LITERATURE REVIEW
MASTERS STUDIES AT DENTAL MEDICINE

EFFECTS OF ANTIOXIDANTS IN ORAL BIOCHEMISTRY

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List of abbreviations.

ROS	reactive oxygen species
DNA	deoxyribonucleic acid
GSH	glutathione
BHA	butylatedhydroxyanisole
BHT	butylatedhydroxytoluene
SOD	superoxide dismutase
AT	alfa-tocopherol
OSMF	oral submucous fibrosis
MDA	malondialdehyde
OSCC	oral squamous cell carcinoma
PEG	polyethylene glycol
CAT	catalase
POD	peroxidase
LMWA	low molecular weight antioxidants
OS	oxidative stress
GCF	gingival crevicular fluid
TBHQ	*tert*-Butylhydroquinone
PG	propylgallate

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Abstract

A paradox in metabolism is that, while the majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (ROS). Thus, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, lipids, and proteins. Maintaining a good balance of oxidants and antioxidants is important for oral and systemic health. Lots of factors can lead to a misbalance of oxidants in oral tissues, such as alcohol, nicotine, pollutants and general stress. It causes an oxidative stress and may damage or kill cells by overly reactive oxygen-containing molecules and chronic excessive inflammation.

Pernicious impacts of ROS are balanced by non-enzymatic and enzymatic antioxidants. Exhausted antioxidant defense mechanisms bringing to oxidative damage to normal tissues and cells, and the breakdown of the fragile balance between oxidants/antioxidants is a cause of carcinogenesis.

In this work, it will be reviewed the processes that lead to the oxidative/antioxidant imbalance, the role of preventing antioxidants and their sources, antioxidants as food additives. The importance of saliva will be highlighted, since it plays an important role in this process and it is a fluid where many biochemical processes are taking place - the process of mastication and digestion of ingested foods promotes a variety of reactions, including lipid peroxidation. It acts as a cleansing solution, an ion reservoir, a lubricant and a buffer. It could constitute a first line of defense against free radical-mediated oxidative stress.

Keywords: antioxidants, antioxidants and oral cavity, oral biochemistry and antioxidants, antioxidative status, antioxidant activity.

Introduction

Nowadays, antioxidants are getting more and more attention as potential preventers of cell damage by free radicals. In fact, it is known that antioxidants may counteract the effects of free radicals in every part of the body. [1]

Free radicals are extremely reactive chemicals that are formed in normal metabolism or are produced by environmental stress and/or radiation. Lipid peroxidation is also a source of free radicals. These chemicals react with cell components, leading to damages and, consequently, mutations in deoxyribonucleic acid (DNA), and chemical modifications in cell lipids and proteins. Both free radicals and antioxidants are produced naturally by the body in order to assist it to stay in a balanced state of health. [2]

In addition, there is a restriction according to the body`s innate supply of antioxidants, and cells are being under constant destruction every day. In this context, there are so-called dietary antioxidants, which are contained in fruits and vegetables or vitamin supplements - they are believed to promote health and to avert cell damage due to the enrichment of the pool of antioxidants that body has. [3]

The main dietary antioxidants are vitamin E, vitamin C and beta carotene. Vitamin E (tocopherol) is a fat-soluble antioxidant. No unequivocal unique function for it has been determined. Though it does act as a lipid-soluble antioxidant in cell membranes, many of its functions can be provided by other synthetic antioxidants. Another vitamin – C, ascorbate, operates in the water-soluble part of tissues. Good sources of vitamin C are strawberries, broccoli and citrus fruits. This vitamin helps to recover levels of active vitamin E in the body. A predecessor of vitamin A, beta-carotene can be found in sweet potatoes, carrot juice and apricots. [2]

Antioxidants tend to work together since the combination of them is more powerful than each substance alone. Vitamin E and Vitamin A (beta-carotene) have been linked to a preventive role in oral cancer. Due to the low oxygen tensions prevalent in the body, beta-carotene is a very effective antioxidant and may reduce the risk of those cancers initiated by free radicals and other strong oxidants. [2]

As was mentioned above, antioxidants include also enzymes. Superoxide dismutase is one of them. [2]

Perspective and prospects.

Proof for the advantageous role of antioxidants in human (and specifically oral health) is restricted. It is not yet fully defined what is the ideal level of antioxidants to avert the damaging effects of free radicals. Also, acceptable levels of vitamins may noticeably vary from person to person, depending on levels of smoking, environmental stress, the ability to absorb the vitamin supplements etc. In addition, it is not clear how antioxidants may influence the prevention of oral cancer and if they truly help to prevent it. Considering antioxidant supplements, this is also controversial, as some scientists believe that they may intervene with the body's natural output of antioxidants, while others defend that our pool of antioxidants is sufficient in the majority of the situations. [4]

Methods

To accomplish this review article a research of the literature was performed, using on-line databases such as Pubmed (http://www.ncbi.nlm.nih.gov/pubmed), Science Direct (http://www.sciencedirect.com), Google Scholar (scholar.google.com), Elsevier (http://www.elsevier.com). Different combinations of the following keywords were used: antioxidants, antioxidants and oral cavity, oral biochemistry and antioxidants, antioxidative status, antioxidant activity. Some limitations ocured due to the fact that some of the articles required a payed access.

