2nd International Conference on Recent Developments in Science, Humanities & Management
Mahratta Chamber of Commerce, Industries and Agriculture, Pune (India)
8th July 2018 www.conferenceworld.in ISBN : 978-93-87793-33-0

Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers

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ABSTRACT

Molecular oxygen was introduced to the early reducing atmosphere of the Earth about 2.7 billion years ago by O_2- evolving photosynthetic organisms, causing the advent of the reactive oxygen species (ROS) as unwanted byproducts. The ROS plays the double role of being the inevitable by-product of aerobic metabolism on one hand and serving as a marker during stressful conditions on the other hand. The damage caused by ROS is extensive and the targets include all biomolecules like lipids, proteins and DNA, damaging the integrity of the cell and ultimately leading to its death. Many antioxidant systems contribute to the regulation of ROS, including superoxide dismutases, catalases and the enzymes of the glutathione redox cycle, which reflects the widespread functional effects of ROS.This review gives an insight into how both arms of the antioxidant machinery; the antioxidant enzymes and the non-antioxidant metabolites, work in conjunction to alleviate the damaging effects of ROS .

Key Words: antioxidants ,catalases, dismutases, molecular oxygen ,reactive oxygen species, superoxide

I.INTRODUCTION

Reactive oxygen species (ROS) are by-products of aerobic metabolism. ROS include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·), all of which have inherent chemical properties that confer reactivity to different biological targets. ROS are often associated with the principle of oxidative stress, which suggests that ROS induce pathology by damaging lipids, proteins, and DNA .However, in the past two decades it has become apparent that ROS also serve as signaling molecules to regulate biological and physiological processes.[1] Reactive oxygen species (ROS) and other free radicals are responsible for many diseases, such as arteriosclerosis, heart diseases, aging process and cancer. They may lead to cell damage through membrane lipid peroxidation and DNA mutations and as a consequence of that many diseases such as

cancer may develop. The importance of reactive oxygen species (ROS) has attracted attention globally over the past decade. [2]

II. OXIDATIVE STRESS AND ANTIOXIDANTS

A role of oxidative stress has been postulated in many conditions, including anthersclerosis, inflammatory condition, certain cancers, and the process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases ischemic diseases hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder alcoholism, smoking-related diseases, and many others. An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and functions.[3] The formation of free radicals or oxidants is a well-established physiological event in aerobic cells. Oxidative stress may occur in tissues injured by trauma, infection, injury, hypertoxia etc. Oxidative stress and oxidative modification of biomolecules are involved in a number of physiological and pathophysiological processes such as aging, artheroscleosis, inflammation and carcinogenesis, and drug toxicity. An imbalance between oxidants and antioxidants leads to oxidative stress which is associated with damage to a wide range of molecules like lipids, proteins, and nucleic acids.[4]

III.OXIDATIVE DAMAGE TO DNA

Damage to DNA is considered to be the most significant consequence of oxidative stress in the body. Specific bases of DNA are oxidized in this damage. Glycols, 8-hydroxy-2-deoxyguanosine and dTG are found to be increased during oxidative damage to DNA. 8-hydroxydeoxyguanosine is the most common marker for oxidative stress. When 8-OHdG levels are elevated, it's important to identify the sources of oxidative stress and assess the primary intracellular antioxidant glutathione. A similar oxidative damage can occur in RNA with the formation of 8-OHG (8-hydroxyguanosine), which has been implicated in various neurological disorders.

IV.LIPID PEROXIDATION

Lipids containing carbon-carbon double bond(s) are easily oxidized by free radicals. Lipid peroxidation occurs on polysaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction producing alkanes, aldehydes, ketones and polymerization products. These products are highly reactive with extracellular matrix and cellular components. Among reactive aldehydes, malondialdehyde (MDA) is a toxic aldehydic end product of lipid peroxidation which mediates the oxidation of cartilage collagen, causing fragmentation, aggregation and changes in protein conformation. Finally this leads to alterations in tissue functioning. Excess binding of these reactive aldehydes to matrix and cellular proteins alters cellular function, membrane permeability and electrolyte balance. This further leads to matrix protein degradation, fibrogenesis, and damage to biological system.[5]

V.OXIDATIVE DAMAGE TO PROTEIN

Proteins are major targets for free radicals due to their abundance and high rate constants for reaction. Proteins containing amino acids such as methionine, histidine cysteine and arginine, seem to be the most vulnerable to oxidation. Activity of receptors, enzymes and membrane transport is affected by oxidative damage of proteins. The oxidised protein products may contain very reactive groups that may contribute to damage of cellular functions. Peroxyl radical is usually considered to be free radical species for the oxidation of proteins. Reactive oxygen species damage proteins and produce carbonyls.[6]

VI.ANTIOXIDANTS

Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS. (Figure 1).

