Free Radicals and Tissue Damage: Role of Antioxidants

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ABSTRACT

Cells generate energy by reducing molecular oxygen to water. During the process, small amounts of partially reduced reactive oxygen forms are produced as unavoidable byproducts which are referred to as Reactive Oxygen Species. An imbalance between free radical-generating and radical scavenging systems results in Oxidative Stress. Reactive nitrogen species and other non reactive derivatives are also involved. These processes lead to tissue damage and contribute to the pathogenesis of many disorders like hypertension, cancer, diabetes, neurodegenerative disorders and others. The human body has several mechanisms to counter the effects of these reactive species by the production of antioxidant enzymes like glutathione and catalase. Antioxidants can also be taken exogenously through the diet. This article provides an overview of the different free radicals known, the mechanism by which they cause tissue damage and the protective role played by different antioxidants.

Keywords: reactive oxygen species, oxidative stress, nitrosative stress, tissue damage, antioxidants.

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DOI: 10.5530/ax.2011.4.2

INTRODUCTION

Oxygen is an indispensible element for the sustenance of living beings and many biological systems. Cells reduce oxygen and generate adenosine triphosphate (ATP) in the mitochondria. Byproducts known as free radicals are created during this process. These free radicals are beneficial in moderate levels but at higher concentrations can damage tissues by oxidative stress.

Since more than half a century the deleterious effects of these reactive species are known but in the last two decades a lot of work has been done in this area. The important role played by anti oxidants in providing protection cannot be underestimated. Antioxidants are increasingly being used to prevent and also repair the damage caused by these free radicals.

Free radical generation and reactive species

A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital. These when formed can be highly reactive and can start a chain reaction. [1] The sources of free radicals can be endogenous and exogenous in nature. Endogenous sources of free radicals are intracellularly generated from auto-oxidation or inactivation of small molecules. Exogenous sources of free radicals are tobacco smoke, certain pollutants, organic solvents, anesthetics and pesticides. The sites of free radical generation encompass all cellular constituents including mitochondria, lysosomes, peroxisomes, endoplasmic reticulum, plasma membrane and sites within the cytosol. [2]Apart from this, certain medications metabolized to free radical intermediate products also cause oxidative damage within the target tissues. Exposure to radiation results in the formation of free radicals within the target tissues.

The term "Reactive oxygen species" (ROS) refers to an array of metabolites derived from molecular oxygen (O_2). Reactive nitrogen species (RNS, e.g. Nitric oxide, $NO^{\bullet}$) play a vital role in the generation of free radicals. $NO^{\bullet}$ is an abundant reactive radical which has a role in diverse physiological processes like neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. Overproduction of reactive nitrogen species is called Nitrosative stress.[3] RNS is abundantly produced during inflammatory processes.

ROS and RNS describe free radicals and other non-radical reactive derivatives. They include radicals such as

superoxide anion ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and lipidperoxyl ($LOO^{\bullet}$). These radicals are becoming increasingly implicated in human diseases. Non-radicals include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone(O_3), peroxy nitrate ($ONOO^-$), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) , lipid peroxide (LOOH).[4]

Mechanism of generation of free radicals

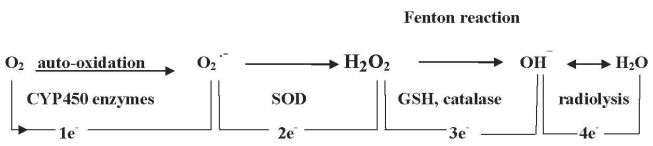
Normally, cell metabolism involves generation of ATP by oxidative process in which biradical oxygen (O_2) combines with hydrogen atom (H) and in the process forms water (H_2O). Oxygen free radicals are the intermediate chemical species having unpaired oxygen in their outer orbit. These are generated within mitochondrial inner membrane where cytochrome oxidase catalyses the O_2 to H_2O reaction. [4]

Three intermediate molecules of partially reduced species of oxygen are generated from enzymatic and non-enzymatic reactions depending on the number of electrons transferred.

Superoxide oxygen ($O_2^{\bullet -}$ one electron): It is generated by direct auto-oxidation of O_2 during mitochondrial electron transport reaction. Alternatively, $O_2^{\bullet}$ is produced enzymatically by xanthine oxidase and cytochrome P450 in the mitochondria or cytosol. [5] $O_2^{\bullet}$ so formed is catabolized to produce H_2O_2 by superoxide dismutase (SOD) a metalloprotein. It is considered to be the least reactive type of ROS and the most commonly produced free radical in humans. Once it is produced it triggers a rapid cascade of events that creates other free radicals.