Oxidative stress

Reactive oxygen species (ROS) contain free radicals and non-radical by-products of oxygen. Free radicals are molecules or molecular fragments that present an unpaired electron in the valence shell (that`s radical) and capable of existing freely. [1]

When the generation of reactive oxygen species in a system outreaches the system`s capacity to counteract and remove them, it causes the condition known as oxidative stress, defined by Sies. There are several reasons of why this imbalance can occur. It can be caused from an absence of antioxidant capacity that appears by disruption in distribution, production or by an excess of ROS from endogenous origin or environmental stressors. Possible endogenous sources include cytochrome P450 metabolism, mitochondria, inflammatory cell activation and peroxisomes. [1]

In the case it is not regulated correctly, ROS can harm cellular proteins, lipids or DNA, like a suppressing signal transduction pathway, and, moreover, normal cellular operation. ROS are by-products of cellular metabolism, produced by electron outflow of enzymes and mitochondrial electron carriers during oxidative phosphorylation or other metabolic events. They are generated as necessary intermediates of metal catalyzed oxidation reactions. Furthermore, other cellular enzymes or enzyme complexes situated in or connected with cellular membranes or organelles were characterized for the production of ROS in nonphagocytic and phagocytic cells. Distinctive outer sources for ROS are redox cycling xenobiotics likewise radiation (for example, ultraviolet light, microwave radiation), environmental agents such as non-genotoxic carcinogens. A diversity of growth factors and cytokines are noted to produce ROS, which via oxidative modification of proteins and/or via alteration of the intracellular redox state induce their transcription factors and mechanisms of operation on signaling components. [5, 6, 7]

Antioxidants and carcinogenesis

Cancer is a result of cell and tissue damage, which may be caused by unsteadiness between antioxidant defense mechanisms and oxidants.
A larger number of free radicals are generated under pathological conditions. [1]

A first line of defense against ROS is defense against their formation, so-called prevention. Metal chelation plays a major role in controlling DNA fragmentation and lipid peroxidation. A scenario of preventive antioxidants as well function by channeling an attacking species into a less harmful product therefore lowering the risk of further damage. The following phase is 4,interception, the act of final deactivation. In this phase would come the approach that will prevent chain reaction of free radicals and formation of non-reactive-end and non-radical products. One more allowance is to switch the direction of radical action from more sensitive target sites to cellular compartments in which oxidative loss would be less harmful. Those intermitting chain-breaking antioxidants are often phenolic compounds. The most effective compound in lipid phase without any doubt is alpha-tocopherol, a non-enzymatic antioxidant. It controls a steady state of peroxyl-radical reduction in the cellular membranes. Carotenoids and oxy-carotenoids can conveniently intercept hydroxyl radical. Valuable detoxifying compounds are three main classes of antioxidant enzymes: superoxide dismutase, catalases and glutathione (GSH) peroxidases. The next step in protection against oxidants can be repair of damage caused by oxidants (lipid peroxidation). [5]

Lipid peroxidation

Peroxidation (oxidation) of lipids is important not only for degradation of foods (rancidity) but also for damages to tissues in vivo, where it can be a reason of cancer, atherosclerosis, aging and inflamatory deseases. The harmful effects are considered to be induced by free radicals (ROO*, RO*, OH*) produced during peroxide decomposition from fatty acids containing methylene-interrupted double bounds. These double bounds are found in the naturally occuring polyunsaturated fatty acids. [1, 5, 6, 8]

Lipid peroxidation is a chain reaction providing an endless supply of free radicals that begin additional peroxidation reactions.
Below it is described as a process:

1) Initiation:

$ROOH + Metal^{(n)+} \rightarrow ROO^* + Metal^{(n-1)+} + H^+$
$X^* + RH \rightarrow R^* + XH$

2) Propagation

$R^* + O_2 \rightarrow ROO^*$
$ROO^* + RH \rightarrow ROOH + R^*$, etc

3) Termination

$ROO^* + ROO^* \rightarrow ROOR + O_2$
$ROO^* + R^* \rightarrow ROOR$
$R^* + R \rightarrow RR$

Lipid peroxidation is a chain reaction with potentionally devastating effects since the molecular predecessor for the initiation process is commonly the hydroperoxide product ROOH. Antioxidants control and reduce lipid peroxidation. In this context, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate are antioxidants used as food additives. [1, 5, 6, 8, 9]

There are also some important naturally existent antioxidants such as vitamin E (tocopherol), which is lipid-soluble, vitamin C and urate, which are water-soluble. Beta-carotene is an antioxidant particularly effective at low pO_2.
Globally, the antioxidants can be divided in two classes: [1, 5, 6, 8]

(1) Chain-breaking antioxidants, which intervene in the propagation steps.
(2) Preventive antioxidants, which reduce the rate of chain-breaking initiation.

