# The Action of the Free Radicals on DNA and Anti-radical Defence Mechanisms

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*The secret to the survival of the organisms, the adaptation to high concentrations of free radicals (FR), consists in finding and adopting oxidative substances. When the radical species are not destroyed properly, there is what the bio-medical literature calls oxidative stress. Adapting to relatively high concentrations of $O_2$ has been done by selection and the incorporation of some anti-oxidant systems, enzymatic and non-enzymatic. If we can talk about the existence of a strategy in the antioxidant action, it consists in sacrificing these substances to protect some molecules of biological importance.*

*Keywords: free radicals (FR), oxidative substances, anti-oxidant systems*

The genetic role of the DNA is the direct consequence of its molecular structure able to stock a huge quantity of hereditary information, to obtain its expression and preservation.

In 1953, Watson and Crick imagined a model of the DNA molecule with two polynucleotide chains, complementarily tied by nitrogenous bases, wrapped plectonemic (the chains are wrapped one around the other and both around a central axis) to form a double propeller.

This model explains the way to stock, preserve and express the hereditary information.

The free radicals can be formed in the organism as a result of the endogenous metabolic activity or the assimilation of some chemical polluting substances locally at cell level from an organ or in more tissues at the same time or gradually.

The normal cell metabolism produces free radicals derived by oxygen. The increase of the partial pressure of the oxygen increases the mitochondrial production of FR.

The free radical is the molecule or atom that has non-pair electrons on the last orbital. The tendency of the odd electron to pair and cancel the magnetic field makes the free radicals to be significant aggressors. It is the case of the superoxide radical, first stage of oxygen reduction, which then leads to the formation of $H_2O_2$ that does not have structure of de radical but it is source of hydroxyl radicals.

When the cells are exposed to oxidative stress, there are frequent prejudices to DNA. The molecular mechanisms that cause these prejudices can include the activity of nucleases and the direct reaction of the hydroxyl radicals with DNA. Several species coming from oxygen can attack the DNA, producing different models of chemical modifications.

By cells undergoing the oxidative stress, severe metabolic dysfunctions can be produced, including the peroxidation of the lipid membranes, breaking the nicotinamide nucleotides, the increase of intracellular concentration of free calcium ions, cell destruction and prejudices on DNA. The latter is often measured by the formation of simple chains, breaking the double chains and chromosome aberrations.

The oxidative stress and the prejudices on DNA appear also when certain cells of mammals are exposed to some factors that cause tumours [1, 2].

The antioxidants are molecules that form the first target in the way of FR, being oxidised or transformed into other less harmless FR.

Haliwell and Ghutteridge [3] define the antioxidants as *any substance that presents in comparatively small concentration with that of an oxidable sub-layer, avoiding significantly or inhibiting the oxidation of that sub-layer.* The definition includes compounds both of enzymatic and non-enzymatic nature.

Due to the very different chemical structure, the antioxidants classify in a simpler way in enzymatic and non-enzymatic, and on their turn, they are lipid and water soluble.

Both the enzymatic and non-enzymatic antioxidants are located in different mediums (membranes, cytoplasm, extracellular liquids). Even though each antioxidant functions according to mechanisms and at different levels in the chain of evolution of the free radicals, the synergy of their action and summing up the combined action to which it is also added the localization in different compartments, offer them a great anti-oxidative efficiency.

## Experimental part

*Enzymatic antioxidants – biomarkers of oxidative stress*

Live organisms have many strategies in order to discomfit the effect of the oxidative stress. Among them, the relevant efficiency is present in the enzymatic systems. The major antioxidant function is covered by two large categories of enzymes:

- enzymes with direct action, of prevention, located in the first line of defence (SOD, hydroperoxide, catalase and glutathione-peroxidases, and other hem- peroxidises, whose intervention depends on the physiological or pathological state [4], to which it is complementarily added

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the specific form of the metal involved in the catalysis - Cu, Zn, Mn, Fe, and Se;

- auxiliary enzymes.

The first step in the control of the oxygen activated is the dislocation $O_2$ and $H_2O_2$ under the action of SOD, which is a ubiquitous enzyme, catalogued initially as form of deposit of Cu with different names: erythro cupreine (RBC), hepatocupreina (liver), cerebrocupreina (brain).

The enzyme is present in all the aerobe cells, as well as in the optionally aerobe bacteria.

