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The role of the prooxidative-antioxidative system in dentistry

Rola układu prooksydacyjno/ antyoksydacyjnego w stomatologii

Agnieszka Markowska-Dyner, BDS

Abstract

During physiological processes, a number of chemical reactions take place in all cells including oxidation and reduction. Those reactions can result in the production of free radicals, ie., atoms or atom groups that have one or more unpaired electrons and therefore extremely high chemical reactivity. Numerous free radicals are formed through natural processes; the levels of free radicals in the cell depends, among others, on physical activity, environmental factors (eg., ionizing radiation from natural or artificial sources, toxins such as exhaust fumes) and lifestyle-related stress. Generation of free radicals is associated with oxidative and nitrosative stress.

Key words: prooxidative-antioxidative system in dentistry

Oxidative stress is defined as a disturbance in cell homeostasis leading to an increase in reactive oxygen species (ROS). It is also a disturbance in the balance between oxidation processes and antioxidant defenses.

In dentistry, oxidative stress is believed to cause periodontal disease, inflammation of gums and bones, neoplastic disease of the oral cavity, xerostomia, Sjögren's syndrome, vitamin C, D and E deficiencies, dental plaque or periapical lesion formation [1, 2, 3]. The major source of ROS are the mitochondria. ROS is generated by the mitochondria respiratory complexes which leak electrons to oxygen producing water and several by-products including the superoxide anion radical ($O_2 \cdot^-$), hydroxyl radical ($HO \cdot$), hydroperoxyl radical ($HO_2 \cdot$), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) [4, 5, 6].

In a healthy organism, 1-5% of oxygen is transformed into reactive oxygen species through excitation or reduction reactions; small concentrations of ROS are indispensable for life processes.

Oxidative stress is associated with nitrosative stress. Nitric oxide (NO) is synthesized from arginine by the enzyme nitric oxide synthase (NOS), which exists in three established isoforms, i.e., neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). Ohashi et al. observed a significant increase of salivary NO in patients with oral mucosal diseases, i.e., lichen planus and recurrent aphthous ulceration compared to healthy controls. They also examined the effect of NO *in vitro* and revealed significant reduction in fibroblast, keratinocyte and epithelial cancer cell line viability. It seems that small concentrations of salivary NO have a protective effect on the oral mucosa but increased concentrations may contribute to oral dysplasia and risk of progression to cancer [7]. The following characteristics of nitric oxide and its metabolites act as tumor promoters: immunocytotoxicity, involvement in angiogenesis, enhanced mutations in the p53 gene and low grade dysplasia transformation into high grade dysplasia, and eventually oral cancer [8,9]. Bahar et al. revealed substantially higher levels of NO, NO₂ and NO₃ in the saliva of patients with oral squamous cell carcinoma. The 8-OHdG marker (an indicator of DNA damage) was also significantly increased while the total antioxidant status (TAS) values were significantly lower [10]. All these findings indicate the importance of oxidative stress in oral carcinogenesis.

Along with macrophages, neutrophils are the predominant phagocytic cells in the blood; neutrophil count and function determine the level of reactive oxygen species production. Neutrophil activation results in the production of large amounts of superoxide anion radical and its derivatives which, under the influence of the enzyme myeloperoxidase (MPO), catalyze strongly reactive oxidizing compounds. Thus, increased MPO activity may evidence enhanced ROS synthesis and neutrophil activation characteristic of neoplastic disease. The elimination and neutralization of ROS are determined based on the total antioxidant status (TAS). Czygier et al. examined MPO and TAS levels in patients with gastric tumor and found a significant increase in MPO compared to healthy controls while TAS levels were significantly decreased [11]. Antoneeva et al. studied MPO levels in advanced stages of ovarian cancer and found that, in the course of tumor progression, there was an increase in the total neutrophil granulocyte count whereas MPO activity decreased [12].

Oxidative stress results in ROS overproduction within mitochondria and resultant metabolic disorders, hypoxia or ischaemia, which may cause DNA, protein, and/or lipid damage leading to genetic mutations [13]. Numerous patients with oral squamous cell carcinoma had somatic mitochondrial DNA mutations (not inherited but arising in the process of neoplastic

transformation). It is believed that mutations which disturb the respiratory chain function may enhance the accumulation of free radicals and thus result in further DNA aberrations.

Reactive oxygen species ultimately lead to metabolic mutations, dysfunctions and aging, which, in turn, trigger inflammatory processes, neoplasms, functional abnormalities of the heart, liver, kidneys, lungs and other organs [8]. Free radicals also participate in the metabolism and cytotoxic actions of several chemotherapeutics including doxorubicin, daunorubicin and analogues thereof used in lung, breast and ovarian cancer.