Chain-breaking antioxidants

The basic chain-breaking antioxidant is superoxide dismutase. It functions in water phase (it is a water-soluble enzyme) to trap superoxide free radicals (O_2*); and Vitamin E, which acts in the lipid phase to trap ROO* radical. [11]

When the enzyme superoxide dismutase (SOD) was discovered in 1969, by Joe McCord and Irwin Fridovich, a new stage in the comprehension of oxidative processes in biological systems began. [11]

Superoxide dismutase (SOD) is an enzyme, known as well as orgotein. It is broadly present in microorganism, animal and plant. Some of the important functions that are performed by SOD include neutralization of the reactive oxygen species (ROS), intervening with the damage produced by the hydrogen peroxide (H_2O_2), and reimbursing the loss made by these ROS. Thus, SOD is not only a primary defense against free radical species, but also avert and restrain the collapse of living organisms. Recently, it was shown that SOD is a primarily protective bioenzyme, it can stand the superoxide free radicals effectively, prolong life range, and control the metabolism of live cells, raise up the organism immune function. [8, 12]

Function of SOD

Initially, SOD was detected in bovine erythrocytes, which were normally extracted from the blood samples of cattle. Due to the SOD's antigenic heterogeneity, and because it is very prone do denaturation, WHO (World Health Organization) declared that the utility of SOD obtained from animal materials should be stopped. Nowadays, many methods have been developed to extract SOD from plant or from bacteria transferred with the corresponding plasmid. [13]

SOD catalyzes the transformation of superoxide to oxygen and hydrogen peroxide. The biochemical reaction is summarized below: [13]

$$M^{3+} + O_2^- \rightarrow M^{2+} + O_2$$

$$M^{2+} + O_2^- + 2H^+ \rightarrow M^{3+} + H_2O_2$$

Certain cellular processes generate O_2^-, so-called superoxide anion free radical. It is one of the transitional products in natural physiological reactions of living cells. This active oxygen species is an important factor of

biological oxygen toxicity and presents a high potential of oxidation. Besides of O_2^-, the superoxide radical, hydroxyl radical, semiquinone radical, alkoxyl radical, hydrogen peroxide (H_2O_2), polyunsaturated fatty acid radical, singlet oxygen $^1O_2^-$, nitrite (NO_2^-) and nitric oxide (NO) are other important and potentially deleterious oxygen derivatives. These radicals have the relative ability of toxicity and oxidation to living cells, even though some of them do not have a single electron. They are degrading and oxidizing biological significant molecules that can react and degrade biomolecules, such as lipids and proteins. [8, 14]

SOD can convert these oxide radicals into hydrogen peroxide. Though the hydrogen peroxide has potential harmful biological activities, it can be fast decomposed into nontoxic products like H_2O and O_2 by catalase and peroxidase that are extensively distributed in living cells. In this way, a complete anti-oxide chain is composed by SOD, CAT (catalase) and POD (peroxidase) in living organisms.[14]

Oral cancer preventers (preventive antioxidants)

Vitamin E

The term vitamin E refers as a mixture of 8 related compounds, which are alpha-, beta-, delta-, and gamma-tocopherol and alpha-, beta-, delta-, and gamma-tocotrienol. 90 percent of vitamin E present in human tissues is in the form of the natural isomer, alfa-tocopherol. Commonly, tocopherols consists of three isoprene units; they have a substituted chromanone nucleus, with a polyisoprenoid side chain of variable length. [8, 10, 15, 16, 17]

Vitamine E functions as a physiological antioxidant, principally a membrane antioxidant, due to the fact that it interacts and inserts in the membrane lipid bilayer. It is the most copious natural antioxidant and, since it is lipid soluble, it is also related with all lipid-containing structures: fat deposits, lipoproteins and membranes, protecting cells from oxidative damage. There is no specific transport protein for it; it is engrossed from the diet with other lipid components. Also, the vitamin E is bounded with lipoproteins in the circulation. [8, 10, 15, 16, 17]

As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state. [8, 10, 15, 16, 17]

Alpha-tocopherol

The most common and most active form of vitamin E is alpha-tocopherol (AT), which is found in margarine, different kinds of oil, dressings and green leafy vegetables, nuts, among others. AT is the only form of vitamin E which is able to meet human ration needs. [7]

AT is stored in the liver and adipose tissue and is excreted primarily in the feces. Vitamin E supplements were associated with a diminished risk for oral and pharyngeal cancer, as well as risk of second primary cancers in head and neck area. [7]

Gamma-tocopherol

Despite the focus on alpha-tocopherol in the medical literature, gamma-tocopherol is the most copious form of vitamin E in the human diet, accounting for about 70% of vitamin E intake. [2, 16]

