

Oxidative stress was first described as "a disturbance in the prooxidant to antioxidant balance in favor of the oxidant species, leading to potential damage"[1]. Under physiological conditions, oxidative stress has beneficial effects in the human body, since reactive oxygen species (ROS) play an important role in the defence against microorganisms (viruses, bacteria, etc.), contribute to cellular homeostasis, modulate various signaling pathways, etc. When high levels of ROS are generated, they exert harmful effects on cellular components (lipids, proteins and nucleic acids), with the alteration of cellular functions[2]. Studies show that oxidative stress may be involved in the pathogenesis of over 100 diseases[1].

ANTIOXIDANT SYSTEMS

When a cell is exposed to conditions of oxidative stress, genes that encode molecules involved in the defence against oxidants are activated. This stimulates the release of enzymes with antioxidant activity and compounds such as glutathione, the concentration of which increases at the cellular level by increasing cystine transport. When the level of ROS is not very high, antioxidant systems manage to restore the balance, but in the cases characterized by very high levels of ROS, different pathological disorders occur[3]. Antioxidants are molecules that interact with ROS, leading to their neutralization and thus protecting cells from their toxic effects[4]. Antioxidant agents are nucleophilic molecules, which, after interacting with oxidizing compounds, electrophilic molecules, transfer one or two electrons to them[5].

Three lines of antioxidant defense are described[4]. The first line inhibits the formation of free radicals. The most important enzymes involved are superoxide dismutase (SOD), catalase (CAT) and gluthation peroxidase (GPx) and as a result of the reactions they catalyze lead to the formation of inactive compounds. The activity of antioxidant enzymes is modulated by several factors. The most important regulatory factor is the oxidative status of the cell. Other factors involved are inflammation, hormones, aging [6]. The second line is represented by compounds that remove free radicals, such as vitamin E, vitamin C, uric acid, albumin, etc. The third line is involved in the repair of the aggressed tissue and the synthesis of new antioxidants, consisting of different proteolytic enzymes such as proteinases, peptidases, etc.[7].

Endogenous antioxidants are classified into two main groups, enzymatic (SOD, CAT, GPx, etc.) and non-enzymatic (glutathione, L-arginine, bilirubin, transferrin, coenzyme Q10, melatonin, etc.). An important role is also played by antioxidants of exogenous origin, such as vitamin C, vitamin E, polyphenols, magnesium, zinc, which cannot be synthesized by humans, being taken from food[8].

ENDOGENOUS ANTIOXIDANTS

Enzymatic antioxidants

Superoxide dismutases, a group of metal enzymes specific for organisms that can survive in the presence of oxygen participate in the conversion of the superoxide anion to oxygen and hydrogen peroxide. These enzymes present metals in their active domains, facilitating the transfer of electrons. Four classes of SOD have been described in various organisms depending on the ions involved in catalyzing the reaction, Cu/Zn SOD, Mn SOD, Fe SOD and Ni SODs. In humans, only the first two classes are found and are located in the cytoplasm, respectively in the mitochondria. The most abundant is the cytosolic form, Cu/Zn SOD. From a structural point of view, they are either dimers or tetramers[9]. In addition, an extracellular, secretory form has been described in humans, located in the interstitial space and in extracellular fluids[8].

Catalases are enzymes that are primarily involved in metabolizing the hydrogen peroxide to water and oxygen. Only aerobic organisms possess these enzymes. Most catalases are homotetramers and have four ferriprotoporphyrin groups per molecule in the active domain. CAT is located mainly in peroxisomes [10] and is one of the most effective enzymes known, so regardless of the level of H_2O_2 the saturation threshold is not reached, a CAT molecule having the capacity to metabolize 6 million molecules of H_2O_2. It has one of the highest turnover rates of all known enzymes[11].

GPx is part of the so-called glutathione system which also includes glutathione, glutathione reductase and glutathione transferase[8]. GPx reduces lipid hydroxides to the corresponding alcohol molecules, using glutathion and hydrogen peroxide to water and oxygen. GPx is a selenium-containing peroxidase and is considered the main enzyme involved in the protection against increased levels of oxidative stress[10]. Five isoforms of GPx have been described in mammals. Their expression is ubiquitous, but the levels differ depending on the type of tissue. For example, GPx-1 (isoform 1) is found mainly in erythrocytes, kidneys and liver[8].

