Reactive Oxygen Species as Mediators of Tissue Protection and Injury

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Abstract. Extensive research efforts during the last three decades resulted in a large body of experimental evidence that suggests an important role of the disbalance between generation and elimination of the oxygen and xenobiotic derived free radicals in physiological and pathological processes Reactive oxygen species (ROS) are generated in many metabolic pathways, and are entering the organisms from exogenous sources, dominantly via airways and gut ROS induced injuries, e g thermal, chemical, radiation, ischaemia/reperfusion, inflammation, hyperoxia, etc , result in diseases like atherosclerosis, ulcerative colitis, autoimmune diseases, asthma, etc The current paper is designed to provide an overview of the effects ROS may exert in various tissues Because of the effective defense systems, the tolerance of viable human cells to ROS is relatively high The oxidant stress induced dysfunction of various systems, such as the gut, airways, nervous, cardiovascular system, etc , involve both direct and indirect mechanisms Understanding of these molecular mechanisms is essential for a rational antioxidant therapy

Key words: Reactive oxygen species, sources — Tissue targets — Diseases — Therapeutic approaches

Introduction

The possibility that O_2 might be toxic was recognized by Joseph Pristley as early as in 1775 when he found this molecule Nevertheless, it was only the discovery of the existence of superoxide dismutase (SOD) activity in mammalian cells (McCord and Fridovich 1969) and the association of the bactericidal activity of neutrophils with the production of the superoxide radical ($O_2^{\cdot-}$) (Babior et al 1976) that, linked free radicals to numerous physiological and pathophysiological processes

The prevalence of O_2 in biological systems means that oxygen centred radicals are found most commonly The terms reduced oxygen species, reactive oxygen

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metabolites, reactive oxygen intermediates (ROI) or reactive oxygen species (ROS) are now generally preferred because except $O_2^{\cdot-}$ and hydroxyl radical ($\cdot$OH), the other forms, i.e. singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxide, hydroperoxide and epoxide metabolites of endogenous lipids and xenobiotics have chemically reactive oxygen containing functional groups but they are not radicals and do not necessarily interact with tissues via radical reactions.

Cellular sources of ROS

In processes that are central to metabolism in aerobic life, O_2 acts as a terminal electron acceptor. Approximately 90% of all oxygen consumed by mammalian cells is catalytically reduced by four electrons to yield two molecules of water. Transfer of one electron to O_2 yields $O_2^{\cdot-}$, which is rapidly dismutated by SOD to H_2O_2. Further reactions require either transition metals to form the extremely reactive $\cdot$OH or myeloperoxidase to produce HOCl and $^1O_2$. Nitric oxide (NO), generated by the endothelium, macrophages, etc., can react with $O_2^{\cdot-}$ to form peroxynitrite ($OONO^-$), which after protonation is unstable and decomposes to $\cdot$OH or nitrogen dioxide (NO_2). ROS found in mammalian tissues are of endogenous (e.g. phagocytes, respiratory chain in mitochondria, enzymatic reactions) and exogenous origin (e.g. radiation, air pollution, cigarette smoke, xenobiotics, drugs) (Fig. 1). They are essential for normal function or metabolism of the majority of mammalian cells. ROS are produced in cell membrane lipid bilayers, in the electron transport system in mitochondria, in cell organelles, like peroxisomes, lysosomes, endoplasmic reticulum, as well as in the cytoplasm by enzymatic and non-enzymatic reactions (Tab. 1).

Reactive oxygen species are destructive unless tightly controlled. Mammalian cells have developed a battery of defenses (catalytic, scavenging, antioxidant, chelating reactions) to prevent and repair the injuries caused by oxidative stress. These
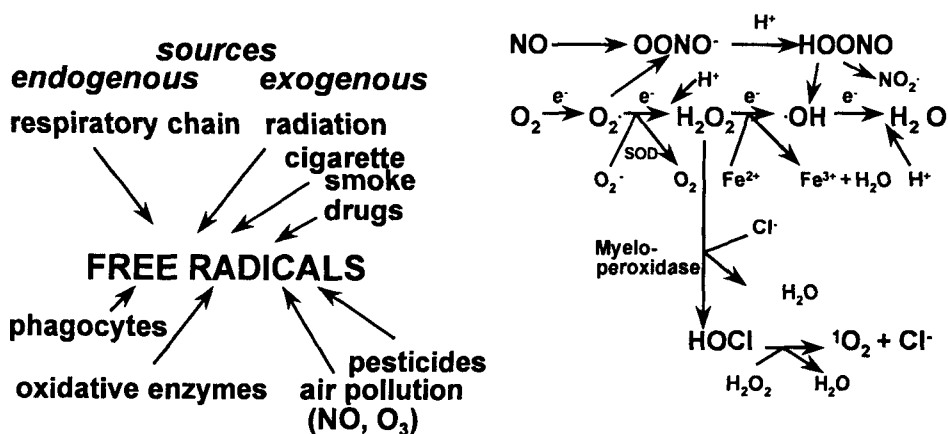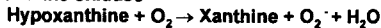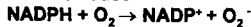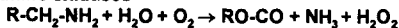