Gamma-tocopherol has a valuable physiologic function, including the inhibition of platelet aggregation, protecting DNA from oxidative damage, and increasing the activity of superoxide dismutase (SOD), a class of enzymes that comprise one of the body's endogenous antioxidant systems (SOD is referred to as a system because there are many forms of this class of enzymes, all of which catalyze the conversion of superoxide into oxygen and hydrogen peroxide). [2, 16]

Cell and animal studies support that the alpha- and gamma-tocopherol forms of vitamin E may play a role in cancer prevention by regulating cell growth and reducing the risk of neurodegenerative diseases (e.g., Alzheimer's disease) by providing antioxidant neuroprotection. [2, 16]

Vitamin A

Vitamin A belongs to a group of fat-soluble vitamins, which are not easily absorbed or extracted from the diet as water-soluble ones. Fat-soluble vitamins are sufficiently reserved and stored in tissues. Vitamin A also functions as a hormone. [2, 15]

The term vitamin A is usually used to refer to three compounds, namely, retinol, retinal and retinoic acid, plus other chemical forms that are converted into retinol within the body (carotenoids). Retinol is called preformed vitamin A, and the carotenoids, of which beta-carotene is the most significant, are called provitamin A. Beta-carotene is turned to all-trans retinal by the activity of beta-carotene dioxygenase in the small bowel. Onward metabolism in the enterocytes produces retinol and retinoic acid. Afterwards it is transported to the liver where vitamin A is guarded as retinol palmitate, which covers more or less one year`s reserve. Dietary sources of retinol are

primarily animal products and include milk, butter, egg, liver and fish. Carotenoids are found in plant-derived foods. [2, 15]

Beta-carotene

Not long time ago, the role of beta-carotene as an antioxidant was actively discussed. Because of the fact that normal epithelial cell growth and differentiation relies on retinoids, and lots of human carcinomas (even those in oral cavity) emerge from epithelial cells, it has been suggested that vitamin A may be an important defense against these diseases. Nevertheless, no direct relationship was already established and antioxidants are not the 'magic bullet' for the treatment of premalignant lesions or the prevention of second primary malignancies. [2, 15]

Vitamin C

Vitamin C or ascorbic acid is a water-soluble antioxidant that is one of the main dietary antioxidants. It is a six-carbon compound chemically related to glucose. The basic biological role of it is as a decreasing agent for the hydroxylation of lysine and proline in protocollagen. It spares vitamin A, vitamin E, and some B vitamins by protecting them from oxidation. Vitamin C turns up to be a biologically important antioxidant. The National Research Council has established that adequate amounts (RDA levels) of antioxidants such as beta-carotene and vitamin C in the diet reduce the risk of cancer. [15, 18]

The chemical name of ascorbic acid is 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol and it subsists in two main dietary forms: L-ascorbic acid, a reduced form and dehydroascorbic acid (DHA), an oxidized form. In the intestine, ascorbic acid is quickly absorbed by active transport. Its major elimination pathway is through urine. [15, 18]

Antioxidant ascorbic acid

Ascorbic acid is a powerful antioxidant or reducing agent that is able to gather free radicals of reactive oxygen and nitrogen species (ROS and RNS) that have possibility to damage nucleic acids and contribute to carcinogenesis. The ascorbate reacts with ROS, extinguishes them and gets transformed into scantily reactive semi-hydroascorbate radical. Thus, ascorbate potentially reduces the *in vivo* damage to proto-oncogenes and tumour suppressor genes, in this way diminishing the risk of cancer by suppressing the oxidative stress caused by reactive free radicals. [19]

Pro-oxidant ascorbic acid

Ascorbic acid represents a double faced character in that it may present a pro-oxidant activity originated from its routine antioxidant feature. Besides diminishing oxidation, it also decreases metal ions like Fe^{3+} and Cu^{3+}, the process during which free radicals are formed. The so-called Fenton reaction in which it is generated highly reactive free radicals in the presence of transition metal ions is written below:

$$2Fe^{3+} + \text{Ascorbate} \rightarrow 2Fe^{2+} + \text{DHA}$$

$$2Fe^{2+} + H_2O_2 \rightarrow 2Fe^{3+} + 2OH^- + 2OH^*$$

These hydroxyl radicals will react with DNA causing its damage by producing breaks in phosphodiester backbone and chemical changes in the DNA bases. This feature of pro-oxidant activity inducing cytotoxicity has been applied in many studies in the prevention and treatment of cancers and it is suggested to be dose-dependent. In addition, there are actual disputes questioning the activity of ascorbic acid, if it is advantageous in the treatment of tumours or counteracts the oxidative stress caused by regular therapeutics by providing antioxidant defense to tumour cells. [19, 20]

Oxidative-antioxidant imbalance

Promotion of oral cancer

Depleted antioxidative defense mechanisms result in oxidative-antioxidant imbalance. Reduced activities of antioxidants with accompanying increased levels of oxidative stress have been linked to different cancers including those from head and neck. In line with this, some researchers have observed lowered antioxidants or antioxidant capacity in blood and tissues of oral cancer and precancer. The explanation can be as the following:

1. Increased utilization of antioxidants to compile ROS/RNS,
2. Lean antioxidant defense system in cancerous environment,
3. Insufficient production of antioxidant enzymes,
4. Magnified extermination of antioxidants by reactive oxygen metabolites.

