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# OXIDATIVE STRESS AS A FACTOR OF DISRUPTED ECOLOGICAL OXIDATIVE BALANCE IN BIOLOGICAL SYSTEMS – A REVIEW

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**Summary**

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Recently, reactive oxygen species (ROS) received a particular attention as they are supposed to participate in some pathological complications and toxic side effects with a still unknown importance. ROS are known to be involved in carcinogenesis, tumour initiation, tumour growth, the triggering and realization of programmed cellular death, apoptosis induced by exterior factors such as chemical agents, environmental pollutants, cytotoxic and hepatotoxic drugs, ionized radiation, UV irradiation etc. In low concentrations, ROS are necessary for numerous physiological processes and normally, there exists an equilibrium between ROS and systemic antioxidants. The state of balance between ROS generation and the protection capacity of endogenic antioxidant defense of biological systems could be specified as ecological oxidative balance (equilibrium) (EOB). In this state, they are maximally protected against toxic oxidative influences. All endogenic and exogenic sources of uncontrolled ROS production result in oxidative stress that impairs the equilibrium to a considerable extent. When EOB is disturbed, the biological systems are not protected against oxidative radical effects because of the impaired interrelationship between the activity and the intracellular levels of endogenic antioxidants and prooxidants, resulting in toxic damage, disease, aging or death of biological systems.

**Key words**: ecologic oxidative balance (EOB), oxidative stress, reactive oxygen species (ROS)

## FREE RADICALS AND REACTIVE OXYGEN SPECIES

### *Chemical nature*

Free radicals (FR) are paramagnetic particles, possessing one or more uncoupled electrons in some of their outer electronic orbits. Their half-life is more or less short and depending on that, FR are accordingly named unstable or stable radicals (Prior, 1979). The reactions with free radicals involve oxidative reductions with the participation of oxygen, peroxides, hydroperoxydes etc. as well as numerous similar biochemical reactions (Valentine *et al.*, 1998). The reactive oxygen species (ROS) – the superoxide radical ($^{\cdot}O_2^-$), the nitric oxide ($NO^{\cdot}$), the hydrogen peroxide ($H_2O_2$), the hydroxyl radical ($^{\cdot}OH.$), the singlet oxygen ($1O_2$) etc. are highly reactive molecules, atoms or ions (Ghosh & Myers, 1998) from both endogenous and exogenous origin (Fig. 1).