Non-enzymatic antioxidants

In the human body, thiol groups can have a dual role. They can act as active ROS removal systems or they can posttranslationally modulate the functions of certain proteins. The oxidation of thiols leads to the generation of -til radicals that react with molecular oxygen or nitric oxide[12]. Thiols are a class of sulfur derivatives and contain sulfur atoms attached to a carbon atom[13]. The most studied thiol compounds are grouped into several classes, namely, low molecular weight compounds (cysteine and acetylcholine), low molecular weight compounds with peptide structure (glutathione) and protein thiols (thioredoxin)[14]. Low molecular weight thiol compounds are rapidly oxidized and have an increased regenerative capacity, being involved in various pathophysiological processes. Glutathione is one of the most important compounds in this category. It should be noted that disulfide bonds are essential in the tertiary structure of a protein. Glutathione has in its structure one molecule of cysteine and is involved in processes such as cytoprotection and transduction[15].

Thiols inhibit the release of pro-inflammatory cytokines and prevent the activation of nuclear factor-kB (NF-kB), including in cases where increased ROS production does not occur, which has raised the hypothesis that the role of thiols is more complex and they are not only involved in ROS inactivation [16]. ROS transfer excess electrons to thiols, leading to the oxidation of thiols and the formation of disulfide bonds[17]. Under oxidative stress conditions, thiols come into contact with prooxidant elements and lead to their transformation into compounds with lower reactivity. The thiol is oxidized and converted to disulfide, a reversible reaction[18].

Glutathione is one of the most important antioxidants. Its synthesis takes place in the cytoplasm where the glutamate and cysteine are transformed into gamma glutamyl cysteine. Subsequently, glutathione is translocated to the mitochondria, endoplasmic reticulum and nucleus. Glutathione can be found in both the reduced (GSH) and the oxidized (GSSG) form. The ratio between the two forms can be considered a marker of oxidative stress. Under conditions of oxidative

stress, the GSSG concentration increases, which will generate an increase in the amount of disulfides, with harmful effects on protein function[19]. Elevated glutathione levels are associated with the inhibition of NF-κB activation which will prevent the release of proinflammatory cytokines. Thus, the GSH is involved in processes such as cell proliferation and apoptosis[20]. Glutathione induces the apoptosis by activating the SAPK/MAPK pathway. Glutathione is involved in detoxification mechanisms, one of the most important being the formation of glutathione S conjugates. Other roles of glutathione are the participation in the regeneration of vitamins C and E, cofactor of some enzymes, the transport of mercury outside the cell etc. Low levels of glutathione have been observed in many conditions such as asthma, chronic obstructive pulmonary disease, hypertension, myocardial infarction, Parkinson's disease, autoimmune diseases, etc.[21].

Melatonin is an indole-amine, which is known as a hormone with the main function of regulating the circadian rhythm of physiological and neuroendocrine processes. Subsequent studies have highlighted its antioxidant role[22]. Melatonin exerts its antioxidant role either directly, participating in the detoxification of ROS and RNS, but also indirectly having a stimulating effect on antioxidant enzymes and an inhibitory effect on prooxidants. Melatonin is found ubiquitously, but the highest amounts are at the mitochondrial level[23]. Melatonin seems to regulate antioxidant enzymatic activity through the interaction with calmodulin[22]. In addition, melatonin increases the efficiency of the electron transport chain at the mitochondrial level, which contributes to the decrease in ROS production[24].

L-arginine is an essential amino acid in humans. L-arginine is the substrate of two enzymes, arginase and NO synthetase[6]. L-arginine stimulates glutathione synthesis and can suppress oxidative stress and stimulate an antioxidant response[25].