Intense studies regarding the regulation of SOD biosynthesis proved that the enzyme is inducible, property that is an advantage to the organism, on taking into account that the biosynthesis is accelerated at high concentrations of $O_2$, and $F^{2+}$ has a repressive effect on the induction produced by paraquat in suspension of E.coli (paraquat is compound redox that produces large quantities of $O_2$).

SOD is tested for the clinical application at potential therapeutic agent in pathological conditions associated with oxidative stress, so that the post ischemic reperfusion of the organs, lung and hepatic affections, acute and chronic inflammations, or of protector in radiotherapy (Montemj and collab., 5). Special results were obtained in diseases where SOD in blood decreases, such as: advanced senescence, diabetes, cancer and leukaemia, brain and vascular diseases, rheumatoid arthritis [4]. Being a protein metabolized with low penetrability at the target place, the administration of SOD imposed the solution to these disadvantages.

Its encapsulation in liposome was used, or the tie to different compounds: polyethylene glycol or SOD synthetic or the form of the complex enzyme Cu $^{24}$-3,5-diizopropyl salicylate.

Cu, Zn-SOD does not protect the bloodstone in the presence of some compounds with oxidative properties such as: nitrites, polyphenols, phenylhydrazine; this behaviour finds its explanation in the oxidation of the oxyhemoglobin, according to the equation:

$$-HbO_2 \rightarrow metHb + O_2$$
$$HbO_2 + O_2 + 2H^+ \rightarrow met\ Hb^+ + 2H_2O_2$$

The observations made prove the production in conditions of oxidative stress and $H_2O_2$, hence the necessity of coupling SOD with catalase, enzyme that decomposes $H_2O_2$. This pair of enzymes is characterised by high speed of action, assuring that the relation is avoided:

$$O_2 + H_2O_2 \rightarrow HO. + HO^- + O_2$$

and supplies the most reactive species of oxygen for which the organism is not equipped with mechanisms of destruction.

Catalase – it is understandable that the aerobe organisms, implicitly the human body, are not equipped with two major classes of mechanisms of defence against $H_2O_2$: catalase and enzymes associated to the glutathione.

Catalase is the protein that contains four groups of ferriporphyrin. It can be highlighted in cytosol [6], but in many tissues it is located in peroxisome and micro-peroxisome. The highest concentration is in liver, and the lowest in the conjunctive tissue; the hepatic cell contains lower quantities, and in mitochondria. In erythrocytes, it is present in free form, and only in traces it is tied to the proteins of the stroma.

Catalase decomposes with great affinity $H_2O_2$

$$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$$

The enzyme has double catalase function, as well as peroxidase. The enzyme does not have an important role in protecting the erythrocytes compared to $H_2O_2$ endogenous; the major way to remove $H_2O_2$ is catalysed by GSH peroxidise [7].

If supplementary sources of formation of peroxides interfere: drugs or ionising radiation, the catalase concentration is very important. In these circumstances the formation of the methemoglobin depends on the catalase concentration, the high calalase activity confers higher resistance to the cells comparing with the oxidative action of $H_2O_2$.

Glutation peroxidase (GPx) – apart from the efficiency in antiperoxide defence, GPx presents some advantages comparing with the other systems of protection: it combines the antioxidant capacities of the thiols; it detoxifies all the types of peroxide existing in the biological environment; it decomposes $H_2O_2$ more efficiently than the catalase; it uses as the second sub-layer GSH, the most mobile thiol compound, present in all the types of animal cells and easy to regenerate by the pentose-phosphoric shunt [8].

GPx protects the cells from the prejudice produced by the radicals directly by reducing the peroxide concentration, useful interference because the peroxide acts as amplifier of the free radical process.

$$\text{Initiation: } RH + \text{initiator} \rightarrow R$$
$$\text{Propagation: } R + O_2 \rightarrow ROO$$
$$ROO\ R + RH \rightarrow ROOH + 2R$$
$$\text{Amplification: } ROOH + Fe^{21} \rightarrow RO + 2OH + Fe^{31}$$

Each peroxide produced is a potential initiator for another stage of initiation. The elimination of the peroxide by reduction GSH- dependent prevents this process, adding another argument to the statement that GSH is a key in the control of the reactions of the free radicals. Contributing to the decomposition of $H_2O_2$, it prevents the initiation of the reaction of formation of one of the most aggressive species reduced by oxygen-OH, for which the organism is equipped with mechanism of shielding it.

The function of the catalase and peroxidise in intracellular regulation of the concentration of $H_2O_2$, being determined by the relative molarity of the enzymes at the place of production, it is considered that GPx has a more important role than the catalase in protecting the target cells.