Figure 1. Reactive oxygen species and their sources in the mammalian organism.

Table. 1. Enzymatic and nonenzymatic sources of reactive oxygen species

a. Enzymatic

Xanthine oxidase
  Hypoxanthine + $O_2 \rightarrow$ Xanthine + $O_2^{\cdot}$ + H_2O
NADPH oxidase
  NADPH + $O_2 \rightarrow$ NADP$^+$ + $O_2^{\cdot}$
Amine oxidases
  R-CH$_2$-NH$_2$ + H_2O + $O_2 \rightarrow$ RO-CO + NH$_3$ + H_2O_2
                                                          H
Peroxidases
  Hypohalous acid formation
    H_2O_2 + X$^{\cdot}$ + H$^+ \rightarrow$ HOX + H_2O
NADH oxidase reaction
  E-Fe^{3+} + ROOH $\rightarrow$ Compound I + ROH
  Compound I + NADPH $\rightarrow$ NAD + Compound II
  Compound II + NADH $\rightarrow$ NAD + E-Fe^{3+}
  NAD + $O_2 \rightarrow$ NAD$^+$ + $O_2^{\cdot}$
Aldehyde oxidase
  R-CHO + $O_2 \rightarrow$ R-COOH + $O_2^{\cdot}$
Dihydroorotate dehydrogenase
  Dihydroorotate + NAD + $O_2 \rightarrow$ NADH + $O_2^{\cdot}$
                                                    + Orotic acid
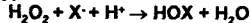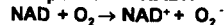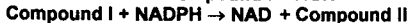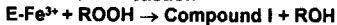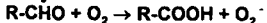
b. Nonenzymatic

Fe^{2+} + $O_2 \rightarrow$ Fe^{3+} + $O_2^{\cdot}$
Hb-Fe^{2+} + $O_2 \rightarrow$ Hb-Fe^{3+} + $O_2^{\cdot}$
Catecholamines + $O_2 \rightarrow$ Melanin + $O_2^{\cdot}$
Reduced flavin
  Leukoflavin + $O_2 \rightarrow$ Flavin semiquinone + $O_2^{\cdot}$
Coenzyme
  Q-hydroquinone + $O_2 \rightarrow$ Coenzyme Q (ubiquinone) + O_2
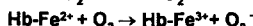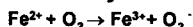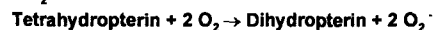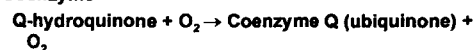 Tetrahydropterin + 2 $O_2 \rightarrow$ Dihydropterin + 2 $O_2^{\cdot}$
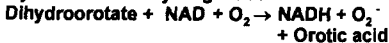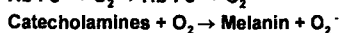
include catalytic ROS removal by enzymes, such as SOD (cytosolic, mitochondrial), catalase (cytosolic, peroxisomal), glutathion peroxidase (cytosolic, mitochondrial), removal of transition metals by endogenous chelators (e.g. ferritin, ceruloplasmin, serum albumin), and the nonenzymatic quenching or scavenging of ROS by antioxidants, involving cholesterol, α-tocopherol, ascorbic acid, β-carotene, and thiol – containing compounds. Recently it has been speculated that uric acid, melatonin, glucose and bilirubin may be physiologically important as antioxidants as early as in the first week of life (Bervoets et al. 1994; Schrod et al. 1997). Since the above mentioned enzymes are only in trace amounts in the extracellular space, ROS production in the interstitium may have a harmful effect on the surrounding living cells.

