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ANTIOXIDANT ENZYMES AND THEIR IMPLICATIONS IN PATHOPHYSIOLOGIC PROCESSES

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1. ABSTRACT

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration. Reactive oxygen is related to both, the arrest of growth and the start of cell differentiation. Low concentrations of reactive oxygen intermediates may be beneficial or even indispensable in processes such as intracellular messaging and defense against micro-organisms, but higher amounts of active oxygen may be harmful to cells and organisms. A wide array of non-enzymatic and enzymatic antioxidant defenses exists, including superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). We describe their main characteristics and how these antioxidant enzymes work together against active oxygen. Small deviations from their physiological values may have a dramatic effect on the resistance of cells to oxidative damage to lipids, proteins and DNA. Consequently, toxic oxygen play a role in aging process as well as in a number of human diseases that we list in this review.

2. INTRODUCTION

Small amounts of reactive oxygen species (ROS), as hydroxyl radicals ($HO^{\cdot}$), superoxide anions ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), are constantly generated in aerobic organisms in response to both external and internal stimuli (1). Low levels of ROS may be indispensable in a plethora of processes, including intracellular messaging (2)- leading among others to proliferation or apoptosis (3)-, immunity (4), and defense against micro-organisms (1). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (5).

The prevention of lipid peroxidation is an essential process in all the aerobic organisms, as lipid peroxidation products can cause DNA damage. Increased lipid peroxidation and decreased antioxidant protection frequently occurs (6): epoxides may spontaneously react with nucleophilic centers in the cell and thereby covalently bind to DNA, RNA and protein (figure 1). Such a reaction may lead to cytotoxicity, allergy, mutagenicity and/or carcinogenicity, depending of the properties of the epoxide in question. Moreover, oxidative events may play an important role in the mechanism of action of ether lipids, and oxidizability may contribute to cellular drug sensitivity (7).

A wide array of enzymatic and non-enzymatic antioxidant defenses exist, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione (GSH), beta-carotene, vitamin A ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) (8). There is a interrelationship between both, the activities, and the intracellular levels of these metabolites, protecting themselves from oxygen toxicity (9).

3. ANTIOXIDANT ENZYMES

3.1. Superoxide dismutase

Superoxide dismutase (EC 1.15.1.1) destroys the free radical superoxide by converting it to peroxide that can in turn be destroyed by CAT or GPX reactions. SOD

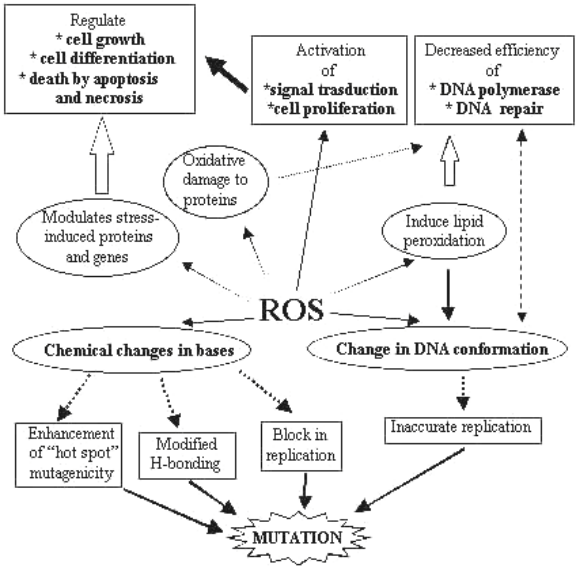

Figure 1. ROS, oxidative damage and human diseases. Interrelationship between the effect of imbalance in the reactive oxygen species (ROS) and their consequences on the cellular growth and the cellular function (top); and between ROS imbalance and the mechanisms and pathways from oxidative damage to mutation (down).

converts the highly reactive superoxide radical to the less reactive H_2O_2.

$$O_2^- + O_2^- + 2\,H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Another function of superoxide dismutase is to protect dehydratases (dihydroxy acid dehydratase, aconitase, 6-phosphogluconate dehydratase and fumarases A and B) against inactivation by the free radical superoxide (10).

Four classes of SOD have been identified, containing either a dinuclear Cu, Zn or mononuclear Fe, Mn or Ni cofactors (11). Fe-SODs and Mn-SODs show homology and posses identical metal chelating residues at the active site, sharing substantial sequence and three dimensional structural homology, while the other superoxide dismutases are structurally unrelated. In humans, there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular-SOD (EC-SOD) (12). SOD catalyses the dismutation of O_2^- by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates (13).