Thus, it has been hypothesized that one of the probable mechanisms serving in the promotion of oral cancer can be lowered capacity to defense ROS/RNS. [1]

Cancer inhibitory actions of antioxidants

Enzymic and nonenzymic antioxidant systems work synergistically with each other to protect cells and organ systems against free radical loss and consequently cancer. Cancer's inhibitory actions of antioxidants are established on:

1. Depression of tumor angiogenesis activity
2. Stimulation of cell differentiation
3. Molecular genetics pathway
4. Immune mechanisms.

In the cancer model it has been indicated that alpha-tocopherol and beta-carotene promote the migration of cytokine-laden macrophages and lymphocytes to the sites of upcoming squamous cell carcinoma. The activity of langerhans cells in carcinogenesis model was found to be stimulated by antioxidant nutrients. They function through induction of cancer suppressor genes, like a wild-type p53 and reduced expression or de-regulation of oncogenes such as H-ras and mutant p53. Antioxidant micronutrients inhibit angiogenesis in tumors by suppressing TGFalpha. Retinoids promote cellular differentiation with resultant apoptosis of neoplastic cells. [1]

Oral cancer develops from precancerous conditions and lesions. If the treatment acted at the beginning of disease than it can result in a high cure rate. Antioxidants have the possibility to avert, inhibit and inverse some of the multiple steps involved in oral carcinogenesis. [21]

So, what is the role of antioxidants in malignant lesions? There are several studies that observed that an oral administration of beta-carotene and vitamin E by patients suffering from oral submucous fibrosis (OSMF) leaded to a decrease in MDA (Malondialdehyde) level alone with clinical improvement of health status. Reports about clinical improvement in OSMF patients after giving retinol, vitamin E, D, and B complex and some minerals were published as well. Alpha-tocopherol seems also to play a role in defense against the harm caused by radiation in OSCC patients treated with radiotherapy. [22]

Antioxidants have also been used to raise the efficiency of cancer treatment by radiotherapy and chemotherapy. Indeed, in oral cancers (which are more solid tumors) where chemo and radiotherapy serve as supplementary therapies, there are evidence that supports that antioxidants do not contradict with the use of these therapies, moreover, they could help in reducing the side effects related to treatment. [22]

Finally, it is important to mention that whereas antioxidant defenses are significant, antioxidant therapy against free radicals should be consumed with prudence as long as its effects rely on the stage at which it is set. It might in

fact induce growth of tumors through raised survival of tumor cells. Also, a pro-oxidant action of some antioxidants should be taken into consideration. It can occur depending on the concentration and environment (oxygen pressure) in which they function. The main area of interest right now is focused on possible anticancer activity of approximately non-toxic antioxidant nutrients like alpha tocopherol, beta-carotenoid and different retinoids. [22]

Antioxidants used as food additives

Beside dietary antioxidants there is a group of antioxidants used as food additives. These may contribute to the increase in the conservation of food, as well as to an enrichment of their nutritional value.

Propyl Gallate

Propyl Gallate is a white to brown crystalline powder with a lightly sour taste and no smell. It is the n-propyl ester of gallic acid (3,4,5-trihydroxybenzoic acid). It is soluble in ethyl ether, ethanol, lard, oil, and water solutions of polyethylene glycol (PEG) ethers of cetyl alcohol, but only lightly dissoluble in water. [23]

Propyl Gallate is generally recognized as a safe (GRAS, American Food and Drug Administration) antioxidant to protect oils, fats, and fat-containing food from rankness that outcomes from the production of peroxides. [23]

Propyl Gallate is consumed when ingested, then methylated, binded, and secreted in the urine. The biological action of Propyl Gallate is steady with its free-radical scavenging capacity**,** with results that involve antimicrobial activity**,** inhibition of the formation of nitrosamines, chemoprotection, enzyme inhibition, inhibition of biosynthetic processes, anesthesia, ionizing/ultraviolet (UV) radiation protection, inhibition of neuromuscular response to chemicals, anticarcinogenesis, antimutagenesis and antitumorigenesis, anticariogenesis, antiteratogenesis. The antioxidant activity is based in its hydrogen-donating hydroxyl groups.

Other names for this ingredient include:

1. PG(Windholz1976),
2. Tenox PG (Windholz 1976),
3. n-Propylgallate(Windholz1976),
4. ProgallinP(Windholz1976),
5. Propyl gallate (RIFM) (Gottschalck and McEwen 2004),
6. Gallicacidpropylester(RTECS2004),

7. 3,4,5-Trihydroxybenzoicacidpropylester (Gottschalck and McEwen 2004).