GPx is an antioxidant enzyme with higher versatility than the one of the enzymes SOD and catalase, first of all because of the wide specificity of sub-layer and secondly it seems to be an inducible enzyme in the lungs and RBC of the smokers. The cigarette smoke contains nitrogen oxides and hydroperoxides that can be used as sub-layer by GPx (4) as well as other polluting substances or oxidised metabolites.

Glutation-S- transferase (GST) are represented by a complex super-family of multi-functional enzymes that catalyse the nucleuphile conjugation of GSH with a large number of electrophile substances, different structurally, of exogenous or endogenous origin.

The enzymes form compounds of important thioether substances of detoxification in the organism [3].

Being multi-functional enzymes, they exercise their actions by different mechanisms, namely:

a. catalyse reactions of conjugation of xenobiotics with GSH, by which in most cases they reduce the toxicity, increase the solubility and speed of elimination;

b. can relate xenobiotics on the surface of the enzyme, inhibiting or not its catalytic activity, and thus inhibiting the interaction of the xenobiotics with target cellular sites (proteins, nucleic acids). GST has this property and is called ligandine [9];

c. GST can form covalent relations between reactive xenobiotics and the active centre of the enzyme; such relations deactivate both the enzyme and the xenobiotic.

GST – they have an anticarcinogenetic activity not only for the conjugation of the genotoxic electrophile, but also by reducing the lipoperoxides and hidroperoxides DNA [10]; the increase of activity GST by oxidative modification by the reactive species of oxygen is concomitant with the induction of the cytochrome P-450 [11].

Tiol-S-transferase – are represented by a group of enzymes that catalyse the reduction of the disulfide with low molecular weight, and mixed protein disulfide in the presence of GSH. Transhydrogenase can remake the thyol function that were target of oxidants [12] and it suggests the important role in conditions of oxidative stress.

*Non-enzymatic antioxidants*

The most important one are: vitamin E (tocopherol) and vitamin C (ascorbic acid) (essential nutriments), to which the glutathione (GSH) is added.

Characteristic for this category of anti-oxidative barriers is sacrificing their structure, being preferred targets of the oxidative actions, transformed in non-reactive radical species, saving the cellular micro-structures or other essential compounds of the organism.

Glutation (γ-glutamyl cysteinyl glycine) (GSH) is an unusual tripeptide, having two structural features with biological importance; it contains a rest ã-glutamyl and a functional grouping thyol belonging to the rest cysteinyl.

Intracellular, GSH is detected in two compartments: in cytosol in significant quantities, and in mitochondria of the different types of cells (heart, liver, kidney, lungs, intestine).

Among the multiple functions attributed to GSH, dominant is the antioxidant action. As main line of direct or indirect antioxidant defence GSH screens the action of a wide range of reactive species, radical or non-radical.

Because of its strong nucleophile character GSH can participate in a variety of defence reactions towards the reactive species of oxygen and chemically toxic, wither direct by means of enzymes GSH dependent as sub-layer or co-sub-layer of the many enzymes of detoxification - GPx and GST.

Detoxification of the hidroperoxides by GSH is carried out according to the equation:

$$2GSH + ROOH \rightarrow 2GSSH + R\text{-}OH + H_2O$$

The reaction is catalysed by two groups of enzymes presented: glutation peroxidises Se-dependent and glutation S-transferase.

Thyol can interact with the following types of radicals:

a. Capturing the radical OH

$$GSH + OH \rightarrow GS + H_2O$$

b. Capturing the secondary radicals:

$$GSH + R \rightarrow GS + RH$$
$$GSH + R_3C \rightarrow GS + R_3CH$$

c. Repairing other protectors:

$$ROO + \text{vit E} \rightarrow ROOH + \text{vit E}$$
$$\text{Vit E} + \text{vit C} \rightarrow \text{Vit E} + \text{vit C}$$
$$GSH + \text{vit C} \rightarrow GS + \text{vit C}$$

d. Reduction of peroxide radicals derived from DNA;

e. Reduction of proteins S-thyol or of other oxide products.

By giving its H to the hydroxyl radical (HO), GSH embezzles one of the most aggressive radicals from its toxic action (a), and by the neutralization of the carbon radical (b), it can determine the repairing of the DNA affected by irradiation or other attack sites of the free radicals (Ward quoted 10).

In this context, it was suggested that the GSH functioning as trap of OH radicals and the carbon radical, it would have an important role in the antimutagenesis and anticarcinogenesis.