3.1.1 Manganese superoxide dismutase

Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit that cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide. The respiratory chain in mitochondria is a major source of oxygen radicals. Mn-SOD is a nuclear-encoded primary antioxidant enzyme that functions to remove these superoxide radical (14). The biological importance of Mn-SOD is demonstrated among others by the following (12): 1) inactivation of Mn-SOD genes in *E. coli* increases mutation frequency when grown under aerobic conditions. 2) Elimination of the gene in *Saccharomyces cerevisiae* increases its sensitivity to oxygen. 3) Lack of expression in Mn-SOD knock-out mice results in dilated cardiomyopathy and neonatal. 4) Tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu, Zn-SOD, CAT or GPX mRNA in various mouse tissues and cultured cells. 5) Transfection of Mn-SOD cDNA into cultured cells rendered the cells resistant to paraquat, TNF and adriamycin-induced cytotoxicity, and radiation induced-neoplastic transformation. 6) Expression of human Mn-SOD genes in transgenic mice protects against oxygen-induced pulmonary injury and adriamycin-induced cardiac toxicity. Thus, the expression of Mn-SOD is essential for the survival of aerobic life and the development of cellular resistance to oxygen radical-mediated toxicity.

3.1.2. Copper,zinc superoxide dismutase

Cu, Zn-SOD (SOD-1) are another class of enzyme conserved throughout evolution, which usually have two identical subunits of about 32 kDa, each containing a metal cluster, the active site, constituted by a copper and a zinc atom bridged by a common ligand: His 61 (15).

Whereas Mn-SOD was found in all tumors, and the ratio between the activities of Cu, Zn-SOD and Mn-SOD was not different from that of the normal tissues, tumors posses less Cu, Zn-SOD than did the more metabolically active tissues (16). Cu, Zn-SOD is believed to play a major role in the first line of antioxidant defense by catalyzing the dismutation of superoxide anion radicals, to form hydrogen peroxide and molecular oxygen. Mice lacking this enzyme exhibited a pronounced susceptibility to paraquat toxicity. Most surprisingly, female homozygous knock-out mice showed a markedly reduced fertility compared with that of wild-type and heterozygous knock-out mice. They exhibited a marked increase in embryonic lethality. These data suggest a role of oxygen free radicals in causing abnormality of female reproduction in mammals (17). Other recent reports involving SOD knock-outs have revealed that Mn-SOD is essential for life whereas Cu, Zn-SOD is not. Cu, Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age (18).

3.1.3. Extracellular superoxide dismutase

EC-SOD is a secretory, tetrameric, copper and zinc containig glycoprotein (with a high affinity for certain glycosaminogycans such as heparin and heparan sulfate) found in the intersticial spaces of tissues and also in extracellular fluids, accounting for the majority of the SOD activity of plasma, lymph, and synovial fluid (19). EC-SOD, is not induced by its substrate or other oxidants (xanthine oxidase plus hypoxanthine, paraquat, pyrogallol, alpha-naphthoflavone, hydroquinone, catechol, Fe^{2+} ions, Cu^{2+} ions, buthionine sulphoximine, diethylmaleate, t-butyl hydroperoxide, cumene hydroperoxide, selenite, citiolone and high oxygen partial pressure) and its regulation in mammalian tissues primarily occurs in a manner coordinated by cytokines, rather than as a response of individual cells to oxidants (20).

3.1.4. Nickel superoxide dismutase

Ni-SOD has been purified from the cytosolic fraction of *Streptomyces sp.* and *Streptomyces coelicolor*. It is composed of four identical subunits of 13.4 kDa, stable at pH 4.0-8.0, and up to 70 Celsius degrees. It is inhibited by cyanide and H_2O_2 but little inhibited by azide. Amino acid composition is different from iron, manganese and zinc-copper SODs. The apoenzyme, lacking in nickel, had no ability to mediate the conversion of superoxide anion to hydrogen peroxide, strongly indicating that Ni^{II} plays a main role in the activity (21).

3.2. Catalase

Catalase (EC 1.11.1.6) is a tetrameric haemin-enzyme consisting of 4 identical tetrahedrally arranged subunits of 60 kDa. Therefore, it contains 4 ferriprotoporphyrin groups per molecule, and its molecular mass is about 240 kDa. Catalase is one of the most efficient enzymes known. It is so efficient that it cannot be saturated by H_2O_2 at any concentration (5).

CAT reacts with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, phenol…) using 1 mole of peroxide in a kind of peroxidase activity:

$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

H_2O_2 is enzymically catabolized in aerobic organism by catalase and several peroxidases. In animals, H_2O_2, is detoxified by CAT and GPX. Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cells type under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (22). The increased sensitivity of transfected enriched catalase cells to adriamycin, bleomycin and paraquat is attributed to the ability of catalase in cells to prevent the drug-induced consumption of O_2. Thus, capturing H_2O_2 before it can escape the cell and converting it to O_2. In this way, catalase can maintain the concentration of O_2 either for repeated rounds of chemical reduction or for direct interaction with the toxin (23).