Propyl Gallate is know to be stable in slightly acidic or neutral environment, however, in mild alcaline or when heated it becomes unstable.[23]

Antioxidant-Related Effects

Propyl Gallate rivals efficiently with trypsin and oxygen for reaction with the eosin triplet state and by this process inhibiting eosin-sensitized photodynamic oxidation of trypsin. It was observed that propyl Gallate decreased the excited eosin to form an oxidized Propyl Gallate form and a semireduced eosin radical. Afterwards, ground state eosin and Propyl Gallate were regenerated using the reverse electron transfer. [4, 9, 23, 24, 25]

Photodynamic activation occurred with the formation of a free radical, and Propyl Gallate acted by inhibiting free-radical formation. [4, 9, 23, 24, 25]

Butylated hydroxyanisole (BHA)

From one of the widely used food antioxidants, it can be pointed out the Butylated hydroxyanisole (BHA; tertiary butyl-4- hydroxyansole). Trading BHA is indeed a composition of two isomers, 3-tert- butyl-4-hydroxanisole (3-BHA, 90%) and 2-tert-butyl-4-hydroxyanisole (2-BHA, 10%). Butylated hydroxytoluene (BTH) is not soluble in water, however, it is highly soluble in oils and fats. The vapor of the BHA is fugacious at frying temperatures; BHA has low melting point. The tert-butyl group ortho or meta to the hydroxyl group suppresses antioxidant activity, that`s why BHA is a “hindered” phenol. The substance has a white color and presents as a waxy solid. The admixture of BHA causes no change in flavor, color or smell. If it will be mixed with the other antioxidants (for example, BHT- Butylated Hydroxytoluene, TBHQ - *tert*-Butylhydroquinone, PG - Propyl gallate) the effect of the substance will increase and the following synergism lead to the considerable antioxidant potency higher than that may be expected from the contribution of each individual antioxidant. [4, 9, 23, 24, 25]

Butylated Hydroxytoluene (BHT)

Butylated hydroxytoluene (BHT; di-tert-butyl-4-methylphenol or 2,6-di-tert-butyl-p-cresol 2,6) is a white crystalline solid with a light phenolic odor. BHT is extremely soluble in oils and fats but not water as well as in propylene glycol. The substance is slightly soluble in glyceryl monooleate, a solvent used for comercial antioxidant formulations. Nevertheless, BHT has lower

effectiveness than BHA. This happens because of the existance of two tert-butyl groups, which suggest better steric hindrance than BHA. The molecule has less carry-through properties and is more fugacious than BHA. The supplementation of BHT causes no changes in odor, flavor or color of a food product and may be utilized in combination with propyl gallate, TBHQ or BHA. [4, 9, 23]

Synergism between BHA and BHT

The effects of antioxidants when they are used in a mixture are usually better than when they are used alone. Synergism may occur because the antioxidants may act as hydrogen donors to the primary antioxidant radicals. Thus, due to the recover of the primary inactivate metal ions or the primary antioxidant, they counteract their prooxidant effects. It was noted that 3-BHA donated a hydrogen atom to peroxy radical to form a phenoxy radical. 3-BHA is being regenerated from the phenoxy radical by a transfer of hydrogen from BHT. BHT is oxidized to quinone methide during the process. A combination of BHT and 3-BHA exibited a higher antioxidant activity than any of them used singly in lard, soybean oil and methyl oleate. [4, 9, 23]

Saliva

As it was mentioned above, saliva is an oral liquid where many biochemical processes take place. Saliva is produced in major and minor salivary glands. Saliva is made up of more than 98% water, a variety of different electrolytes, over a thousand proteins, including the major glycoprotein mucin, nucleic acids, immunoglobulins, plasma-derived-albumin, hormones, the nitrogenous products urea and ammonia, digestive enzymes such as alpha- amylase, lysozyme, among others. [3, 26, 27]

Saliva has different actions – especially implicated in lubrication, physicochemical defense, buffering effect, sustenance of tooth continuity, antimicrobial protection and wound cure, taste and, moreover, taking part in an early digestion process. Saliva itself is significant in biofilm formation on tooth surfaces, bacterial adhesion, crystal growth homeostasis, may be a considerable source of information for genetic and forensic profiles and keeps mucosal continuity of the oral and upper gastrointestinal mucosal surfaces. [3, 26, 27]

Saliva includes regionally produced substances and other molecules obtained from the systemic circulation, like serum products, electrolytes, gingival crevicular fluid (GCF), microorganisms and other external agents. Interestingly, even markers for immunological, toxicological, hormonal,

infectious diseases can also be found in saliva. Thus, saliva can be an alternative diagnostic tool for checking the oral and systemic health. [3, 26, 27]