Hydrogen peroxide (H_2O_2, two electrons): H_2O_2 is reduced to water enzymatically by catalase (in the peroxisomes) and glutathione peroxidase (both in the cytosol and mitochondria).The enzyme glutathione peroxidase also breaks down any peroxides that form on lipids within the body.

Hydroxyl radical (OH^-, three electrons): It is the most reactive of the free radical molecules. It damages cell membranes and lipoproteins by lipid peroxidation. Damage to lipoproteins in low density lipoprotein plays an important role in atherosclerosis. OH^- is formed by radiolysis of water and by reaction of H_2O_2 with ferrous (Fe^{++}) ions; the latter process is termed as Fenton reaction.[5]

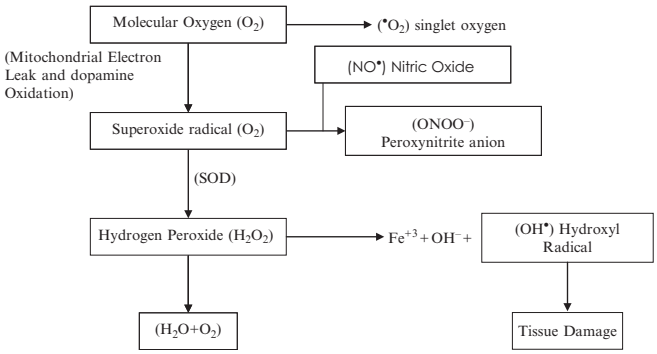


Process of formation of reactive oxygen species by four electron step reduction of oxygen.

In addition to the above a few other active oxygen free radicals are formed in the body.

- Nitric oxide (NO), a chemical mediator generated by various body cells (endothelial cells, neurons, macrophages etc), combines with superoxide and forms peroxynitrate (ONOO) which is a potent free radical.
- Halide reagent (Chlorine / Chloride) released in the leukocytes reacts with superoxide and forms hypochlorous acid (HOCl) a cytotoxic free radical.

Representation of Generation of Oxygen Free-Radical[7]



Most of the oxygen taken up by the cells of our body is converted into H_2O during mitochondrial respiration. However, less than 5% of oxygen is converted into ROS. These substances are highly toxic in nature and if allowed to accumulate, they can destroy all the macromolecules of the cells like lipids, proteins, mitochondrial and nuclear DNA molecules causing severe oxidative stress. Oxidative stress results in a series of events which deregulate the cellular functions leading to various pathological conditions and play a major role in the development of chronic and degenerative ailments. The majorities of free radicals is produced under normal physiological conditions, and are rapidly sequestered by antioxidant enzyme systems within the mitochondria. Mitochondrial dysfunction causes excessive production of these ROS leading to oxidative stress and cell death. [8] It is caused by an imbalance between the production of reactive oxygen and biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

In human diseases, an increase in free radical activity occurs as a consequence of either primary (excess radiation exposure) or secondary (tissue damage by trauma) mechanism. ROS react with most cellular macromolecules,

including proteins, lipids, and DNA. ROS–induced oxidation of proteins can lead to changes in the protein's three-dimensional structure as well as to fragmentation, aggregation, or cross-linking of the proteins. Finally, protein oxidation often will make the marked protein more susceptible to degradation. ROS are a major source of DNA damage, causing strand breaks, removal of nucleotides, and a variety of modifications of the organic bases of the nucleotides which can lead to permanent changes or damage to the DNA, with potentially detrimental effects for the cell.[9]

Oxygen-free radical (OFR) or more generally, ROS and RNS are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. [9]

Lipid peroxidation

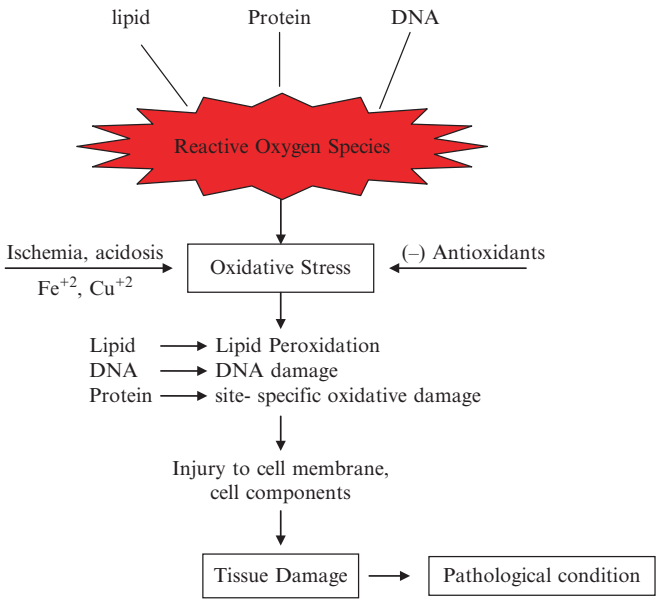
Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells and cell structures. Free radicals in the presence of oxygen may cause degradation (peroxidation) of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen derived free radicals particularly by OH. The lipid free radical interactions yield peroxides, which are themselves unstable and reactive and an autocatalytic chain reaction called propagation ensues which can result in extensive membrane, organellar, and cellular damage.(4) Oxidative destruction of polyunsaturated fatty acids by lipid peroxidation is damaging because it may alter the integrity of cell membranes. [10]