The severity of free radical mediated pathological processes in an organism has been evaluated based on malondialdehyde (MDA) and lipid peroxides concentrations. These compounds are end-products of lipid peroxidation, one of the most well known and well-studied free radical chain reactions. MDA and lipid peroxide concentrations in blood plasma and other body fluids and tissues increase along with enhanced ROS production. End-products of lipid peroxidation are considered second messengers of free radicals [14,15,16,17]. Salzman et al. investigated plasma levels of MDA in patients with squamous cell carcinoma of the oral cavity and oropharynx. They observed an increase in MDA levels in the study participants with T3-4 tumors and those who manifested recurrence [18].

Beevi et al. evaluated the magnitude of oxidative stress and levels of nitric oxide in patients with oral cavity cancer by analyzing the levels of lipid peroxidation products, antioxidants and nitric oxide products. ROS induce peroxidation of membrane lipids resulting in the generation of lipid peroxides which, in turn, are metabolized to MDA, lipid hydroperoxides (LHP) and nitric oxide products like nitrite (NO_2^-), nitrate (NO_3^-) and total nitrite (TNO_2^-). The patients with oral cavity cancer had significantly elevated levels of the above mentioned lipid peroxidation products while enzymatic and non-enzymatic antioxidants were significantly lowered. Enhanced lipid peroxidation with a concomitant decrease in antioxidants is indicative of a close relationship between lipid peroxidation and oral cavity cancer. Increased nitric oxide production induces pathogenic processes [19].

Protection from factors facilitating ROS generation is one way of defense against oxidative stress. The other is the antioxidative barrier which consists of two antioxidative systems: main and auxiliary.

The main antioxidative system comprises the following elements:

-enzymatic: superoxide dismutase (Cu/ZnSOD – cytosolic, MnSOD – mitochondrial), catalase (FeCAT – peroxisomes), glutathione peroxidase (SeGPX – cytosol, mitochondria), glutathione transferase (cytosol);

-extracellular metalloproteins (albumin-Fe, Cu, transferrin-Fe, ceruloplasmin-Cu) and intracellular metalloproteins (ferritin-Fe, metallothionein-Cu);

-molecular: α -tocopherol, β -carotene, ascorbic acid, glutathione, ubiquinone, urates, carnosine, anserine;

The auxiliary system consists of the following components:

-enzymatic: glucose-6-phosphate dehydrogenase, glutathione-disulfide reductase;

-molecular: bilirubin, biliverdin, cysteine, adenosine, histidine, lipoic acid, linolenic acid [9,20,21,22].

The antioxidative barrier has the following functions:

1. preventive, ie., suppression of free radical formation,
2. radical scavenging, ie., suppression and / or breaking free radical chain reactions,
3. removal of oxidatively modified proteins from the cell.

The antioxidative barrier is characterized by a coherent action. All its responses are interrelated and cannot occur separately (Figure 1).

Fig. 1. Interrelations between the enzymatic antioxidant defense [23]

Preventive antioxidants, which belong to the main antioxidative system, suppress the formation of ROS and prevent lipid peroxidation. Ceruloplasmin, ferritin, transferrin and albumin are plasma antioxidants, proteins, which bind to transition metal ions, eg., copper and iron, which usually have unpaired electrons. Thus, generation of free radicals does not take place [8, 24]. *In vitro* studies revealed that albumin protected against erythrocyte membrane lipid peroxidation through binding copper ions and thus prevented hydroxyl radical formation from hydrogen peroxide. Iron ion binding proteins, ie., ferritin and transferrin, prevent excess ion accumulation while ceruloplasmin takes part in superoxide anion radical transformation and ferrous ion oxidation. Meucci et al. found that total protein concentration and uric acid level showed good correlation with total antioxidant capacity of the saliva of hemodialyzed patients [14].

The first line of defense, ie., preventive antioxidants, consists of closely interrelated enzyme actions. The enzymes involved are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx). According to L.W. Oberley and T.D. Oberley, superoxide dismutase is the key enzyme in cell differentiation [25]. They believe that malignant transformation can only occur in stem non-differentiating cells which appear to have lost the ability to undergo SOD induction. Tumor cells produce the superoxide anion radical but, due to diminished amounts of SOD, remain unprotected against free radical actions. SOD catalyzes dismutation of the superoxide anion radical resulting in the formation of hydrogen peroxide; the latter is then broken down to oxygen and water in the presence of catalase or glutathione peroxidase. Tumor cells react to oxidative stress by diminishing the activity of antioxidative enzymes [26], and especially MnSOD and CuZnSOD. Manganese SOD protects the mitochondrial genome, most vulnerable to free radicals. Low levels of this antioxidant can initiate carcinogenesis whereas its elevation might evidence tumor progression or malignant transformation [27]. SOD levels and gene expression differ depending on the organ, cancer stage and even the distance of a metastasis from the primary lesion. SOD levels in patients with laryngeal cancer were lower compared to control participants. However, its levels in stage 3 and 4 tumors were markedly decreased compared to stages 1 and 2 [28]. Similar results were observed in patients with gastric cancer although those with other stomach