3.3. Glutathione peroxidase

The selenium-containing peroxidases, being the more important example glutathione peroxidase (EC 1.11.1.19), catalyze the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH, thereby protecting mammalian cells against oxidative damage.

$$ROOH + 2\,GSH \xrightarrow{GPX} ROH + GSSG + H_2O$$

There are at least five GPX isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type. *Cytosolic and mitochondrial glutathione peroxidase* (cGPX or *GPX1*) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione. GPX1 and the *phospholipid hydroperoxide glutathione peroxidase GPX4* (or PHGPX) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. PHGPX can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidised lipoproteins (24). GPX1 is predominantly present in erythrocytes, kidney, and liver, and GPX4 is highly expressed in renal epithelial cells and testes. Cytosolic *GPX2* (or GPX-G1) and extracellular *GPX3* (or GPX-P) are poorly detected in most tissues except for the gastrointestinal tract and kidney, respectively. Recently, a new member, *GPX5*, expressed specifically in mouse epididymis, is interestingly selenium-independent (25).

GPX (80 kDa) contains one selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity (26). Although GPX shares the substrate, H_2O_2, with catalase, it alone can react effectively with lipid and other organic hydroperoxides. The glutathione redox cycle is a major source of protection against low levels of oxidant stress, whereas CAT becomes more significant in protecting against severe oxidant stress (27). In animals cells, and specially in human erythrocytes, the principal antioxidant enzyme for the detoxification of H_2O_2 has for a long time been considered to be GPX, as catalase has much lower affinity for H_2O_2 than GPX (28).

Cells depleted of glutathione peroxidase were more sensitive to the toxicity of paraquat and adriamycin than untransfected parental cells from which they derived but not more sensitive to bleomycin, menadione, or phenazine methosulfate. In fact that the mildly increased sensitivity to paraquat and adriamycin was the consequence of the diminished cellular content of glutathione peroxidase was confirmed by the increase in sensitivity of untransfected cells after treatment with buthionine sulfoximine, an agent which depletes cells of glutathione. These and other data strongly suggest that the enzymatic action of GPX protects cells from the toxicity of paraquat and adriamycin. The toxin that these agents engender is likely to be hydrogen peroxide or another hydroperoxide upon which glutathione peroxidase acts (29).

GPX equally protects against the oxidation of dihydrorhodamine 123 (an indicator dye) by peroxynitrite, requiring GSH as reductant. Thus, there is also a function of GPX and potentially of other selenoproteins containing selenocysteine or selenomethionine, in the GSH-dependent maintenance of a defense line against peroxynitrite-mediated oxidations, as a peroxynitrite reductase (30).

4. REACTIVE OXYGEN SPECIES IN PATHOPHYSIOLOGIC PROCESSES

ROS generated during metabolism can enter into reactions that, when uncontrolled, can became impaired and affect certain processes leading to clinical manifestations (31). Direct effects include peroxidative changes in membranes and other cellular components, including oxidative DNA damage (32). Normally, the body is protected by a wide range of fluids by metal-binding macromolecules. SOD, GPX, and CAT within cells remove

Table 1. Antioxidant enzymes and human diseases

Disease	Specification	Main key enzyme/s
Allergy	Intolerance to aspirin	GPX (43)
Allergy	Intolerance to other drugs	SOD (44)
Allergy	Intolerance to some foods	GPX (45)
Allergy	Reaction in skin tests	SOD (46)
Cancer	Bowel	CAT, GPX, SOD (47)
Cancer	Breast	GPX (1)
Cancer	Colorectal	COX-2 [a] (48)
Cancer	Kidney	CAT, GPX, SOD (49)
Cancer	Leukemia	CAT, GPX, SOD (50)
Cancer	Liver	CAT, GPX, SOD (47)
Cancer	Skin	GPX (51)
Cardiological and vessels injuries	Ischemia	SOD (39)
Cardiological and vessels injuries	Atherosclerosis	SOD (52)
Infectious disease	Arthritis	COX-2 [a] (53)
Infectious disease	Helicobacter pylori	SOD (34)
Infectious disease	Hepatitis	GPX (54)
Infectious disease	HIV	GPX (55)
Infectious disease	Influenza virus	CAT, GPX, SOD (41)
Genetic disorder	Chronic granulomatous disease	CAT (56)
Genetic disorder	Down's syndrome	SOD (57)
Metabolic malfunction	Diabetes	CAT, SOD (27)
Neurodegenerative disease	Allergic encephalomyelitis	NOS [b] (58)
Neurodegenerative disease	Alzheimer's disease	SOD (59)
Neurodegenerative disease	Amyotrophic lateral sclerosis	SOD (60)
Neurodegenerative disease	Huntington's disease	SOD (36)
Neurodegenerative disease	Parkinson's disease	GPX (60)
Neurodegenerative disease	Prion disease	SOD (36)
Ophthalmologic problem	Cataract	CAT, SOD (27)

