

The prooxidative-antioxidative system components

(Składowe układu prooksydacyjno-antyoxydacyjnego)

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Abstract – The authors have presented respective elements of the prooxidative-antioxidative system. Thus they discussed the formation of free oxygen radicals and noted their biological effect. Then they presented antioxidants in more detail, dividing them into physiological antioxidants and synthetic compounds. The subsequent part of the paper is devoted to preventive antioxidants and free radical scavengers.

Key words - free radicals, biological effects, antioxidants.

Streszczenie – Autorzy przedstawili poszczególne elementy układu prooksydacyjno-antyoxydacyjnego. Omówili więc powstawanie wolnych rodników tlenowych i zwrócili uwagę na ich biologiczne działanie. Następnie szczegółowiej przedstawili antyutleniacze dokonując podziału na antyutleniacze fizjologiczne oraz związki syntetyczne. Kolejną część artykułu poświęcili antyoksydantom prewencyjnym i zmiataczom wolnych rodników.

Słowa kluczowe - wolne rodniki, biologiczne skutki, antyoksydant.

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I. FREE RADICALS

According to the definition, a free radical is an atom or a molecule which is capable of independent existence and has one or a few unpaired electrons on the valence shell (e.g. H₂O₂ – hydrogen peroxide, O₂⁻ – superoxide radical, OH⁻ – hydroxyl radical). This state is energetically unfavourable due to high reactivity and short lifetime of free radicals.

Stable energy state is achieved through taking over an electron from surrounding molecules and creating an electron pair. The molecule from which the electron has been taken becomes a free radical itself and a series of such transformations leads to a chain oxidation-reduction reaction [1,2].

In a living organism, the main source of free oxygen radicals are respiratory processes in cells. In processes catalysed by such enzymes as NADPH oxidase, xanthine oxidase, aldehyde oxidase, lipoxygenase, cyclooxygenase, radicals called reactive oxygen species (ROS) are formed. The autoxidation process of biologically active compounds, e.g. hydroquinones, epinephrine, haemoglobin and thiol compounds is also the source of ROS. The effect of ionizing radiation and microsomal hydroxylation of exogenous compounds such as adriamycin, nitrofurantoin, paraquat or carbon tetrachloride are another sources of large quantities of free radicals [3-5].

Apart from mitochondrial system of electron carriers, free radicals can originate from the reaction of autoxidation of reduced compounds (e.g. adrenaline, ferredoxin, flavin), from the first phase of biotransformation of some chemical compounds involving the cytochrome P-450 of

endoplasmic reticulum (the compounds include aromatic and aliphatic hydrocarbons, heavy metals, and others), from the so-called respiratory burst of activated phagocyte cells of neutrophils, eosinophils and macrophages. In the right conditions, free radicals are quickly inactivated by the cell's antioxidative system [6,7].

If this does not occur, a chain reaction of radical-forming processes starts, e.g. the reactions of a radical with a molecule cause the formation of another radical. Harmful effect of free radicals becomes noticeable when their concentration in body fluids, tissues and cells exceedingly increases. The accumulation of a large quantity of free radicals leads to their interaction with the elements of interior milieu and the damage of the latter [8-11]. Harmful effects of the influence of ROS on our organism are presented in Table 1.

Table 1. Selected effects of reactive oxygen species influence on cells and cell components [1,11,12]

Reactive oxygen species	Biological effects of reactive oxygen species
	• Oxidation of low-molecular-weight compounds
	• Degradation of collagen, the loss of the gelation ability
	• Depolymerisation of hyaluronic acid
	• Deterioration of the pulmonary surfactant function
	• Haemoglobin oxidation
	• Inactivation of enzymes
	• Inactivation of transport proteins
	• Proteoglycan synthesis disorders
	• Breaks of DNA strands, damage to DNA bases, ribose degradation
	• Damage to chromosomes
	• Peroxidation of lipids
	• Lysis of erythrocytes
	• Inhibition of oxidative phosphorylation in mitochondria
	• Ca ²⁺ haemostasis disorders
	• Cytoskeleton structure disorders
• Modification of antigenic properties of cells	
• Platelet aggregation	
• Changes in the cell morphology (like membrane blebbing)	
• Formation of mutations	
• Malignant transformation of cells	

Free radicals differ principally in terms of activity. The most active free radical is the hydroxyl radical (OH*), which can potentially react with any molecule

and is presently considered the main reason for the oxygen toxicity. Less active radicals but potentially cytotoxic as well include e.g. the peroxy radical ROO*, the superoxide radical O₂⁻, the hydroxyperoxy radical OOH*, hydrogen peroxide H₂O₂, the alkoxy radical RO*, the hydroxyperoxy derivative ROOH, the peroxide derivative ROOR, the aroxy radical ArO* [3,6].