disease, eg., gastric ulcer, were found to have elevated CuZnSOD [29]. On the other hand, Malafa et al. observed an increase in MnSOD expression in metastatic gastric cancer and considerable differences between MnSOD levels in the primary and metastatic lesions [30]. MnSOD levels were elevated in human colorectal neoplasms compared to adenomas and normal mucosa, and continued to increase along with disease progression (marker of gradual neoplastic transformation in the human colorectum) [31]. SOD and MnSOD activity was increased in women with neoplastic breast disease depending on disease stage; it was also closely related to the activity of other enzymatic antioxidants [24]. MnSOD levels were also increased in the serum of patients with epithelial ovarian cancer and were proportional to tumor malignancy [32] while patients with lung cancer showed diminished SOD, CAT, GPx and GST activities [33].

Due to its specific physicochemical, enzymatic and microbiological characteristics and frequent exposure to potentially risky exogenous factors, the oral cavity is a site of ROS generation. The latter play an important role both in physiological and pathological processes. The role of ROS in the pathogenesis of periodontal disease is well established. Saliva is the body fluid which constitutes the first line of oral cavity defense against ROS; however, the activity of antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase in saliva is much lower than in the blood. The main components of saliva involved in the defense against free radicals are small-molecule antioxidants, ie., glutathione, ascorbic acid, uric acid, albumins and the salivary peroxidase. Antioxidative enzymes and small-molecule antioxidants exhibit different activities in the serous secretions of the parotid glands and mucous/mixed secretions of the submandibular and sublingual glands [34,35,36]. Several study reports published in Head Neck documented elevated levels of MnSOD, CAT, GPx and MPO. MnSOD expression increased significantly along with tumor progression. GPx and MnSOD are considered potential predictors of survival in patients with buccal mucosal squamous cell carcinoma. Gokul et al. studied the oxidant-antioxidant status in patients with oral squamous cell carcinoma. They found that malondialdehyde and nitric oxide were significantly elevated in the blood and tumor tissue specimens of OSCC patients. Tumor tissue levels of SOD and CAT were significantly reduced while, in the erythrocytes, catalase levels were significantly reduced and the SOD levels were higher in OSCC group compared to healthy controls [37]. Gurudath and Ganapathy estimated SOD and GPx levels in patients with oral submucous fibrosis, oral leukoplakia and oral cancer. They observed a decrease in the levels of the examined enzymes, which was the most pronounced in patients with oral cancer [38]. Huo et al. investigated SOD, CAT, MDA and NO levels in the blood and tumor tissue of patients with oral squamous cell carcinoma. They observed a significant increase in MDA and NO levels in the blood and tumor tissue and a decrease in SOD and catalase in tumor tissue. Serum SOD and catalase were increased and decreased, respectively. [39]

The second line of defense consists of active radicals scavenging. Small-molecule antioxidants, including vitamins A, C and E, compete with the target species (biomolecules or other compounds) for the oxidizing agent. ROS reaction with an antioxidant generates a free radical which is markedly less reactive than radicals produced as a result of, eg., lipid peroxidation. Free radical chain reaction is then broken and peroxidation of biologically active compounds stopped.

The group of non-enzymatic antioxidants includes ascorbic acid which inhibits early stages of carcinogenesis (ie., initiation and promotion). It acts as a ROS scavenger or reacts with active

forms of oxygen converting them to forms which are less toxic to the cell. Anti-carcinogenic effects of ascorbic acid also consist of immune system stimulation, inhibition of nitrosamine formation (nitrosamines are extremely toxic chemical compounds), inhibition of neoplastic cells infiltration through involvement in the synthesis of collagen and decreasing the level of lysosomal glycosides which promote cancer spread [40, 41]. Another representative of non-enzymatic antioxidants is vitamin E, which is oxidized to tocopheroxyl radicals and then, through reduction by ascorbate, recycled back to biologically active tocopherol. Vitamin E comprises a group of eight hydrophobic compounds, four tocopherols and four tocotrienols that occur in α , β and γ forms. Alpha-tocopherol is the most active form that constitutes about 90% of total vitamin E. This vitamin is a potent scavenger of organic peroxy radicals; it inhibits lipid peroxidation and affects the growth and differentiation of cancer cells through the inhibition of protein and nucleic acid synthesis. Vitamin E also acts as an efficient quencher of singlet oxygen.