Antioxidants reduce oxidative stress induced carcinogenesis either by direct scavenging of free radicals or by inhibiting cell proliferation. Vitamin C is helpful in preventing cancer. This may be attributed to it's antioxidant effect, blocking of formation of nitrosanimes, enhancement of the immune response and acceleration of detoxification of liver enzymes. β-carotene is another antioxidant. By way of it's photo protective properties it may be helpful against UV induced carcinogenesis. It may also act as anticarcinogen by altering the liver metabolism. Vitamin E, an important antioxidant, plays a role in immunocompetence. It increases humoral antibody protection and provides resistance to bacterial infections. Besides, it enhances T-lymphocytes tumor necrosis factor production and inhibits mutagen formation. Thus vitamin E may be useful in cancer prevention and inhibit carcinogenesis by the enhancement of immune response.[7]

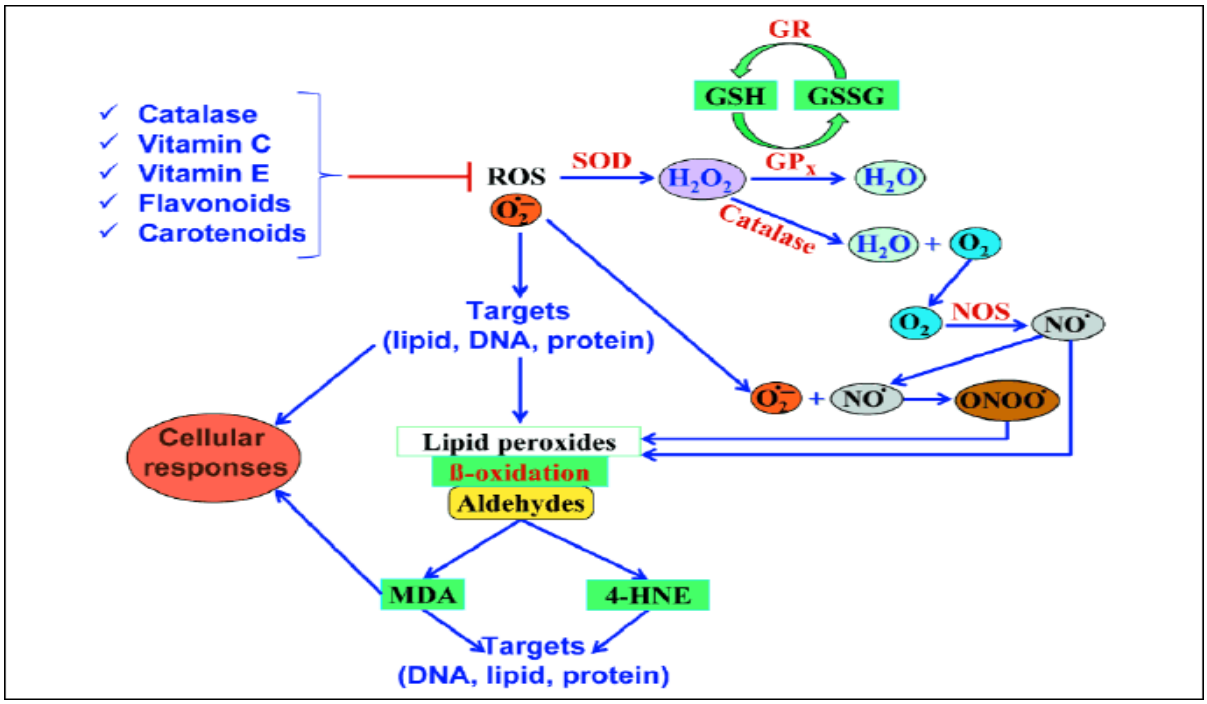

Figure 1:ROS targets and defense systems.

VII.MECHANISM OF ACTION OF ANTIOXIDANTS

Antioxidants may exert their effect on biological systems by various mechanisms including metal ion chelation, electron donation, co-antioxidants or by gene expression regulation Antioxidants work by two main mechanisms:

The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems.