EXOGENOUS ANTIOXIDANTS

After iron, zinc is the most important microelement in the human body[26]. The main source of zinc is red meat, followed by dairy products and cereals. Metallothioneins, a group of cysteine-rich metal-binding proteins, play a very important role in zinc homeostasis. Thus, stress and inflammation, processes that affect metallothioneins will also affect the action of zinc. Cysteine residues in the structure of metallothioneins will be reduced when zinc is sequestered and will be oxidized when it is released[27]. Zinc plays a very important role in maintaing the balance betweeen oxidants and antioxidants, being part of the SOD, one of the most important enzymes involved in the oxidative stress. Another mechanism by which zinc intervenes in oxidative stress is the stabilization of sulfhydryl proteins. Thus, the main enzymes protected by zinc against the harmful effects of oxidative stress are d-aminolevulinate dehydrase, alanyl-tRNA synthetase, tubulin and dihydroorotase[26]. Zinc has antioxidant action by inhibiting NADPH oxidases, enzymes that catalyze the production of O_2^-. Zinc also binds the OH^- ion by increasing the expression of metallothioneins that have cysteine residues in their structure. Moreover, it competes with iron and copper to inhibit the generation of the OH^- ion[28].

Vitamin C cannot be synthesized by humans, being one of the most important water-soluble vitamins. It seems that the antioxidant effect of vitamin C is potentiated by vitamin E. Vitamin C can eliminate free radicals, such as peroxyl radical[29]. It acts as a cofactor for hydroxylases and monooxygenases, enzymes involved in the synthesis of collagen, carnitine and neurotransmitters[30]. It can recycle certain antioxidant molecules such as alpha-tocopherol or glutathione[31]. Its antioxidant action is mainly based on its ability to donate electrons. By donating its electrons, vitamin C prevents the oxidation of other compounds. Thus, the ascorbate redox form of vitamin C interacts with free radicals, reducing them and leading to the formation of the ascorbyl radical, which is less reactive[32].

Vitamin E is found mainly in nuts, sunflower seeds and vegetable oils[33]. The vitamin E family includes four tocopherols and four tocotrienols, all forms with antioxidant activity. Alpha-tocopherol is the dominant form found in tissues and has an important role in neutralizing the peroxyl radical. Although alpha tocopherol is the most investigated, there are studies showing that tocotrienol has a superior activity in removing this radical[34,35]. Alpha tocopherol prevents the oxidation of the polyunsaturated fatty acids in lipoproteins or cell membranes. Vitamin E intervenes in the reaction with the peroxyl radical resulting in the tocopherol radical which will interact with vitamin C or glutathione to return to the reduced form[35].

Polyphenols are compounds composed of one or many aromatic rings with one or more hydroxyl groups in their structure. It has been shown that thousands of phenolic and polyphenolic compounds result from plant metabolism. Flavonoids represent one of the most important groups[36]. Polyphenols are found mainly in fruits and juices. They have antioxidant action through phenolic groups can accept unpaired electrons resulting in stable phenoxyl radicals[37].

ANTIOXIDANTS AND VIRAL INFECTIONS

Many studies have shown that there is a link between oxidative stress and viral infections[38,39]. ROS can modulate the viral replication and influence the spread of the infection. In most cases, once the virus is detected by the immune system and inflammatory cells are attracted to the site of infection and activated, NADPH oxidase activity is up-regulated and nitric oxide synthesis increases. Pro-inflammatory cytokines play an important role in the production of ROS. In the early stages of infection, the production of ROS is a host defense mechanism against the virus. However, as the infection progresses, ROS accumulate exerting harmful effects such as the perpetuation of an inflammatory process and cell death[40]. Viruses are able to induce ROS-generating enzymes, such as xanthine oxidase and alter the oxidant-antioxidant balance. The increase in the production of ROS has been highlighted in numerous viral infections (human immunodeficiency virus, hepatitis B and C viruses, Epstein Barr virus, herpes simplex viruses, etc)[41]. Antioxidants influence gene transcription, modulate signaling pathways involved in the host's immune response, and exert direct antiviral effects[42]. A recent study highlights the possible mutagenic role of ROS, especially in the case of RNA viruses. It is known that RNA viruses have a higher mutation rate than DNA viruses. These viruses could be more susceptible to the action of ROS[43].

It is well known that nutritional deficiencies are associated with increased susceptibility to infectious agents. Vitamins A, B6, B12, C, D, E, and trace elements (zinc, iron, selenium, magnesium, and copper) participate in innate and adaptive immunity. These compounds are involved in processes such as the proliferation and differentiation of immune cells, production of antimicrobial agents, chemotaxis, phagocytosis, etc.[44].