Saliva is reckoned to be the first line of protection against oxidative stress (OS), the key event for many systemic and oral diseases. It is needed to be admitted that saliva is a rich source of endogenous antioxidants: albumin, enzymes, uric acid, ascorbate. All of them contribute to the antioxidant potential of saliva. [3, 26, 27]

In general, normal saliva contains a diversity of oxidants which might change the redox status and the integrity of oral structures, due to the fact that oral cavity is a portal of entry for colonizing microorganisms, xenobiotics and nutrients. [3, 26, 27]

Potentional biomarkers of oral cancer

Oxidants as biomarkers in oral cancer and precancer

Discovering of ROS/RNS in biologic systems is frequently problematic. Their half-life is very short (seconds) and there are effective systems to scavenge them. [1]

At cellular level specific ROS can be personally evaluated in tissue or quantification of the oxidative damage of biomolecules in blood, urine or saliva is another option of gauging these biomarkers. Stable, specific, or non-specific derivatives of these substances can be measured, such as amino acid oxidation products (meta-tyrosine, ortho-tyrosine, hydroxyl-Leu, dityrosine), lipid peroxidation products (isoprostanes), peptide oxidation products (oxidized glutathione). Changes in their color, luminescence or fluorescence are making possible to measure these end products. Long-term follow-up studies of these biomarkers can lead to a better comprehension of the mechanisms of oral carcinogenesis as well as to develop new therapeutic approaches.[1]

The biomarkers of oral squamous cell carcinoma were studied. There are several big groups of them, including cellular ones, humoral, and in vivo-specific. [28]

Conclusion

This work reviewed the published information about biochemical processes, which take place in oral cavity and are related to oxidative reaction in the organism. All these structures are connected by the oral liquid, saliva. Oxygen, which human body uses as a source of living, can cause damages in the tissues, leading to different pathological processes. Free radicals produced by oxidative stress are responsible for this, and antioxidant compounds control and reduce the harmful effects caused by them. The protection is achieved by the sequestration of transitional metal ions into complexes, quenching or scavenging free radicals and other ROS and RNS, breaking chain reactions and restoring damaged molecules.

In this work it was discussed naturally subsistent exogenous antioxidants, which include vitamin C, A and E, as well as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate as food additives. Among endogenous antioxidants, it was highlighted the role of the enzyme superoxide dismutase.

Antioxidants serve as preventers of carcinogenesis. But if the antioxidant treatment is started in the beginning of the disease, there is a higher chance of recovering, because antioxidants have the possibility to avert, inhibit and inverse some of the multiple steps involved in oral carcinogenesis.

Finally, antioxidants and oxidized molecules may serve as biomarkers of oral cancer and precancer, and saliva may be used, in the future, as an important diagnostic tool for their assessment.

References.

1. Choudhari S. K., Chaudhary M., Gadbail A. R., Sharma A., Tekade S. Oxidative and Antioxidative Mechanisms in Oral Cancer and Precancer: A Review. *Oral Oncology* 50.1 (2014): 10-18.
2. Shrihari T.G, Vasudevan V, Kailasam S, Devaiah D, Manjunath V, Jagadish G.R. Antioxidants: Are We abusing It*? J Indian Aca Oral Med Radiol* 2012;24(4):306-310.
3. Ginsburg I, Kohen R, Shalish M, Varon D, Shai E, et al. (2013) The Oxidant-Scavenging Abilities in the Oral Cavity May Be Regulated by a Collaboration among Antioxidants in Saliva, Microorganisms, Blood Cells and Polyphenols: A Chemiluminescence-Based Study. PLoS ONE 8(5): e63062. doi:10.1371/ journal.pone.0063062
4. Nanditha B, Prabhasankar P. Antioxidants in bakery products: a review. *Critical Reviews In Food Science And Nutrition* [serial online]. January 2009;49(1):1-27
5. Brenneisen P. et al. Selenium, oxidative stress, and health aspects:review. *Molecular Aspects of Medicine* 26 (2005) 256–267
6. Silvia E., Martínez-López E., Jiménez P., María-Mojica P., García-Fernández A.J. "Effects of Heavy Metals on Biomarkers for Oxidative Stress in Griffon Vulture (Gyps Fulvus)." *Environmental Research* 129 (2014): 59-68.
7. Baynes J. W. Oxygen and life. *Medical Biochemistry*. By John W. Baynes, Marek H. Dominiczak. Philadelphia: Elsevier Mosby, 2005. 499-506.
8. Mayers, P. A. "Biological Oxidation." *Harper S Illustrated Biochemistry*. By Harold Anthony. Harper, Robert K. Murray, Daryl K. Granner, and Victor W. Rodwell. Estados Unidos: Lange Medical Publications, 2006. 86-91.
9. Williams G.M, Iatropoulos M.J., Whysner J. "Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxytoluene as Antioxidant Food Additives." *Food and Chemical Toxicology* 37.9-10 (1999): 1027-038.
10. Chaney, S. G. "Principles of Nutrition II: Micronutrients." *Textbook of Biochemistry: With Clinical Correlations*. By Thomas M. Devlin. New York: Wiley-Liss, 2002. 1139-158.
11. Fridovich I. "Superoxide Radical and Superoxide Dismutase." *Accounts of Chemical Research* 5.10 (1972): 321-26.
12. Singh V. P., Poon J., Engman L. "Turning Pyridoxine into a Catalytic Chain-Breaking and Hydroperoxide-Decomposing Antioxidant." *The Journal of Organic Chemistry* 78.4 (2013): 1478-487