The second mechanism involves removal of reactive oxygen species or reactive nitrogen species initiators by quenching chain-initiating catalyst.

Antioxidants acting in the defense systems act at four different levels such as:

- Preventive
- Radical scavenging
- Repair and de novo
- Adaptation.

The first line of defense is suppression of the formation of free radicals (preventive). The exact mechanism and site of radical generation in vivo are not well elucidated. However, the metal-induced decompositions of hydrogen peroxides and hydroperoxides must be one of the main sources. In order to suppress these reactions, certain antioxidants reduce hydroperoxides and hydrogen peroxide before hand to water and alcohol, respectively. Besides, some proteins quench metal ions which prevents formation of free radicals.[8]

Phospholipid hydroperoxide glutathione peroxidase (PHGPX), glutathione peroxidase, glutathione-s-transferase, and peroxidase are known enzymes that decompose lipid hydroperoxides to alcohols. Catalase and Glutathione peroxidase reduce hydrogen peroxide to water. PHGPX reduces hydroperoxides of phospholipids integrated into biomembranes which makes it unique.

The second line of defense is scavenging or quenching of the active free radicals. The quenching of these radicals leads to suppression of chain initiation. This in turn prevents the chain propagation reactions. Several endogenous radical-scavenging antioxidants have been identified. Some of them are hydrophilic and others are hydrophobic. Vitamin C, bilirubin, uric acid, albumin and thiols are hydrophilic while vitamin E and ubiquinol are hydrophobic free radical scavengers. Vitamin E is the most potent radical-scavenging lipophilic antioxidant. The third line of defense is the repair and de novo antioxidants. These include proteolytic enzymes, proteinases, proteases, and peptidases. These are present in the cytosol and in the mitochondria. These recognize, degrade, and remove oxidatively damaged proteins, thereby preventing the accumulation of oxidized proteins. DNA repair systems also play a vital role in defense system against oxidative damage. Enzymes like glycosylases and

nucleases repair the damaged DNA. There is another important function called adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site[9].

VIII.OXIDATIVE STRESS, HUMAN DISEASES, AND ANTIOXIDANTS

Free radicals, or reactive oxygen species (ROS), are constantly generated in the human body and include free radicals such as superoxide ($\cdot O_2^-$) and hydroxyl (·OH), nitric oxide ($NO^{\cdot-}$) and non radicals such as hydrogen peroxide (H_2O_2). ROS are produced in normal cellular metabolism and are well established in their roles as being both beneficial and harmful to living systems. ROS at low or moderate levels play beneficial roles in living systems, such as the defensive responses to infections and the functions in cellular signaling pathways. This balance between beneficial and harmful effects of ROS, which is controlled by redox regulation, has great importance to living organisms. Living organisms can be protected from various oxidative stresses by the process of redox regulation. Redox homeostasis is controlled by regulation of redox status in vivo. However, when ROS are produced excessively in biological systems, there results an imbalance between ROS and the activity of enzymatic and non-enzymatic antioxidants in the defense system. This imbalance, so called oxidative stress, causes potential biological damage to cellular lipids, proteins, or DNA, which negatively affects their functions in the human body. Under mild oxidative stress, cells can be protected by the defensive systems, but under severe oxidative stress, cells are damaged and lead to death by apoptotic or further necrotic mechanisms. Because of this, oxidative stress has long been considered to be involved in the pathogenesis of human diseases such as atherosclerosis, inflammation, cancer, diabetes, central nervous system disorders as well as cardiovascular diseases.[10]

IX.REACTIVE OXYGEN SPECIES (ROS) AND HUMAN DISEASES

ROS can be generated both endogenously and exogenously. ROS, present in the atmosphere as pollutants, are generated by exogenous sources such as radiations (X-rays, γ-rays, and UV light irradiation), xenobiotics, metals, ions, chlorinated compounds, and environmental agents. In the human body, ROS are endogenously produced by catalyzed reactions and various other mechanisms in mitochondria as well as by neutrophils and macrophages during inflammatory cell activation. As mentioned above, ROS include both free radicals and non-free radicals. Superoxide, hydroxyl, and nitric oxide are major free radicals and hydrogen peroxide is a major non-free radical. ROS produced endogenously is examined and explained briefly. Superoxide ($\cdot O_2^-$) is reactive radical and produced mostly in cell mitochondria by leakage of a small number of electrons during energy transduction. The hydroxyl (·OH) radical is the neutral form of the hydroxide ion, and ·OH reacts with all components of the DNA molecule. This radical is highly reactive and making it a very dangerous radical. Nitric oxide (NO·-) acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. Hydrogen peroxide (H_2O_2), produced in mitochondria, is not a free radical but acts as a ROS precursor to superoxide. Even though H_2O_2 in low concentration may be poorly reactive, high

concentration of H_2O_2 can attack several cellular energy-producing systems and form ·OH in the presence of transition metal ions; $\cdot O_2^-$ facilitates this reaction.[11]