However, single-electron reactions (typical for the formation of free radicals) have to take place, since they condition proper functioning of the organism. In such a process of transformations, e.g. prostaglandins or nitric oxide (NO*) can be synthesised. Free radicals play also a significant role in intracellular signalling, responsible for e.g. initiating the synthesis of some proteins [6,12-14].

II. ANTIOXIDANTS

In the homeostasis of the organism, the influence of ROS is balanced by antioxidants. They are substances which significantly inhibit the level of molecule oxidation, while occurring in relatively low concentrations. Antioxidants can be divided into physiological (natural) antioxidants and synthetic compounds. In both these groups, there are antioxidative enzymes, preventive antioxidants and free radical scavengers [1,14].

Antioxidative enzymes include, among others:

Superoxide dismutase (SOD): A metalloenzyme occurring in two forms: in the intracellular form – which divides into the mitochondrial form with manganese in the active centre (MnSOD) and the cytoplasmic form with copper and zinc (Cu/Zn SOD) – and the extracellular form. This enzyme decomposes the superoxide radical [3,4].

Catalase (CAT): A haemoproteinase with peroxidase properties. It catalyses the reactions of hydrogen peroxide reduction. It shows the greatest activity in the liver, kidneys and erythrocytes [5,7].

Glutathione peroxidase (GPx): It is a metalloenzyme and takes part in the reduction of hydrogen peroxide while at the same time reduced glutathione is transformed into its oxidised form. Three forms of this enzyme are known: intracellular, membrane and extracellular (present in blood). The first two forms contain selenium. The molecule of the membrane form is a so-called lipid hydroxide peroxidase. Supplementation with selenium increases its activity. Glutathione peroxidase reduces dehydroascorbates to vitamin C [5,7].

Preventive antioxidants include, i.a. [8]:

Transferrin: The main iron-transporting protein in the organism

Lactoferrin: it binds iron

Caeruloplasmin: it binds copper

iron and copper, which belong to the transition metals, contain unpaired electrons and they usually take part in free radical reactions as substrates for the formation of highly reactive hydroxyl radicals (Haber-Weiss reaction)

Free radical scavengers include, among others [1,2,4,5,7]:

Vitamin E (tocopherol): The strongest fat-soluble antioxidant. Peroxide radicals react with vitamin E 120 times faster than with multisaturated fatty acids. As a result of this reaction, inactive tocopherol radicals are formed, which move onto the surfaces of cell membranes. There, in reaction with vitamin C, they are reduced to α -tocopherol.

Vitamin C (ascorbic acid): As it is water-soluble, it maintains appropriate oxidation-reduction potential in a cell and it is the main antioxidant of the extracellular fluids.

Vitamin A: As an antioxidant, it has immunogenic properties and therefore it is used in the prevention of viral infections, malaria, tuberculosis. There are two principal dietary sources of vitamin A: easily absorbed retinyl palmitate in food of plant and animal origin and slightly absorbed carotenoids from the food of plant origin.

Carotenoids: They comprise a group of about 50 compounds which protect cell and cytoplasmic membranes against the influence of ROS.

Ubiquinol-10: A reduced form of the coenzyme Q10, fat-soluble. It occurs in lipoproteins in relatively low concentrations but it is capable of regenerating tocopherol from the tocopherol radicals and increasing its antioxidative efficiency.

Flavonoids (phytoestrogens): A group of compounds present in fruit, vegetables, tea, and red wine. They also include isoflavones, which occur in soya products. Genistein – a phytoestrogen occurring in soya beans – has antioxidative properties; it "catches" the peroxide anions, singlet oxygen, the hydroxyl radicals and the lipid peroxide radicals. The last property is related with inhibiting effect of genistein on the in vitro oxidation of LDL cholesterol. Approximately 3-4% of genistein is

built into the LDL molecule, where it protects tocopherol contained therein against oxidation and prolongs its effect. Genistein protects cells against cytotoxic influence of the ox-LDL. In large concentration, this occurs through the antioxidative effect and in small concentrations – through direct protection of cells.

Nicotinamide: A natural antioxidant necessary for the synthesis of NAD and NADP – the compounds which are cofactors of many enzymes that catalyse oxidation-reduction reactions.