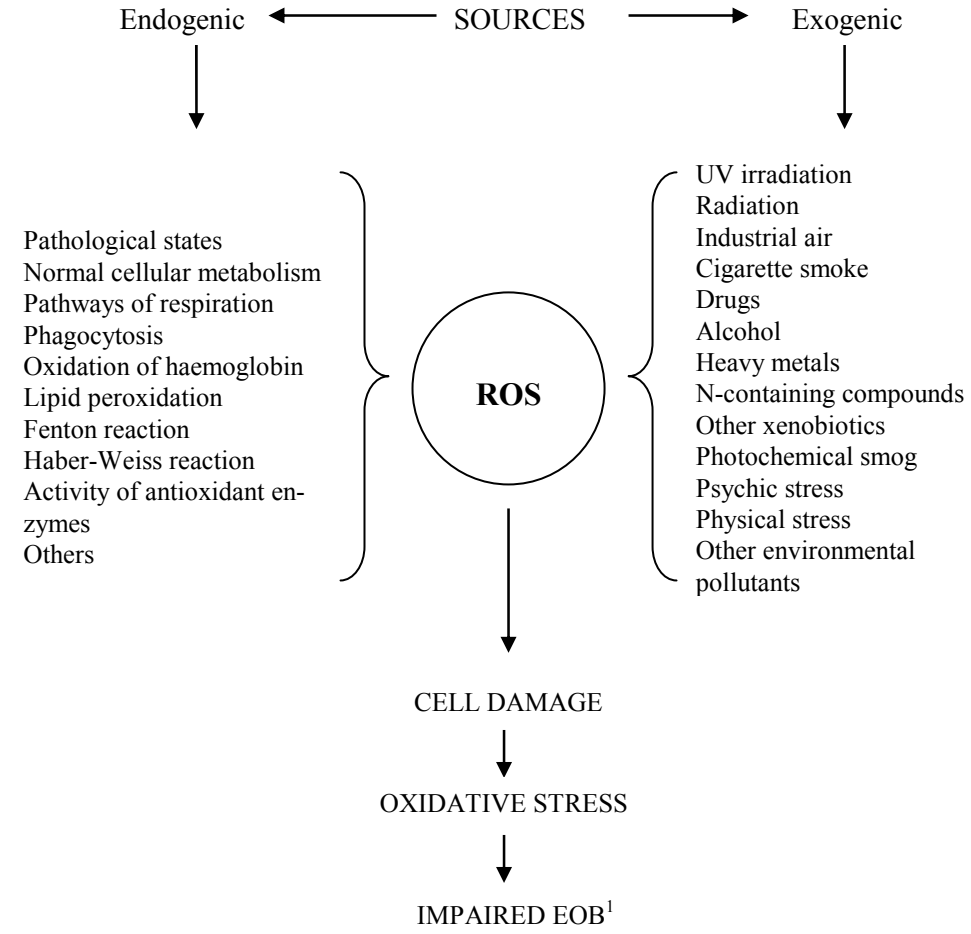


**Fig. 1.** Reactive oxygen species (ROS) sources and some of their effects; [1]EOB=ecological oxidative balance.

*ROS sources*

*Endogenous sources* of ROS are:

- Biochemical processes of oxidative reduction occurring as a part of the normal cellular metabolism (Ghosh & Myers, 1998);
- The pathways of cellular respiration – the reduced CoQ in the electron transport chain is the principal source of oxygen radicals;
- The oxidation of haemoglobin – about 1–3 % of the total amount of oxygen that enters into the erythro-

cytes, is reduced to $\cdot O_2^-$ (Gillham *et al.*, 1997);
- Phagocytosis – during the oxygen burst, numerous phagocytizing cells produce a huge amount of $\cdot O_2^-$ as an element of their function (Marks *et al.*, 1996);
- Others (Fig. 1).

*Exogenous sources*, contributing to ROS generation in biological systems, are: UV irradiation, gamma radiation, environmental pollutants ($O_3$, $NO_2$), the mutagenic, carcinogenic and toxic influence of N-containing substances (such as nitroso-, nitro-, azoderivatives and hydroxylamines) and other toxic xenobiotics (including drugs), alcohol intoxication, cigarette smoke, herbicide intoxications, the toxic effect of oxygen on anaerobe microorganisms, the toxic effect of ozone and peroxides, physical and psychic stress etc. (Albano *et al.*, 1992; Poli, 1993; Nordmann & Ronach, 1995; Baykov, 2000) (Fig. 1).

*Biological role of ROS*

In low concentrations, ROS are essential for multiple normal physiological processes as cell differentiation (Tatla *et al.*, 1999; Abe *et al.*, 2000), apoptosis (Ghosh & Myers, 1998), cell immunity (Golub and Descamps-Latscha, 1985) and cellular defense against microorganisms (Lajarin, *et al.*, 1999). Recently**,** ROS are paid a particular attention because they are presumed to be involved in complications of pathologic processes and in adverse side effects (Vladimirov, 2004) with unknown significance (Jornot *et al.*, 1998; Mills *et al.*, 1998). It was found out, that with aging, the systemic free radical content is increasing and the autoimmune processes are more common (Harman, 1981). ROS participate in cancerogenesis, tumour initiation, the triggering and realization of programmed cellular death, apoptosis induced by exterior factors such as chemical agents, environmental pollutants, cytotoxic and hepatotoxic drugs, ionized radiation, UV irradiation etc. (Manome *et al.*, 1993; Jacobson, 1996). ROS are also involved in the mechanism of $NO^\cdot$ toxicity (Moncada *et al.*, 1991). In fact, the peroxynitrite anion ($ONOO^-$), formed during the interaction of $NO^\cdot$ and $\cdot O_2^-$ is strongly toxic (Yabuki *et al.*, 1999). The increased $NO^\cdot$ production is believed to promote a number of diseases as cancer, heart strokes, diabetes, sepsis, pulmonary diseases etc. (Vladimirov, 2004; Castranova, 2004). Thus, $NO^\cdot$ levels could be useful markers for the appearance of inflammations or the development of pathological alterations and diseases (MacMicking *et al.*, 1997).