Highly concentrated ROS can play an important role as mediators of cell structure, and cause nucleic acids, lipids, and protein damage. Consequently, tissue injury itself can cause ROS generation, which may contribute to worsening of the injury. These oxidative damages lead to permanent modifications of genetic material and may cause mutagenesis, carcinogenesis, and aging. Furthermore, metal-induced generation of ROS also causes an attack on DNA, as well as on the other cellular components including the polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation. Oxidative stress (imbalanced production of ROS) has been implicated in various pathogeneses involving cardiovascular disease, cancer, neurodegenerative disorders, diabetes, ischemia/reperfusion damage, aging as well as male infertility. These diseases are divided into two groups: 1) mitochondrial oxidative stress conditions causing cancer and 2) diabetes mellitus and inflammatory oxidative conditions causing atherosclerosis and chronic inflammation, ischemia, and reperfusion injury. The process of aging is caused by the damaging consequences of free radical action, which results in lipid peroxidation, DNA damage, and protein oxidation. Inflammatory cells may also increase DNA damage by activating pro-carcinogens to DNA damaging species. Cancer can be considered a degenerative disease of old age, related to the effects of continuous damage over a life span by toxic oxygen.[12]

X.ROLES OF ANTIOXIDANTS AGAINST OXIDATIVE STRESS

When free radicals from various sources are exposed, organisms develop their own series of defense mechanisms. Defense mechanisms against free radical-induced oxidative stress include preventative mechanisms, repair mechanisms, physical defenses, and antioxidant defenses. Potentially damaging ROS are dealt with by enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase, and catalase. Non-enzymatic antioxidants include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), glutathione, carotenoids, flavonoids, and other antioxidants. Antioxidants are able to stabilize or deactivate free radicals before they attack the cells. ROS can be eliminated by a number of enzymatic and non-enzymatic antioxidant mechanisms. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is very important to organisms' health and survival. However, under oxidative stress conditions, enzymatic antioxidants may not be sufficient, and non enzymatic antioxidants (dietary antioxidants) may be required to maintain optimal cellular functions. Enzymatic antioxidants Enzymatic antioxidants are endogenously produced in the human body. These endogenous antioxidants play a critical role in maintaining optimal cellular functions. The most efficient enzymatic antioxidants in endogenous antioxidants involve glutathione peroxidase, catalase, and superoxide dismutase.[13]

Glutathione peroxidase

Glutathione peroxidase is present in the mitochondria and cytosol. Glutathione peroxidase is one of the most important antioxidant defense mechanisms present in the cells. It is generally thought to be more important than

catalase in the removal of hydrogen peroxide (H_2O_2) in humans. This enzyme associates with glutathione, which is present in high concentrations in cells and catalyzes the conversion of hydrogen peroxide (H_2O_2) or organic peroxide to water or alcohol. Glutathione peroxidase has a competitive reaction with catalase for hydrogen peroxide as a substrate, and is the major source of protection under the low levels of oxidative stress[14].

Catalase

Unlike glutathione perxidase and superoxide dismutase, catalase is located in peroxisome of aerobic cells and is very efficient at converting hydrogen peroxide (H_2O_2) to water and molecular oxygen. In animals, catalase and glutathione peroxidase detoxify hydrogen peroxide. Cells are protected by catalase from hydrogen peroxide generated within the cells.[15]

Superoxide dismutase

Similar to glutathione peroxidase, peroxide dismutase is distributed in the mitochondria and cytosol. This enzyme is one of the most effective intracellular enzymatic antioxidants and converts O_2^- to hydrogen peroxide (H_2O_2) and then hydrogen peroxide to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria. Under normal conditions, the high levels of SOD keep superoxide concentrations at low levels and prevent formation of peroxynitrite. SOD neutralizes superoxide ions during the process of successive oxidative and reductive cycles.