Glutathione (γ -glutamyl-cysteinyl-glycine, GSH): Renders all forms of ROS harmless, protects protein thiol groups against irreversible inactivation caused by ROS, eliminates effects of harmful activity of ROS, e.g. taking part in the decomposition of lipid peroxides – the products of lipid damage. Apart from antioxidative protection, glutathione takes part in electrophile compounds detoxication, metabolism of leukotrienes and prostaglandins, it reduces dehydroascorbic acid to ascorbic acid, takes part in the reduction of methaemoglobin, it is a form of cysteine transport, it influences the activity of the glycolysis enzymes. The main place of glutathione synthesis is the liver, from where it migrates to other tissues haematogenously. The cells capable of absorbing GSH have γ -glutamyl transpeptidase on their surface. The main consumers of GSH are kidneys, the brain, lymphocytes, lungs and intensively working muscles.

The antioxidative defence system of the organism is three-stage. The first line of defence consists in preventing the formation of free oxygen radicals and their reaction with biologically active compounds. Antioxidative enzymes and proteins that bind ions of transition elements take part in this defence system. The second line of defence comprises reactive oxygen species scavengers. In the water environment, these are vitamin C, uric acid and glutathione, and in the lipophilic environment – vitamin E, carotenoids and ubihydroquinone. These compounds stop the free-radical-forming chain reactions as well as oxidation reactions. The third line of antioxidative defence is responsible for the elimination of the effects of the reaction of ROS with biomolecules. This involves recreating proper structure of damaged molecules, e.g. by means of the enzymes which repair damaged DNA [14].

The efficiency of the antioxidative potential of the organism is evaluated with certain analytical methods. The parameters which comprehensively describe the antioxidative state of the organism include, among others, the total free radical scavenging ability – TRAP (Total Radical-Trapping Antioxidant Parameter) and the total antioxidative potential of plasma/serum – TAS (Total Anti-

oxidant Status). It is also possible to determine the concentration of vitamins E, A, C and coenzyme Q10 in serum and the activity of glutathione peroxidase and peroxide dismutase. The concentration of the final product of lipid peroxidation – malondialdehyde (MDA) – is also an indirect indicator of antioxidative evaluation [15].

III. REFERENCES

- [1] Bartosz G. *Druga twarz tlenu*. PWN, Warszawa, 2003.
- [2] Chevion S. *et al.* The antioxidant status of patients subjected to total body irradiation (TBI). *Biochem. Mol Biol Int* 1999; 6:1019 - 1027.
- [3] Giacosa A, Filiberti R. Free radicals, oxidative damage and degenerative diseases. *Eur J Cancer Prev* 1996; 5: 307 – 312.
- [4] Haramaki N, Packer L. Oxidative stress indices in exercise. W: *Exercise and Oxygen Toxicity*. Sen CK, Packer L, Hanninen O (red.). New York; Elsevier, 2014 : 77 - 87.
- [5] *Insler MS, Helm CJ. Molecular Cell Biology. New York; Scientific American Books, 2012.*
- [6] Davson H. *Textbook of general physiology*. New York; Churchill, 2013.
- [7] Karp G. *Cell Biology*. New York; McGraw-Hill Book Company, 2013.
- [8] Racek J. *et al.* Free radicals in immunology and infectious diseases. *Epidemiol Mikrobiol Imunol* 2001; 50 (2): 87 – 91.
- [9] Klahorst S. Exploring the Antioxidant Frontier. The world of food ingredients. March 2002, [www.google.pl/?gfe_rd=cr&ei=NBICVbeaNcuF8QfTzID4BA&gws_rd=ssl#q=\[9\]%09Klahorst+S.+Exploring+the+Antioxidant+Frontier.+The+world+of+food+ingredients](http://www.google.pl/?gfe_rd=cr&ei=NBICVbeaNcuF8QfTzID4BA&gws_rd=ssl#q=[9]%09Klahorst+S.+Exploring+the+Antioxidant+Frontier.+The+world+of+food+ingredients)
- [10] Pokorny J, Yanishlieva N, Gordon M. *Antioxidants in foods, practical applications*. Cambridge; Woodhead Publishing Ltd., 2001.
- [11] Chun T-H., Itoh H, Saito T, Yamahara K, Doi K, Mori Y. *i wsp.* Oxidative stress augments secretion of endothelium-derived relaxing peptides, C-type natriuretic peptide and adrenomedullin. *J Hypertens* 200.; 1: 575–580.
- [12] Halliwell B, Gutteridge J. *Free radicals in Biology and Medicine*. Oxford; Oxford Press, 2012.
- [13] Commission of the European Communities: Nutrition and energy intakes for the European Community. Reports of the Scientific Committee for Food: 31st series. Luxembourg 1993.
- [14] Bloch RS, Gartner S. *Physiologische Chemie*. Stuttgart; Aufl. Enke, 2013.
- [15] Pawłowska-Góral K. *i wsp.* Całkowity potencjał antyoksydacyjny, metody pomiaru, przydatność kliniczna. *Diagn Lab* 2003; 39: 327 – 338.