Review Article

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Oxidative Stress and Antioxidants

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ABSTRACT

The first reaction between food components and biological tissues occurs in the oral cavity. Saliva modulates the ecosystem in the oral cavity and plays a critical role in oral homeostasis. In addition, saliva is a first line of defense against free radical-mediated stress. Salivary antioxidant defense mechanisms seem to be very important. In this study we discuss in detail free radicals (FR) and reactive oxygen species, oxidative stress, oxidative stress and disease, antioxidants, factors influencing antioxidant efficacy and level, antioxidants and disease prevention, antioxidants and disease treatment, classification system and finally salivary antioxidants.

INTRODUCTION

Antioxidants are present in all body fluids and tissues and protect against endogenously formed free radicals, usually produced by leakage of the electron transport system. The nature and activity of antioxidants in body fluid have been extensively characterized.[1, 2] This review discusses in detail the oxidative stress and antioxidants because it is important for dental and oral researcher to identify and study the relation between dental and oral problems and antioxidants.

HISTORY

Plants, between 50 and 200 million years ago, produced many antioxidant pigments which evolved as chemical defenses against reactive oxygen species (ROS) produced during photosynthesis. In the late 19th and early 20th centuries, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fueling of internal combustion engines.[3, 4]

FREE RADICALS (FR) and REACTIVE OXYGEN SPECIES (ROS)

The reduction in ratio of molecular oxygen to water in biological systems is accompanied by a large free energy releasing and producing FR or ROS.[5–7] ROS, otherwise called "partially reduced oxygen products", are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can damage complex cellular molecules such as fats, proteins, or DNA. There are a few main sources of ROS in our body.[8] If the ROS are not inactivated, their high

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chemical reactivity can damage all types of cellular macromolecules susceptible to oxidation.

Lipids, proteins, carbohydrates and DNA are all capable of reacting with ROS and can produce various human disorders.[9]

ROS, in addition to superoxide anions and hydroxyl radicals, include oxygen-centered radicals of organic compounds (peroxyl, ROO, alkoxyl and RO) together with other non-radical reactive compounds such as hydrogen peroxide (H_2O_2) and singlet oxygen.[7,10]

ROS might be major contributors to the pathogenesis of several chronic degenerative diseases. In the last 2 decades, over 80 clinical conditions have been identified in which involvement of FR and ROS has been suggested. The most important FRs in biological systems are radical derivatives of oxygen (e.g., O2.-, OH., OOH., RO., ROO., RCOO., RCOOO., ArO. , ArOO., etc); many other FR/ROS exist: nitric oxide and nitric dioxide, thiol radicals, and carbon-centered radicals.

Oxidation is defined as a loss of electrons and therefore, an oxidant or an oxidizing agent is a substance that accepts electrons and causes another reactant to be oxidized. An antioxidant may be defined as a substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly prevents or delays a pro-oxidant initiated oxidation of the substrate.[11]

OXIDATIVE STRESS

Oxidative processes consist of normal cellular events and there is a balance in ROS production and antioxidant defenses in vivo. When concentrations of ROS exceed physiologic levels potential cellular damage might occur.[12]

Most cells can tolerate a mild degree of oxidative stress because they have sufficient antioxidant defense capacity and repair systems for recognizing and removing damaged molecules.[9]

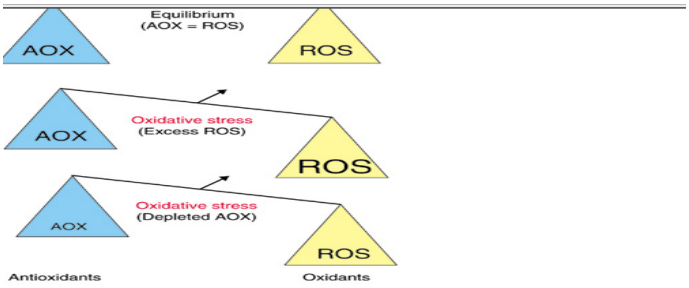

Fig.1: Oxidative stress and the balance between antioxidants and oxidants

Oxidative stress originates from internal and external sources: the internal sources include the action of enzymes, metabolism, and cells that generate oxygen radicals and other ROS; external sources include air pollutants, radiation and various type of foods such as oxidized fat.[13] Several conditions, including viral and bacterial infections, hyperthermia, ionizing and UV irradiation, and environmental pollutant can cause oxidative stress.[14]

OXIDATIVE STRESS AND DISEASE

Oxidative stress and toxic ROS have been shown to be involved in the etiology and pathogenesis of degenerative diseases, medical conditions and dental and oral problems,[6,15] such as heart disease, cancer, ageing, caries and periodontal disorders.[16,17] This data was suggested after many animal and epidemiological studies and clinical intervention findings.[18–21]

ANTIOXIDANTS

Antioxidants are found in every living cell and all biological species and scavenge reactive oxygen within cells. [22] Therefore, biological antioxidants in human diet, within intracellular antioxidants, and enzyme system protect against the potentially harmful effects of excessive oxidative stress and prevent various pathologic diseases. [22, 23] Antioxidants also maintain structural and tissue integrity. [10]