Non-enzymatic antioxidants

As mentioned above, under the oxidative stress conditions, enzymatic antioxidants may not be sufficient and, therefore, non enzymatic antioxidant (dietary antioxidants) may be required to maintain optimal cellular functions. Even though some dietary compounds do not contribute to neutralize free radicals, antioxidants may enhance the endogenous antioxidant activities.

It is well known that fruits and vegetables are good sources of many antioxidants. It has also been reported that diets rich in fruit and vegetables are associated with reduced risks of chronic diseases such as cancer and heart diseases. Therefore, a healthy diet may maintain nonenzymatic antioxidants as well as exogenous antioxidants at or near optimal level, thus lowering the risk of tissue damage. In general, non enzymatic antioxidants involve vitamins E, C, glutathione, carotenoids, and flavonoids.[16]

Vitamin E and Vitamin C

Vitamin E is fat-soluble and includes 8 different forms. The main function of vitamin E is to protect against lipid peroxidation. It inhibits lipid peroxidation by effectively scavenging the peroxyl radical in cell membranes. Among 8 different forms of vitamin E, α- tocopherol is the most active form in vivo and the major membrane bound antioxidant employed by the cell in human body. Additionally, α-tocopherol and vitamin C (ascorbic acid) function together during the antioxidant reactions. Vitamin C acts to regenerate α-tocopherol from α-

tocopherol radicals in membranes and lipoproteins, and increases intracellular glutathione levels, thus playing an important role in protein thiol group protection against oxidation. Vitamin C is water-soluble and an important and powerful antioxidant working in aqueous environments of the body. As mentioned above, this vitamin is a partner with Vitamin E in scavenging radicals. In addition to working with vitamin E, it cooperates with carotenoids as well as with the antioxidant enzymes. [17]

Glutathione

Glutathione is a major thiol antioxidant and has multiple functions as an intracellular antioxidant. It is the major water soluble antioxidant in these cell compartments and is present at high levels in the cytosol, nuclei, and mitochondria. The main protective roles against oxidative stress are to act as a co-factor for several detoxifying enzymes, to scavenge hydroxyl radical and singlet oxygen directly, and to regenerate vitamin C and E to their original active forms.[18]

Carotenoids

Carotenoids, present in plants and microorganisms, are mainly color pigments and contain conjugated double bonds. Their antioxidant activity arises due to the ability to delocalize unpaired electrons with resonant stabilization. Carotenoids can quench singlet oxygen and react with free radicals. They can prevent damage in lipophilic compartments by scavenging peroxy radicals. Even though it has been reported that β-carotenoids in high concentration can cause an increase in lipid peroxidation due to the adverse role as a pro-oxidants, many studies have epidemiologically revealed that the consumption of diets rich in carotenoids is correlated with a lower risk of age-related diseases.[19]

Flavonoids

Flavonoids are a large group of polyphenols which include phenolic acids and flavonoids. Over 4000 flavonoids have been identified and are divided into several groups such as flavonols (quercetin and kaempherol), flavanols (catechin), anthocyanidins, and isoflavones (daidzein and genistein) according to their chemical structures. [20]Flavonoids, present in food mainly as glycosides and polymers, contain a substantial fraction of dietary flavonoids. They are a broad class of low molecular weight, ubiquitous plant metabolites, and are integral parts of the human diet. There are factors that determine whether a flavonoid will act as an antioxidant or as a modulator of enzyme activity; these biological properties include the nature and position of the substituents and the number of hydroxyl groups on the flavonoid. Flavonoids are mostly reported as being antioxidants that protect against oxidative stress due to their abilities to scavenge peroxyl radicals, effectively inhibiting lipid peroxidation, and by chelating redox-active metals, preventing catalytic breakdown of hydrogen peroxide. [21]

XI.CONCLUSION

ROS are unavoidable by products of normal cell metabolism. ROS are generated by electron transport activities of chloroplast, mitochondria, and plasma membrane a byproduct of various metabolic pathways localized in

different cellular compartments. Under normal growth condition, ROS production in various cell compartments is low [22]. Enhanced level of ROS causes oxidative damage to lipid, protein, and DNA leading to altered intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein crosslinking, inhibition of protein synthesis, DNA damage, ultimately resulting in cell death. In order to avoid the oxidative damage, living organisms possess a complex antioxidative defense system comprising of nonenzymatic and enzymatic components. Although rapid progress has been made in recent years, there are many uncertainties and gaps in our knowledge of ROS formation and their effect on plants mainly due to short half-life and high reactivity of ROS.

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