Antioxidants such as vitamin C and vitamin E are involved in host immune response. It has been shown that the administration of vitamins and micronutrients improves the course of HIV infection[45]. It has been shown that elevated vitamin C levels correlate with increased proliferation of T lymphocytes and stimulation of immunoglobulin synthesis. In addition, vitamin C stimulates the activity of NK cells[46]. Research has shown that intravenous administration of vitamin C has beneficial effects on the evolution of postherpetic neuralgia[47]. Moreover, vitamin C deficiency correlates with an increased susceptibility to severe respiratory infections. Vitamin C may improve the course of SARS-CoV-2 infection. A reduction in the number of T lymphocytes was identified among these patients[48]. In addition, the consumption of juices which contain high amounts of vitamin C was associated with a better evolution of patients with hepatitis C receiving antiviral therapy[49]. Vitamin E participates in important immune processes such as the augmentation of the phagocytosis capacity of alveolar macrophages and the increase in the production of IL-2[46]. Vitamin E stimulates T-cell-mediated immunity, and a recent study found that subjects who received vitamin E supplements had a lower risk of developing upper respiratory tract infections[50].

The study by Pugliese et al. showed that HIV-infected children have inadequate serum levels of copper and selenium[51]. In HIV patients, selenium deficiency has been shown to correlate with low T lymphocyte counts, disease progression and an increased risk of death[41]. It has been shown on animal models with selenium deficiency that coxsackievirus and influenza virus exhibit an increased virulence due to changes in the viral genome. It should be noted that selenium is associated with antioxidant enzymes, such as glutathione peroxidase; selenoenzymes GPX1 and GPX4 are ubiquitously expressed and are key enzymes of the antioxidant system[52]. A study performed on glutathione peroxidase-1 knockout mice showed that they developed myocarditis after being infected with a normally amyocarditic strain of coxsackievirus B3 and the viral sequencing revealed seven nucleotide changes when compared to the stock virus[53].

Other important trace elements are zinc and iron. Zinc participates in maintaining the integrity of the main physical barriers represented by skin and mucous membranes. Zinc deficiency is associated with an exaggerated proinflammatory response, increasing the production of proinflammatory cytokines. Zinc also stimulates the activity of regulatory T lymphocytes. Studies have

revealed a reduction in the severity of respiratory infections among groups of patients receiving zinc-based supplements. In addition, it has been shown that zinc exhibits antiviral effects on Dengue virus and coronavirus[48]. Iron enhances the immune response by contributing to the synthesis of enzymes such as ribonucleotide reductase, a key enzyme involved in immune cell proliferation and induces T cell differentiation[54].

Melatonin has proinflammatory action by modulating several transcription factors, NFκB, inducible hypoxia factor, nuclear erythroid factor 2-related factor 2. Melatonin also mediates the immune response by acting on the thymus. In fact, it regulates the activity of the thymus indirectly, in a zinc-dependent manner. As mentioned above, zinc is a key element in the body. It has been shown that melatonin increases immunoglobulin synthesis and IL-2 production. Based on these observations, melatonin is one of the drugs recommended among patients infected with SARS CoV-2[55].

Recent research has shown that adjuvant antioxidant therapy in patients with hepatitis C virus infection (HCV) attenuates the protein oxidation and increases serum glutathione levels[56]. Groenbaek *et al.* administered antioxidant supplements to a group of 23 patients with HCV infection and analyzed the effects on liver transaminases, viral load and markers of oxidative stress. They observed an increase in GPx activity and plasma levels of vitamin C and alpha tocopherol. However, no changes in liver transaminases and viral load were observed[57].

CONCLUSION

The human body is endowed with numerous lines of defense against pathogens, among which oxidative stress plays an important role. Antioxidants are key molecules in maintaining the local homeostasis, preventing the negative effects of oxidative stress, caused by the accumulation of ROS, a phenomenon that may occurs during viral infections. At the same time, antioxidants modulate the immune response and can be used as adjuvants in the treatment of viral infections. In conclusion, it should be mentioned that "Cantacuzino" National Medico-Military Institute for Research and Development produces natural SOD, an antioxidant dietary supplement. Natural SOD contains vitamins, minerals and essential amino acids obtained from green barley. The product increases the body antioxidant capacity through the activity of superoxide dismutase and catalase. It is a valuable product that can be used in various pathological conditions related to the disturbance of the balance between oxidants and antioxidants, but also in maintaining the local homeostasis.

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