[a]COX-2: cyclooxygenase-2; [b]NOS: nitric oxide synthase

superoxide and peroxides before they react with metal catalysis to form more reactive species. Finally, peroxidative chain reactions initiated by reactive species that escaped enzymatic degradation are terminated by chain-breaking antioxidants, including water-soluble ascorbate, lipid-soluble vitamin E and ubiquinone. To optimize performance, oxidative stress must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate reactive oxygen species (33).

An unbalanced production of reactive oxygen intermediates has been postulated to play a role in the pathogenesis of a number of clinical disorders such as ischemia/reperfusion, atherosclerosis, neurodegenerative diseases, allergy and cancer. Besides it has been established its relationship with other specific pathologies: Alzheimer's disease, Parkinson's disease, allergic encephalomyelitis, chronic granulomatous disease, Down's syndrome, hepatitis, arthritis, HIV infection, diabetic complications, cataract formation and ulcer (table 1). Helicobacter pylori generated substantial amounts of superoxide radicals. H. pylori infection has a different effect on mitochondrial and cytoplasmic SOD in the gastric mucous, reflected by a pronounced increase in the cytokine inducible Mn-SOD and a marginal decrease in the constitutive Cu, Zn-SOD (34). In a similar fashion, linkage studies have revealed that mutations in Cu, Zn-SOD are responsible for 10-15 % of cases of the fatal motor neuron disease familial amyotrophic lateral sclerosis (35). These patients have mutations in the gene encoding cytosolic Cu, Zn-SOD, and multiple lines of evidence from cell culture and transgenic models indicate that these mutations cause Cu, Zn-SOD to acquire toxic properties (36).

ROS have been implicated in many lung diseases including those associated with exposure to asbestos, nitrogen dioxide, ozone, paraquat, hyperoxia, carbon tetrachloride, and the anticancer drugs bleomycin and adriamycin. Phagocytic cells have been implicated in the generation of ROS during inflammation (37).

Preservation of leukocyte SOD inducibility appears to correlate with longevity in elderly individuals and may be of value in predicting resistance to malignancy or fatal cardiovascular events (38). Oxygen species are key participants in damage resultant from ischemia/reperfusion. Brain and heart tissues are protected from this oxidative injury by antioxidant enzymes as SOD and GPX. Overexpression of both enzymes confers significant protection against both infarction and brain edema in transgenic mice (39). Transgenic mice with overexpression of human SOD-1 are studied along with matched nontransgenic controls. In the transgenic hearts with overexpression of SOD-1 the burst of superoxide generation was almost totally quenched. This event was accompanied by a 2-fold increase in the recovery of contractile function, a 2.2-fold decrease in infarct size, and a greatly improved recovery of high energy phosphates compared with that in nontransgenic controls. These results demonstrate that superoxide is an important mediator of postischemic injury and that increasing intracellular SOD-1

dramatically protects the heart from this injury. Thus, increasing intracellular SOD-1 expression may be a highly effective approach to decrease the cellular injury that occurs following reperfusion of ischemic tissues (3). Concerning MnSOD, results exist suggesting that its messenger RNA maybe induced by oxygen radicals or by other chemical mediators, such as cytokines (40).

The pathogenesis of influenza virus infections of the lungs is also mediated by oxidative stress. Influenza infections cause airway epithelial inflammation and oxidant-mediated damage. In this setting, cellular antioxidant enzymes may protect airway epithelial cells against damage resulting from toxic oxygen radicals produced by activated leukocytes (41). Such infections might therefore be expected to induce expression of stress-response genes and genes encoding antioxidant enzymes and to activate transcriptional regulatory proteins.

5. PERSPECTIVE

As a conclusion, abnormalities in the cellular regulation and expression of antioxidant enzymes have a role in cell division cycle and in the balance of life (42). This understanding illustrate the importance of the antioxidant defense system in maintaining normal cellular physiology and fighting against diseases. Besides, as described above, active oxygen intermediates scavenging has been proposed as one of the mechanism to promote immunity (4).

Thus, when antioxidant, free radical scavenging systems are overwhelmed, pathologic conditions may result. Defense systems against free radical in human are a proof of the main role of antioxidant enzymes in blood cells detoxification, showing the coordinated enzymatic mechanism, and the interrelationships between all these enzymatic activities. Undoubtly, we bet for the therapeutic implications of antioxidant activities and their possible future clinical application. Finally, we want to emphasize the importance of the study of all the regulation mechanisms at molecular level.

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