Pathogenesis of tissue damage

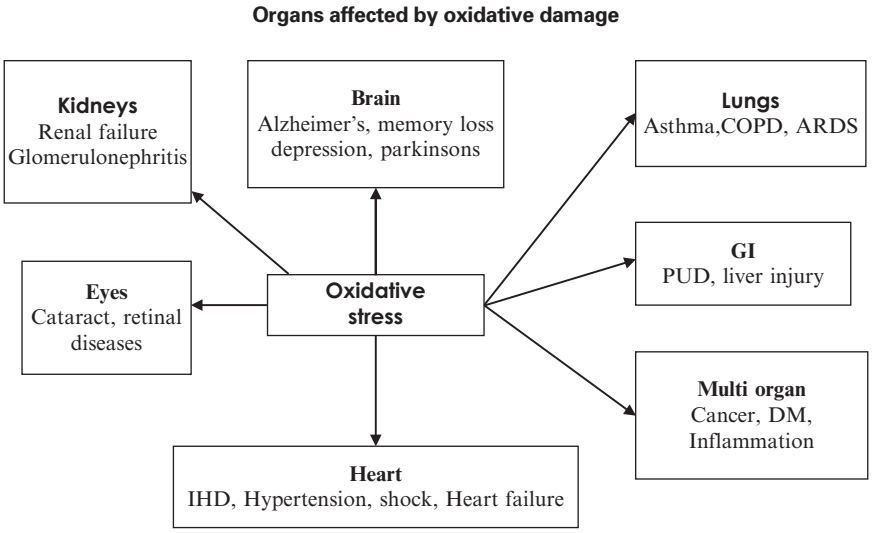
Free radicals which are formed endogenously act intracellularly within the cell and are released into the surrounding area.[2] Prime targets for free radical reactions are the unsaturated bonds in membrane lipids. Consequent peroxidation results in a loss in membrane fluidity and receptor ailment and potentially in cellular lysis. Free radical damage to sulphur containing enzymes and other proteins culminates in inactivation, cross linking and denaturation. Nucleic acids can be attacked. Subsequent damage to the DNA can cause mutations. Oxidative damage to carbohydrates can alter any of the cellular receptor function including those associated with hormonal and neurotransmitter responses. [2] Consequences of oxidative stress are adaptation or cell injury, i.e., damage to DNA, proteins and lipids; disruption in cellular homeostasis and accumulation of damaged molecules.[11] Prolonged exposure to free radicals, even at low concentration , may result in the damage of biologically important molecules and potentially lead to DNA mutation, tissue injury and disease.[12]

Injury of tissue and its healing represents a sequence of various events, depending on the injurious cause itself, e.g. infection, inflammation, ischemia, and on other factors, such as the intensity of damaging agent, type of tissue, condition of whole organism, etc.[13] As soon as there is tissue injury, healing process starts. An effort is made to eliminate injurious action and restore tissue integrity and its function by remodeling impaired structures. These responses are mediated by a variety of messengers released during the injurious/ healing process. During this period immune system is activated, phagocytes produce cytotoxic agents ,which not only prevent the spread of infection but also remove host cellular particles that are damaged. Activation of phagocytes depends largely on their high consumption of oxygen, called oxidative burst, during which ROS are produced which are likely to serve as mediators of injury. [14]

Oxygen derived free radical reactions have been implicated in the pathogenesis of over 200 clinical conditions.



Pathogenesis of tissue damage



Protection against toxic effects

Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, lipids, and protein) and other small antioxidant molecules.[11] There are many components which act against free radicals to neutralize them. Antioxidants may exert their activity by suppressing the production of active species, by reducing hydroperoxides, by sequestering metal ions, and by scavenging free radicals and terminating the chain reaction and also by clearing the damage of the cell. Some antioxidants also induce the biosynthesis of other antioxidants.[4] They can be endogenous enzymatic antioxidants, non enzymatic, metal binding proteins like ferritin, ceruloplasmin and also phytoconstituents and nutrients.