Vitamin A, which occurs in two forms, i.e., A1 (retinol) and A2 (3-dehydroretinol), should also be included in the group of non-enzymatic antioxidants. These compounds play a vital role in cell growth and differentiation, and especially epithelial and bone cells. Vitamin A suppresses epithelial and epidermal metaplasia and hence can be used in the treatment of early-stage neoplastic processes within the mucous membrane, in particular the epithelial lining of the mouth, alimentary canal and urinary system [42,43].

The third line of defense consists of repair enzymes including glutathione, thioredoxin and melatonin. Glutathione exists in the reduced form (GSH) or the oxidized form (GSSG). GSH participates in the breakdown of endogenous hydrogen peroxide whereby it is oxidized to glutathione disulphide (GSSG) by glutathione peroxidase which catalyzes this reaction [44].

Along with reductase and glutathione peroxidase, glutathione is a component of the antioxidative system that reduces hydrogen peroxide by NADPH. Its reduced form (GSH) may also react with superoxide anion radical and hydroxyl radical. Glutathione is a thiol antioxidant whose effects are comparable to those of α -lipoic acid. Being water and fat soluble, the acid can act as an antioxidant in both the cytosol and plasma membrane. It is readily absorbed from food and transformed to a reduced form, i.e., dihydrolipoic acid (DHLA). Both forms scavenge reactive oxygen species, chelate transition metal ions, reduce other antioxidants and restore protein function lost as a result of oxidative stress. Another broad spectrum non-enzymatic antioxidant is a neurohormone melatonin secreted by the pineal gland. Melatonin prevents DNA, cell membrane and protein damage. Unlike other antioxidants, melatonin, once oxidized, cannot be reduced to its former state and has therefore been referred to as a suicidal or terminal antioxidant [45].

Until 1970s, the medical literature comprised few reports on the role and importance of free radicals. It was McCord and Fridovich, who emphasized the fact of superoxide dismutase being present in and protecting mammalian body cells from the toxic effects of superoxide free radicals. being present in almost all mammalian body cells [46]. Babior revealed that neutrophil functions were associated with free radical generation [47] while Granger observed that several disease processes were related to imbalance between mechanisms responsible for free radical production and antioxidative defense mechanisms [48]. High levels of ROS in cancer cells may induce high rates of cell division, further DNA aberrations and genomic instability; they may also evoke resistance to some chemotherapeutic agents. On the other

hand, oxidative stress to cancer cells may prove useful in the search for new treatment strategies.

Further studies on oxidative stress in cancer cells should lead to the identification of tumor types responsive to drugs that might eliminate cancer cells by a ROS-mediated mechanism and/or inhibition of antioxidative responses. The studies on antioxidative barrier and especially SOD, GPx, GR and GST activities are aimed at the identification of a tumor marker that would indicate the presence of a tumor, metastases or even survival duration. Aurer et al. concluded that oxidative stress influenced the function of the immune system, caused its hyperactivation and thus resulted in enhanced release of inflammatory factors and increased susceptibility to periodontal diseases.[49] – wpisane do referenc

Considering an association between *Streptococcus anginosus* infection with head and neck cancer, Sugano et al. assessed the salivary levels of this bacterium in patients with periodontitis [50]. In the light of contemporary medical knowledge, chronic bacterial infection can markedly increase the risk of developing certain cancers. During inflammation, ROS production may cause DNA damage leading to gene mutation and neoplastic transformation. The head and neck contain a lot of cavities and spaces where tumors can develop with no evidence of disturbances to the function of a particular organ. Hence, diagnosis of cancer is delayed or a metastatic lesion is diagnosed earlier than the primary malignancy.

Squamous cell carcinoma (SCC) accounts for 40% of all head and neck malignancies and 90% of all oral cancers. In the case of early detection, survival rate varies between 80 to 90%. However, early SCC does not frequently cause bothersome symptoms. There is no screening for SCC and patients are seen when the disease is advanced. According to the National Cancer Registry of the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw, the number of diagnosed in-situ SCC of the oral cavity, oesophagus and stomach diagnosed in the years 1999-2011 was as low as 123. The development of quick and non-invasive screening tests using a saliva sample seems an important target of future investigations to improve diagnosis of head and neck diseases. Secondary prophylaxis with early detection of lesions based on screening tests may help decrease mortality and increase survival time. Saliva is not just a digestive secretion. It contains several substances that may serve as clinically important biomarkers. Saliva testing is non-invasive, painless, simple and low cost. Hence, considering low rates of early head and neck SCC diagnosis, undertaking research in this area is of top priority.

Easy access to the oral cavity allows meticulous examination of tumorous lesions. and drawing pertinent conclusions based on pharmacotherapy effects

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