*Mechanisms of the toxic influence of ROS*

For now, it is assumed that ROS are causing a severe toxic damage upon important biological structures via:
- Lipid peroxidation of polyunsaturated fatty acids in cell membrane phospholipids (Freeman & Grapo, 1982);
- Covalent binding to proteins (Esterbauer *et al.*, 1992);
- Disturbance of intracellular free calcium homeostasis;
- DNA chain breaks. DNA is an important target of ROS, related to aging, inflammations and cell transformation. As a result of the radical damage of DNA, chain breaks do occur (Imlay & Linn, 1988).

Oxidation of proteins by ROS resulting in modification of amino acid side groups, oxidative degradation of peptide bonds or formation of covalent bonds with oxidized products of lipids or carbohydrates (de Zwart *et al.*, 1999).

## OXIDATIVE STRESS

When ROS production exceeds the detoxication capacity of systemic endogenic antioxidant defense, oxidative stress occurs. The oxidative stress causes cellular damage and reduces the endogenic antioxidant defense of biological systems (Marks *et al.*, 1996; Chopra and Wallace, 1998). The decrease in the activity of antioxidant enzymes could have a dramatic effect upon the resistance of cells to oxidant-induced damage of cell genome and cell death rate (Anderson, 1999). The overall process of free radical damage in cells is called oxidative stress (Reiter *et al.*, 1997).

### *Lipid peroxidation – a marker of oxidative stress*

Lipid peroxidation is among the best predictors of the level of ROS-induced systemic biological damage (Saygili *et al.*, 2003). Lipids are most easily oxidized and thus are the preferred substrate to free radical damage via lipid peroxidation (Comporti, 1985). The lipid peroxidation is a sequence of oxidative chain reactions of polyunsaturated fatty acids of membrane phospholipids, resulting in phospholipid degradation, membrane injury and formation of saturated hydrocarbons (ethane, pentane), aldehydes etc. (Day, 1996; Saygili *et al.*, 2003). One of lipid peroxidation endproducts, malondialdehyde (MDA) (Fig. 2) is isolated in urine, blood and tissues and is used as biomarker of radical damage, resp. oxidative stress (Day, 1996). It is known that in disease states, the lipid peroxidation in biological organisms is activated, although it is not fully elucidated whether it triggers the pathological processes or is resulting from them (Tvetkov & Bochev, 1996). A categorical answer to the question how the free radical-induced lipid peroxidation injuries cell membranes is still lacking. According to Popova & Popov (2002), lipid peroxidation labilized cellular organelles via degradation, affecting structural elements of biological membranes.

### *Oxidative stress and ecological oxidative balance*

Under normal physiological conditions, there is a balance between ROS generation and the detoxicating capacity of endogenous systemic antioxidant defense. This state could be named ecological oxidative balance (EOB) (Georgieva & Gadjeva, 2005), where biological systems are maximally protected against oxidative challenge. Elevated ROS concentrations