The cells contain important defense systems against free radicals termed as antioxidant enzymes. The main enzymatic scavengers responsible for the prevention of ROS formation and oxidation are Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and glutathione reductase (GRx). SOD catalyses the dismutation of superoxide to hydrogen peroxide and is the body's primary defense as it prevents further generation of free radicals. Catalase and glutathione peroxidase detoxify oxygen reactive radicals by catalyzing the formation of H_2O_2. The selenoprotein GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG (oxidized glutathione), with NADPH as a source of reducing power.[15] The range of antioxidant defenses available within the cell and extracellularly should be adequate to protect against oxidative damage. However, the balance can be lost because of overproduction of free radicals, by exposure to sources that overwhelm the oxidant defenses, or by inadequate intake of nutrients that contribute to the defense system.[16]

Different types of Free radicals and their Defense system [7]

Types of free radical (or) oxidants	Defense system
Superoxide anion (O_2)	Superoxide dismutase
Hydroxyl radical ($OH^{\bullet}$)	SOD, Mn-SOD, Cu, Zn-SOD, glutathione
Peroxyl radical ($ROO^{\bullet}$)	Tocopherols, Ubiquinone
Singlet oxygen ($O_2^{\bullet}$)	Carotenoid
Hydrogen peroxide (H_2O_2)	Catalase, Se Glutathione peroxidase
Hydroperoxides (ROO^{-})	Glutathione peroxidase, Glutathione reductase
Transition Metals (Fe^{+2}, Cu^{+2})	Chelators

Anti-oxidants and their role

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated insitu (endogenous), or externally supplied through foods (exogenous). The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention.[15] Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization

of oxidative stress. Each nutrient is unique in terms of its structure and anti-oxidant function. [18]

Classification of antioxidants

I Enzymatic (Endogenous): SOD, Catalase, GPx, and GRx

II Non Enzymatic

a. Metabolic antioxidants (endogenous) glutathione, L-arginine,CoQ10,melatonin,uric acid, Transferring
b. Nutrient antioxidants (exogenous) Vitamin E, Vitamin C, carotenoids, trace elements(selenium, Zinc, Manganese), flavonoids, omega 3 and omega 6 fatty acids

Some of the important antioxidants are reviewed here briefly.

Vitamin E: Vitamin E is a non enzymatic endogenous fat-soluble vitamin with high anti-oxidant potency. It's a chiral compound with eight stereoisomers: α, β, γ, δ tocopherol and α, β, γ, δ, tocotrienol. But α tocopherol is the most bioactive stereo-isomer in humans which safeguards cell membranes from damage by free radicals. Its antioxidant function mainly resides in the protection against lipid peroxidation. It has been proposed for the prevention against many cancers, cardiovascular diseases, ischemia, cataract, arthritis and certain neurological disorders.

Vitamin C: It is a non enzymatic endogenous antioxidant water-soluble vitamin. It works synergistically with vitamin E to quench free radicals and generates the reduced form of vitamin E. It also has anti-atherogenic, anti-carcinogenic, immunomodulator properties. [19]

Beta-carotene: It is a fat soluble member of the carotenoids which is considered as provitamin as it can be converted into active vitamin A. It is a strong antioxidant and is the best quencher of singlet oxygen.

Lycopene: Lycopene, a carotenoid, possesses antioxidant activity and was proved to possess antiproliferative property.

Selenium (Se): It is an exogenous nutrient antioxidant and a trace mineral found in soil, water, vegetables (garlic, onion, grains, nuts, and soybean), sea food, liver, and yeast. It forms the active site of several anti-oxidant enzymes including glutathione peroxidase.

Flavonoids: Flavonoids are polyphenolic compounds which are present in most plants. These are classified into flavanols, flavanones, flavones, iso-flavones, catechins, anthocyanins, proanthocyanidins which possess potent antioxidant activity.

Omega-3- and omega-6 fatty acids: These are essential long-chain polysaturated fatty acids which are found in fat fish (salmon, tuna, halibut, sardines, and pollock), krill, algae, walnut, nut oils and flaxseed. There are three major dietary types of omega-3 fatty acids: eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and alpha-linolenic acid (ALA) which serves as antioxidants. [20]

CONCLUSION

Free radicals play a significant role in pathogenesis of tissue damage, consequently having implications in many clinical conditions. Both endogenous and exogenous antioxidants play a protective role in repairing the damage caused by the free radicals. Exogenous antioxidant supplementation is increasingly used to fight against oxidative stress. A lot of research is being undertaken to identify new plant resources which have no or low side effects and potent antioxidant activity.

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