Biological targets of ROS

The toxicity of many xenobiotics is now associated with formation of free radicals, which may be involved in different pathologic conditions. ROS act either extracellularly (e.g. inflammatory reactions, cataractogenesis, atherosclerosis), intracellularly (e.g. ageing, ischaemia-reperfusion, cancer) or both (e.g. immune reactions, xenobiotic induced reactions, senile dementia). As a consequence of having one or more unpaired electrons in outer orbital, ROS have an increased reactivity with other molecules in a variety of biological systems. These species produce damage of lipids, proteins and DNA (Fig. 2). The developed membrane impairments, altered gene expressions, and accumulation of intracellular free Ca^{2+} result in activation/deactivation of various enzymes and lead to cell injury or death.

Reactive Oxygen Species

Lipids	Proteins	DNA

Oxidation of vitamin E ⟷ Carbonyl formation Oxidation of thiols   DNA damage

Lipid peroxidation

Damage to Ca^{2+} and other ion transport systems

Altered gene expression   Poly ADP ribosylation

Membrane damage

Instability to maintain ion gradients and Ca^{2+} accumulation

Depletion of ATP and NAD(P)(H)

Activation/Inactivation of enzymes

Cell injury

Figure 2. Mechanism of reactive oxygen species induced cell injury

Clinical conditions associated with ROS production induced tissue damage

There is a consensus that free radicals are definitely responsible for oxygen toxicity and tetrachlormethane induced liver damage In the perinatal period, the fetus and newborn, and especially premature infants are more exposed to oxidative injury than adults (e g oxygen-, photo-, NO-therapy), and are particularly susceptible to free radical injury because of a relative deficiency in endogenous antioxidants (Inder et al 1994, Robbins et al 1995) Recent clinical and experimental evidence suggests that oxygen toxicity rising from disbalance between production and elimination of ROS is a major factor in the pathogenesis of life-threatening complications of toxicity of xenobiotics, drugs and participates in the development of diseases of various tissues and systems not only in very low birth weight infants but also in adults

There are different pathologic conditions where ROS are at least partially involved in the pathophysiological mechanisms Granger et al (1981) introduced a hypothesis on the role of ROS in reperfusion injury after intestinal ischaemia ROS affect the gut, airways and vessels either directly or indirectly via their innervation, epithelium or endothelium (Van der Vliet et al 1989, Bauer et al 1997) The epithelium and endothelium, which are the most vulnerable components of smooth muscle tissues, are frequently exposed to ROS of both exogenous (ozone, nitrogen oxides, different particles, etc) and endogenous origin (inflammatory cells, peroxisomes, metabolites of xenobiotics, etc) An intact epithelium or endothelium is

generally sufficient to protect the smooth muscle from ROS-induced spasms. In the presence of injury, however, ROS can produce prolonged smooth muscle contractions or motility disturbances. Several pathological conditions of the digestive system (e.g. ischaemia-reperfusion injury, inflammation, Crohn's disease, ulcerative colitis, ulcer disease, xenobiotic- and drug-related diseases) are characterised by the formation of ROS (Parks et al. 1983; Mansbach et al. 1986; Suematsu et al. 1987; Ramage et al. 1988; Van der Vliet et al. 1989; Nilsson et al. 1994; Papparella et al. 1997). Conditions in which the function of the smooth muscle is affected involve also injuries and diseases of the airways, such as normobaric hyperoxic injury, bronchial hyperreactivity, respiratory distress syndromes (ARDS, NRDS), bronchopulmonary dysplasia, idiopathic pulmonary fibrosis, emphysema, asthma, diseases induced by inhalation of pollutants, xenobiotic-related diseases, pulmonary hypertension, etc. (Evans et al. 1986; Mathru et al. 1994; Plaza et al. 1995; Bracci 1997). The heart, vessels and their endothelium, as well as the epicardium, are also targets of ROS (e.g. ischaemia-reperfusion injury after infarction or transplantation, effects of chemicals as ethanol or doxorubicin, xenobiotic-related diseases, atherosclerosis and hypertension, selenium deficiency, vasculitis). Due to their action, the vascular resistance and tone of the vessel wall is significantly affected (Rubanyi 1988; Wolin 1991; Bharadwaj and Prasad 1997). Several diseases of the brain (hyperbaric hyperoxic injury, hypoxic ischaemic encephalopathy of infants, Parkinson's disease, effect of neurotoxins, vitamin E deficiency, neuronal lipofuscinoses, traumatic injury, inflammation, ischemia-reperfusion, Alzheimer's disease), of blood (effects of chemicals as phenylhydrazine, primaquine, sulphonamides, or lead, protoporphirine photooxidation, malaria, anaemias – sickle cell or favism), the liver (ischaemia-reperfusion, effects of halogenated hydrocarbons, quinones, iron, acetaminophen, ethanol, or endotoxins), the kidney (autoimmune nephrosis, inflammation, effects of aminoglycosides or heavy metals), the eye (prematurity-, diabetic-, photic-retinopathy, cataracts), the skin (solar or ionizing radiation, thermal injury, effects of photosensitisers as tetracyclines, contact dermatitis, porphyria), of the striated muscle (muscular dystrophy, multiple sclerosis, exercise), and others (ageing, age-related diseases, cancer, rheumatoid arthritis and other autoimmune diseases as lupus erythematodes, inflammation in general, developmental toxicity, alloxan-induced diabetes, effects of radiation, radiosensitizers, iron overload) are thought to be related to the action of ROS. Direct action of ROS on various tissues is aggravated by modification of the local circulation, which may participate in tissue injuries such as myocardial reperfusion injury of infants and adults or it may participate in pathological processes accompanying ageing, diabetes, etc. (Kehrer 1993; Stohs 1995; Fantel 1996).