A highly complex antioxidant protection system protects and controls free radical formation in cells and organs of the body.[24] Antioxidants stabilize and deactivate free radicals before they attack cells.[25,26] Antioxidants are necessary for maintaining optimal cellular and systemic health.[27,28]

Lipids, proteins and DNA bases are destroyed during oxidant–antioxidant challenge, leading to many diseases.[29] Therefore, any compound that can prevent damage to lipids, proteins, DNA and other macromolecules may be defined as an antioxidant.[13]

Antioxidant defense system is a powerful and complex mechanism in the cells that limits free radicals produced from internal and external stressors by prevention of FR formation, oxidants removal, ROS transformation and deletion, membrane stabilization and removal of FR catalysts. [13, 21]

Some chemical elements, such as selenium, have no antioxidant action alone but are required for the activity of some antioxidant enzymes. Selenium is required for the synthesis of glutathione peroxidase; zinc and copper are the cofactors of SOD. Some, such as iron, aid antioxidant defense by preventing catalysis of FR formation. [30, 31]

FACTORS INFLUENCING ANTIOXIDANT EFFICACY AND LEVEL

The following factors influence antioxidant efficacy and level: production and amount of free radicals, destruction rate of free radicals, antioxidant potency and concentration, gene expression, dietary intake, smoking habits, physical activity, hormones, ageing, stress, antioxidant food intake, trace elements supplementation (selenium, zinc, copper etc), concentration of antioxidants, the environment and lifestyle conditions, proper function of other members of the antioxidant system and some other unknown factors.[6,30,32]

ANTIOXIDANTS AND DISEASE PREVENTION

Although oxidative stress leads to the onset and development of many degenerative and inflammatory diseases, nutrients and non-nutrient dietary constituents, such as vitamins C, E and carotenoids can affect pro-oxidant/antioxidant balance and prevention of certain degenerative disease.[6,33] Best protection depends not only on adequate intakes of vitamins C, E and carotenoids but also on achieving and maintaining the correct balance of fatty acids, vitamins A, B (B_6, B_{12}, and folic acid), trace elements (Zn, Cu, Mn, and Se) and non-essential nutrients.[6]

Several studies have shown that diets rich in fruits and vegetables provide protection against cardiovascular disease, several common type of cancer, and other chronic diseases. [34]

People who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases, and there is evidence that some types of vegetables, and fruits in general, protect against some cancers. Since fruits and vegetables are good sources of antioxidants, suggesting that antioxidants might prevent some types of diseases. [35, 36]

ANTIOXIDANTS AND DISEASE

Free radical attack on the oral mucosa leads to various alterations, ranging from infection to lethal cancer.[14] Antioxidant function and its deficiency can lead to many diseases such as diabetes, AIDS, ulcerative colitis, Crohn's disease, meningitis, CVD, colorectal, lung and breast cancer, coronary heart disease, cataract, and ageing.[34] Oxidative stress also is thought to contribute to the development of a wide range of diseases, including Alzheimer's disease,[37] Parkinson's disease,[38] the pathologies caused by diabetes,[39,40] rheumatoid arthritis,[41] neurodegeneration in motor neuron diseases,[42] and cardiovascular disease. Low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally in cardiovascular disease.[43]

ANTIOXIDANTS AND DISEASE TREATMENT

The brain might suffer from oxidative injury due to its high metabolic rate and elevated levels of polyunsaturated lipids and is the target of lipid peroxidation. Therefore, antioxidants are commonly used as medications to treat various forms of

brain injury. Superoxide dismutase and sodium thiopental and propofol are used to treat reperfusion injury and traumatic brain injury.[44,45]

Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. [46, 47]

CLASSIFICATION AND TYPE OF ANTIOXIDANTS

There are different classification systems for various groups of antioxidants, all of which are presented in Table 1. [5, 13, 30, 48] Among them the last classification that is based on the way they act is the best one:

A) Preventive antioxidants; they suppress the formation of FR (e.g. SOD, CAT, GSHPx and S-transferse, carotenoids, transferin, albumin, haptoglubin, and caeruloplasmin)

B) Radical-scavenging; antioxidants that scavenge radicals to inhibit chain initiation and break chain propagation (e.g. albumin, bilirubin, carotenoids, ubiquinol, uric acid, vitamins A, C, and E)

C) Repair and "de novo" enzymes that repair the damage and reconstitute membranes (DNA repair enzymes, lipase, protease, transferase) and other reactants. [7]

Examples of enzymatic antioxidants are catalase, glutathion peroxidase, glutathione redutase, superoxide dismutase, peroxiredoxins, peroxidase, and thioredoxin. [5]

Examples of non-enzymatic or molecular antioxidants include vitamins E, C, and A, melatonin, uric acid (UA) and glutathione.