13. Li C., Daxiang C. "Superoxide Dismutase, A Potential Theranostics Against Oxidative Stress Caused by Nanomaterials." *Nano Biomedicine and Engineering* 4.4 (2012): 195-206.
14. Oberley L.W, Buettner G. R. “Role of Superoxide Dismutase in Cancer: A Review”. *Cancer Res* 1979;39:1141-1149.
15. Bose K. C. S., Vyas P., Singh M. Plasma non-enzymatic antioxidants – vitamin C, E, beta-carotenes, reduced glutathione levels and total antioxidant activity in oral dub mucous fibrosis*. European review for medical and pharmacological sciences. 2012*; 16: 530-532
16. Dixon, S. "Vitamin E - What We Know." Ed. D. Pravikoff. Cinahl Information Systems, EBSCO, 2014.
17. Dominiczak M. H., Broom I. Micronutrients: Vitamins and Minerals. *Medical Biochemistry*. By John W. Baynes, Marek H. Dominiczak. Philadelphia: Elsevier Mosby, 2005. 128-131.
18. Stillway L. W. Bioenergetics and Oxidative Metabolism.*Medical Biochemistry*. By John W. Baynes, Marek H. Dominiczak. Philadelphia: Elsevier Mosby, 2005. 93-97
19. Putchala M. C., Ramani P., Sherlin H. J., Premkumar P., Natesan A. "Ascorbic Acid and Its Pro-oxidant Activity as a Therapy for Tumours of Oral Cavity – A Systematic Review." *Archives of Oral Biology* 58.6 (2013): 563-74
20. Sigusch B. W. "The Role of Vitamin C (ascorbic Acid) in the Prevention and Therapy of Oral Diseases." *Archives of Oral Biology* 58.8 (2013): 905-06.
21. Chandran V., Anitha M, Avinash SS, Rao G.M., Shetty B.V., Sudha K. Protein oxidation: A potential cause of hypoalbuminemia in oral cancer. *Biomedical Research 2012; 23 (2): 227-230*
22. Bairati, I., F. Meyer, M. Gelinas, A. Fortin, A. Nabid, F. Brochet, J.-P. Mercier, B. Tetu, F. Harel, B. Masse, E. Vigneault, S. Vass, P. Del Vecchio, and J. Roy. "A Randomized Trial of Antioxidant Vitamins to Prevent Second Primary Cancers in Head and Neck Cancer Patients." *JNCI Journal of the National Cancer Institute* 97.7 (2005): 481-88.
23. "Final Report on the Amended Safety Assessment of Propyl Gallate." *International Journal of Toxicology* 26 (2007): 89-118.
24. Iverson F. "Phenolic Antioxidants: Health Protection Branch Studies on Butylated Hydroxyanisole." *Cancer Letters* 93.1 (1995): 49-54.
25. Iverson F. "In Vivo Studies on Butylated Hydroxyanisole." *Food and Chemical Toxicology* 37.9-10 (1999): 993-97.
26. Battino M, Ferreiro M.S, Gallardo I, Newman H.N, Bullon P: The antioxidant capacity of saliva. *J Clin Periodontol 2002; 29: 189–194*
27. Ginsburg I., Koren E., Shalish M., Kanner J., Kohen R. Saliva Increases the Availability of Lipophilic Polyphenols as Antioxidants and

Enhances Their Retention in the Oral Cavity. *Archives of Oral Biology* 57.10 (2012): 1327-334.

28. Masumi Tsuda and Yusuke Ohba (2012). Functional Biomarkers of Oral Cancer, *Oral Cancer*, Dr. Kalu U. E. Ogbureke (Ed.), ISBN: 978-953-51-0228-1, InTech

Appendix.

DECLARAÇÃO

Monografia de Investigação/Relatório de Atividade Clínica

Declaro que o presente trabalho, no âmbito da Monografia de Investigação/Relatório de Atividade Clínica, integrado no MIMD, da FMDUP, é da minha autoria e todas as fontes foram devidamente referenciadas.

27/05/2014

Ekaterina Spiridonova

O / A investigador(a)

PARECER

(Entrega do trabalho final de Monografia)

Informo que o Trabalho de Monografia desenvolvido pelo(a) Estudante Ekaterina Spiridonova

com o título: Effects of antioxidants in oral biochemistry,

está de acordo com as regras estipuladas na FMDUP, foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

27/05/2014

O(A) Orientador(a)

Maria Rodrigues