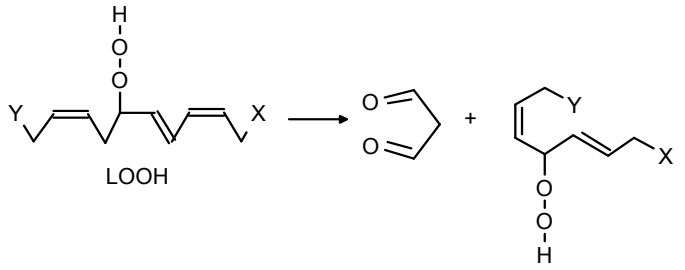


**Fig. 2.** Scheme for lipid peroxidation endproducts formation.

or their inadequate elimination when their formation exceeds the rate of their removal by cellular defense (reduced antioxidant defense) result in impaired biological EOB consequent to the occurring oxidative stress. According to our hypothesis (Georgieva & Gadjeva, 2005), the oxidative stress and the considerably impaired EOB, states when biological systems are not protected against oxidative damaging effects are probably the sources of pathological changes (Georgieva & Gadjeva, 2005).

*The role of oxidative stress in diseases and pathological states*

ROS formed during multiple normal processes in tissues and cells as well as their excessive formation in state of oxidative stress play an important role in the pathogenesis and various diseases. It is well known that toxic ROS and oxidative stress are of primary importance in immune and inflammatory mechanisms involved in the majority of diseases (Tatla *et al.*, 1999). The oxidative stress is a crucial factor for determination of the individual risk for occurrence of numerous diseases (Anderson *et al.*, 1999). The oxidative stress, manifested by unbalanced ROS production and/or impaired endogenic antioxidant defense is related to aging as well as to several diseases such as cancer, atherosclerosis, rheumatoid arthritis, renal diseases, uraemia, diabetes, Alzheimer's and Parkinson's diseases, the acquired immunodeficiency syndrome (AIDS), pulmonary inflammations etc. (Del Maestro, 1980; Cerutti, 1985; Bornnemann *et al.*, 1997; Das *et al.*, 1997; Walubo *et al.*, 1998; 1999; Mates & Sanchez-Jimenez, 2000; Hristozov *et al.*, 2001). It is assumed that oxidative stress is also involved in the activation of resistance to chemotherapy (Toyokuni *et al.*, 1995).

In parasitic diseases, changes in the host levels of some low-molecular antioxidants (vitamins A, C and E) as well as increased oxidative stress is reported (Chuenkova *et al.*, 1989; Evans & Halliwet, 2001). In chickens infected with *Ascaridia galli* and rats infected with *Fasciola hepatica* there is evidence about antioxidant disbalance manifested with deficiencies of vitamin C, vitamin E and trace elements (Gabrashanska *et al.* 2003). The studies of Dede *et al.* (2000; 2002) on endogenic antioxidant vitamins C, E and A revealed a particularly impaired metabolism of vitamin E in sheep and goats, infected with inestinal parasites. In protozoa infections, the levels of free radicals, producing oxidative stress in the host are increased (Ozer *et al.*, 1995).

*Oxidative stress and drug effects*

It is known that many drugs as tuberculostatics, anticancer and ionophore antibiotics, analgetics and anaesthetics generate ROS in the course of their metabolism, that are consiquently leading to oxidative stress and a numebr of toxis side effects as hepatotoxicity, cardiotoxicity, cytotoxicity, pulmoary fibroses etc. (Marks *et al.*, 1996; Hug *et al.*, 1997; Walubo *et al.*, 1998; Mates *et al.*, 1999). Thus, the drugs used for chemotherapy of various diseases contribute to the remission but the produced oxidative stress impairs the biological EOB and hence is one of the probable causes for their toxic oxidative damaging effects.

Although the application of isoniazid (INH) in the chemotherapy of tuberculosis healed a huge number of patietns, the significant amount of experimental and clinical information allowed to reveal its hepatotoxic effect (Byrd & Nelson, 1972; Farrer *et al.*, 1977; Vidal Pla *et al.*, 1991; Johansson *et al*., 1995; Saraswathy *et al.*,