Table 1: Different classifications of antioxidants

Classification 1	Classification 2	Classification 3	Classification 4	Classification 5	Classification 6
endogenous in origin	enzymes,	enzymatic	protection at cellular level (mainly by enzymes)	soluble in water (hydrophilic)	Primary or preventive a.o.
exogenous in origin	small molecules that are synthesized in human body other molecules derived from the diet	non-enzymatic	protection at cellular level (mainly by enzymes)	soluble in lipids (hydrophobic)	secondary scavenging or chain breaking repair system

TOCOPHEROLS AND TOCOTRIENOLS (VITAMIN E) ALSO HAVE PRO-OXIDANT ACTIVITIES

In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma [ascorbic acid (vitamin C), glutathione, lipoic acid, uric acid], whereas lipid-soluble antioxidants protect cell membranes from lipid peroxidation [carotenes, α-tocopherol (vitamin E), ubiquinol (coenzyme Q]. [49] It is important to note that various metabolites and enzyme systems have synergistic and interdependent effects on one another.[50]

SALIVARY ANTIOXIDANTS

Saliva is a complex fluid in the oral cavity, composed of a mixture of secretary products from the major and minor salivary glands. [51] Saliva has multifunctional roles in the oral cavity, [52] and is very important for maintaining oral health. [53, 54] Therefore, the saliva research field is rapidly advancing. [55]

About 99% of saliva is water.[27,51,54–57] The remaining 1% is a complex of organic and inorganic molecules, such as electrolytes, mucins, antiseptics, immunoglobulins, proteins and various enzymes.[58] Although the main component of saliva is water, it plays key roles in lubrication, mastication, taste perception, prevention of oral infection, and tooth decay.[29,51,59,60]

Saliva has various defense mechanisms such as immunological and enzymatic defense systems against bacteria, viruses, fungi, protection of mucosa; it also promotes healing.[23,61] One of the important defense mechanisms is antioxidant system.[61] Antioxidants have many health benefits that make their evaluation in disease process very popular.[62]

The first reaction between food components and biological tissues occurs in the mouth.[60] Saliva modulates the ecosystem in the oral cavity and plays a critical role in oral homeostasis; saliva is also the first line of defense against free radical-mediated stress.[10,52] Saliva antioxidant defense mechanisms seem to be very important. There are few studies on the relationship between antioxidants of saliva with dental, gingival and oral diseases.

Saliva is the first biological medium confronted by external materials that are taken into our bodies as part of food, drink, or inhaled volatile ingredients.[63] However, saliva has received less attention and research remains limited. Whole saliva is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluids released from salivary glands, of which the parotid, submandibular and sublingual are the three major sources.[64]

All of the enzymatic and molecular antioxidants are present in saliva in variable amounts. Among them the most important are UA, vitamin C, albumin and prox enzymes. The water-soluble UA is the major antioxidant of saliva (70% of salivary TAC) while vitamin C has a secondary role. The lipid-soluble antioxidants transported by proteins are found in low concentrations in saliva (10% of salivary TAC).[65]

Uric acid concentration in saliva is similar to that in serum.[63] The peroxidase found in the oral cavity is a very important salivary antioxidant enzyme.[66] This oral peroxidase (OPO) is composed of two peroxidase enzymes, salivary peroxidase (SPO) and myeloperoxidase (MPO). The SPO secreted from the major salivary glands, mainly the parotid gland, [66] contributes 80% of OPO activity, while MPO, produced by leukocytes in inflammatory regions of the oral cavity contributes the remaining 20% of OPO activity.[67–69] Despite the importance of peroxidase in saliva, it accounts for only 0.01% of the total salivary proteins. OPO plays a dual role: (1) it reduces the level of hydrogen peroxide (H_2O_2) excreted into the oral cavity from the salivary glands by bacteria and by leukocytes, and (2) it increases specific antibacterial activity by inhibiting the metabolism and proliferation of various bacteria in the oral cavity.[70]

However, in contrast to the oral antibacterial characteristics of OPO, which have been studied thoroughly, the possible anticarcinogenic role of OPO against the most prevalent and lethal cancer of the oral cavity, squamous cell carcinoma (SCC), has scarcely been mentioned and never investigated. Antioxidant production is higher in stimulated saliva than in the resting state.[25]

Stimulated saliva contains a lower concentration of antioxidants but when flow rates are taken into account, antioxidant capacity is higher than in unstimulated saliva.[70] When conducting analysis of saliva for antioxidants, whole saliva is more relevant as it contains gingival crevicular fluid, immune cells and tissue metabolites.[71] Unstimulated flow represents the major intra-oral condition, which would provide a more accurate account of the oral environment and saliva antioxidant composition for analysis.[72]

SUMMARY

Salivary antioxidant systems have very important roles in the oral cavity. They have many health benefits on oral, gingival and dental health. Many different types of salivary antioxidants have different mechanisms in prevention and also progression of many inflammatory and degenerative diseases in the oral cavity. More studies are required in this field to find the effect of antioxidants on disease and also disease on antioxidants to find out if dietary rich antioxidants have preventive effect on oral common problems, such as periodontal disease and tooth decay, or not.

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