The tocopherols (vitamin E) – the name tocopherols includes a family of phenolic compounds derived from the croman hetero-cycle (benzopiran) where the radicals are grafted –OH, one or more groups of methyl in different positions and a rest of 16 atoms of C.

There are several types of tocopherols: α -.β-,γ-,δ-tocopherols. The tocopherol with the most intense potency is the α- tocopherol.

The capacity of the vitamin to participate in reaction of oxido-reduction is due to the phenolic function in position 6 of the chroman nucleus.

α- tocopherol is a nutriment with obligatory character in the diet, whose action in the sphere of oxidative metabolism is in most cases closely related to other non-enzymatic antioxidants.

In virtue of the antioxidant action, the α- tocopherols, as the most efficient compound of lipid phase [3] assures the preservation of the integrity of the membrane integrity of the lipids, transforming itself into a tocopheryl radical (cromanoxil).

The interference of the vitamin E in the defence of the antioxidant implies a peroxil radical and group phenolic hydroxyl of the α- tocopherol generating hidroperoxide and tocopheryl radical:

$$\text{L-OO} + \text{vit E-OH} \rightarrow \text{LOOH} + \text{vit E-O}$$

Even if a *radical gives birth to another* [3], the new radical, the cromanoxil radical (vit E-O) is less stable, less aggressive, unable to initiate oxidative reactions, but it can react with a new molecule of peroxide radical.

The reversibility of the tocopheryl radical is assured by the reaction with donators of hydrogen soluble in water. They are the ascorbate and thyols.

Ascorbic acid (vitamin C), a compound closely related to glucose, it is necessary to carry out different physiological functions in the live organisms. The interference of the ascorbate in various functions is due to its two roles, diametrically opposed, based on the redox properties and unanimously accepted to act as reducer with advantageous role in the cell metabolism and as capro-oxidant (anti-infectious, detoxifying, antioxidant role).

The liber radical of ascorbyl can be produced by self-oxidation of ascorbate catalysed by transitional metals ($Fe^{3+}$ or $Cu^{2+}$) or by the interference of the ascorbate in antioxidant mechanisms.

**Results and discussions**

On taking into account the special importance that the macro-molecules of DNA have in maintaining the anatomic and functional integrity of the organism, in order to achieve all the vital processes, in one work to assure the integrity of the genetic information, it is understood the special interest that specialists offered lately to the modifications that appeared in the DNA molecule after its exposure to oxidative stress.

By different experiments that used various systems of producing free radicals, it was proved that they can determine a large range of prejudice at all the levels of organization of the genetic material, among which in the literature are mentioned:

- chain breaking, simple and double;
- tansformation of the purine and pyrimidine bases, obtaining products that induce aberrations in the processes of transcription and translation;
- obtaining some new radicals, derived from different components of DNA that can have increased harmfulness.

The structural modification of DNA leads to the error codification of the genetic information, hence the possibility to appear mutagenesis, carcinogenesis, acceleration of the phenomenon that follow the process of cell degeneration and aging.

The responses of the organism stress were aspects of great interest for biologists.

Maybe the most attractive and interesting research refers to the transitory adapting modifications of the systems that assure the resistance to oxidative stress.

The human body, during its evolution, selected both the repairing system of DNA, and the defence of ADN, the latter opposing to the prejudice produced by the oxidative stress.

The solutions adopted by the body, by synthesis or use of some substances of exogenous origin with antioxidant action, mean to face the ever higher levels of free radicals, produced after the irradiation, administration of different chemical substances, the presence of polluting substances in different environment, etc.

Modulating the reactivity of the free radicals implies a complex interactive process between the generation of free radicals and enzyme and non-enzyme systems located in the hydrophilic and hydrophobic cell microclimate.

## Conclusions

The antioxidant systems, varied as structure and way of action, with large capacity of modulation of the production of free radicals, protect the cell constituents, especially the DNA.

The antioxidants are molecules that form the first target in the way of free radicals.

The non-enzyme antioxidants sacrifice their own structure, being oxidised or transformed into harmless radicals or with low toxicity. Unfortunately, a series of studies proved, apart from the antioxidant character, even a pro-oxidising action of some substances.

Recognizing this duplicity stimulates the research in view of establishing more exactly the conditions and factors that embezzle their beneficial action.

That's why the better knowledge of the physio-pathology of the oxygen metabolism, the effects on the genetic system is important not only theoretically, but it is also necessary because it allows the development of an efficient therapy.

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