1998; Attri *et al.*, 2001; Campos-Outcalt, 2003), that is a serious therapeutic problem. According to Lauterburg (1985) the formation of toxic metabolites was postulated in the hepatotoxicity of isoniazid. The treatment with INH inhibits the biosynthesis of mycolic acid in the cellular wall of *Mycobacterium* from one part (George *et al.*, 1995; Betts *et al.*, 2003), and results in ROS generation during INH metabolism, from the other (Albano & Tomasi, 1987; Johansson *et al.*, 1995; Wang *et al.*, 1998). Via the ROS production, isoniazid injuries the DNA, the proteins etc. and this is probably one cause for its adverse side effects, including its oxidative hepatotoxicity. The mechanism of INH hepatotoxicity is relative to oxidative stress produced after treatment (Sodhi *et al.*, 1997; 1998). Our studies showed that the treatment with isoniazid impairs the oxidants/prooxidants EOB in favour of prooxidants. In an *in vivo* experiment with albino mice treated with INH, increased concentrations of MDA (marker of oxidative stress) and reduced activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were found. Those results demonstrate oxidative radicals-induced damage and diminished antioxidant protection in experimental mice following INH-produced oxidative stress (Georgieva *et al.*, 2004). Our data support the hypothesis (Georgieva & Gadjeva, 2005) about decreased endogenic defense of biological systems in impaired EOB, provoked by ROS generated throughout the metabolism of some chemotherapeutic drugs.

Numerous *anticancer drugs* exert their cytotoxicity via ROS-mediated resp. oxidative stress-mediated mechanisms. Such drugs are the anthracyclines as well as bleomycin, vincristin, cyclophosphamide and hydroxyurea (Reszka *et al.*, 1988). It is shown that the anthracyclines doxorubicin and daunorubicin undergo a single electron reduction to semiquinone radicals. In the presence of oxygen, these radicals are rapidly oxidized to formation of $^{\cdot}O_2^-$, that consecutively leads to formation of $H_2O_2$ and $HO^{\cdot}$ (Reszka *et al.*, 1988; Benchekroun, *et al.*, 1993). The ROS generated by the cascade of free radicals are believed to be the principle cause for anthracycline-induced peroxide damage of membrane lipids and DNA (Benchekroun *et al.*., 1993; Hyang-Suk Kim *et al.*, 2001). In the opinion of Gadzheva *et al.* (1994) the cumulative toxicity of nitrosoureas (anticancer chemotherapeutics) is also related to formed free radicals and their damaging effect on cellular membranes and biological molecules.

*The ionophore antibiotics* – monensin, salinomycin, lasalocide, narasin, maduramycin and the newest member of this group – semduramycin, are widely used in the control of coccidiosis, a common avian disease. In turkeys, experimentally infected with *Eimeria adenoeides* and treated with coccidiostatics, toxic side effects and histopathological changes in kidneys, the cardiac muscle and the liver apart the changes in the intestinal mucosa are reported (Lozanov & Koinarski, 1985).

During *halothane anaesthesia*, ROS causing adverse hepatotoxic events, are also produced because the intermediate metabolites of halothane possess a free radical activity. The mechanism of halothane hepatotoxicity is directly related to oxidative stress and immune-mediated tissue damage. This commonly used anaesthetic is known to cause hepatotoxicity in 20% of patients, mainly through ROS generated during the anaerobic metabo-

lism of halothane, acting directly on liver and damaging the hepatocytic membranes via lipid peroxidation (Yamazoe *et al.*, 1998). There are repots for decreased antioxidant systemic defense in dogs submitted to 2-hour halothane anaesthesia that is attributed to lipid peroxidation, probably caused by ROS produced during the metabolism of halothane (Giuri, 2002).

The cited examples about the adverse events of many drugs, being a small part of the numerous existing data, are supporting the hypothesis for impaired systemic EOB (Georgieva & Gadjeva, 2005) following oxidative stress caused by uncontrolled ROS production during the metabolism of some drugs. In the state of impaired EOB and oxidative stress, the biological systems are the most vulnerable against oxidative damage, resulting in toxic injuries, diseases, aging or death of biological systems.

The consequences of impaired EOB following the occurring oxidative stress depend on systemic capacity to re-establish the equilibrium either independently, or with the assistance of appropriate means.

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