Potential therapeutic approaches

During the last 25 to 30 years, a large body of experimental evidence has accumulated from pharmacological intervention studies suggestive of the important role exerted by ROS in mammalian tissues under normal and pathological conditions.

The drugs which may potentially be used in antioxidant therapy have different chemical structures and mechanisms of action. They are inhibitors of the synthesis of ROS (e.g. allopurinol), agents supporting and complementing enzymatic protective systems used in substitutive therapy (e.g. SOD), antioxidant substances containing thiol groups (e.g. glutathion), vitamins (e.g. A, C, E), drugs interfering with iron and copper metabolism (e.g. desferoxamine), and scavengers of $O_2^{\cdot-}$ (e.g. flavonoids), of $^{\cdot}OH$ (e.g. manitol) and substances which eliminate H_2O_2 (e.g. N-acetylcysteine). Inhibition of $O_2^{\cdot-}$ formation by captopril and nitrosopine and elimination of $^{\cdot}OH$ by thiourea protects the endothelium of the rat aorta from the deleterious effect of the $^{\cdot}OH$ produced by $FeSO_4$ and H_2O_2. It is well known that the oxidatively modified LDL plays a prominent role in endothelial damage and development of atherosclerosis. The illuminated nifedipine, called nitrosopine, which is avoid of Ca^{2+} channel blocking properties, acts as a ROS scavenger. In contrast to nifedipine, it significantly antagonizes the oxidized LDL-induced lipid peroxidation and attenuation of acetylcholine-induced relaxation. Many natural antioxidants, like Kampo Medicines, bisbenzylisoquinoline alkaloids from Mahonia Aquifolium, etc. are not only potent inhibitors of lipid peroxidation but also protect acetylcholine induced relaxation of the rat aorta from the deleterious action of ROS (Sotníková et al. 1994; Bauer et al. 1999). Thus our own results also indicate that SOD, catalase, dimethyl-sulfoxide, ascorbic acid, stobadine, nitrosopine, bisbenzylisoquinolines, Kampo Medicines, ACE inhibitors, etc. appear to be useful and promising antioxidants.

Though the first trial of the efficacy of α-tocopherol in the prevention of O_2 toxicity in preterm newborns was performed in 1949, the clinical efficacy of antioxidants in neonatology has remained a controversial issue. Several of them, e.g. allopurinol, α-tocopherol, SOD, catalase, propentofylline, deferoxamine, indomethacine, D-penicillamine, were investigated at least in part successfully in preterm or term animals and infants to determine their ability to prevent complications of neonatal intensive care, which may be associated with oxidative injury (e.g. Oroszlán et al. 1992; Palmer et al. 1994; Papparella et al. 1997; Penn et al. 1997; Numagami et al. 1998). The therapeutic relevance of antioxidant therapy varies in different diseases and pathological conditions. There are promising results in some (ischaemic injuries, LDL oxidation, platelet aggregation, pulmonary injuries, central nervous system injuries, ulcerative colitis, chemical poisonings and oxygen stress) (Rice-Evans and Diplock 1993; Hall 1997; Evans et al. 1998), and conflicting results in others, such as bronchial asthma, rheumatic arthritis, cancer, cerebral vasospasms, etc.

Our present knowledge suggests that the toxic effects of ROS may be either the sole cause of tissue injury, partially involved in the pathological mechanisms of diseases, or they may accumulate only as a phenomenon accompanying the pathologic processes.

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