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The role of saliva in the process of oxidative stress – review of literature

Rola śliny w procesie stresu oksydacyjnego

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Streszczenie

Ślina stanowi pierwszą linię obrony przeciwko wolnym rodnikom stresu oksydacyjnego. Podczas procesów zapalnych dziąseł czy w trakcie żucia, zmienia się skład śliny w związku z produktami reakcji zapalnej, wywołanymi mikro uszkodzeniami w obrębie jamy ustnej. Autorzy dokonali przeglądu aktualnego piśmiennictwa dotyczącego reaktywnych form tlenu, przeciwutleniaczy I pro-utleniaczy w ślinie i metod służących do oceny zdolności przeciwutleniającej śliny.

Porównując procesy antyoksydacyjne śliny u mężczyzn i kobiet, stwierdzamy na znaczące różnice w składzie śliny związane z płcią. Aktualne dane wskazują na znaczne wzmożenie procesów antyoksydacyjnych śliny np. w młodzieńczym idiopatycznym zapaleniu stawów. Również pacjenci z przewlekłą niewydolnością nerek, cukrzycą i hemodializowani pokazują wzrost intensywności procesów związanych ze stresem oksydacyjnym zarówno w surowicy jak i w ślinie. Stwierdzenie obniżonych poziomów peroksydazy w obrębie jamy ustnej u osób palących może być jedną ze składowych mechanizmu inicjacji i progresji chorób jamy ustnej związanych z dymem papierosów, takich jak rak jamy ustnej. Wyniki ostatnich badań wskazują, że całkowita ilość przeciwutleniaczy w ślinie jest obniżona u dzieci z zakażeniem HIV.

Wnioski: Wskaźniki procesów oksydacyjnych w ślinie mogą być stosunkowo łatwo monitorowane. Może to stanowić narzędzie do rozwoju i monitorowania nowych strategii leczenia. Nieinwazyjne oznaczanie stężenia ślinowych przeciwutleniaczy, takich jak

dysmutazy ponadtlenkowej (SOD) i kwasu moczowego (UR) pozwala na ocenę zdolności obronnych błony śluzowej jamy ustnej. Nadal istnieje potrzeba normalizacji metod pobierania próbek śliny oraz wypracowania jednolitych protokołów przeprowadzania oznaczeń.

Abstract

Background: Saliva constitutes a first line of defence against free radical-mediated oxidative stress, since the process of mastication and digestion promotes lipid peroxidation. During gingival inflammation, gingival crevicular fluid flow increases the change of saliva composition with products from the inflammatory response, modulating oxidative damages in the oral cavity. Authors review the current literature concerning the reactive oxygen species, oxidants, pro-oxidants and antioxidants in saliva, and methods for assessing the antioxidant capacity of saliva.

Comparison of salivary antioxidant status in male and female subjects reveales a significant gender-related difference in saliva composition. The current data demonstrate a significant enhancement of the salivary antioxidant system in juvenile idiopathic arthritis patients. Also patients with chronic renal failure, diabetes and on hemodialysis show increase oxidative stress burden in both serum and saliva. The finding of reduced oral peroxidase levels in smoking subjects may represent a contributory mechanism for initiation and progression of cigarette smoke-related oral diseases such as oral cancer. The results of recent studies indicate that the total antioxidant capacity of saliva decreased in children with HIV infection.

Conclusion: Whole saliva may contain simply measured indicators of oxidative processes. This may provide a tool for the development and monitoring of new treatment strategies. A non-invasive determination of the salivary concentrations of antioxidants such as superoxide dismutase (SOD) and uric acid (UR) allows the evaluation of the defensive capacity of the oral mucosa. Still, there is a need for standardization of methods for saliva sampling and testing protocol.

Key words: saliva, oxidative stress, antioxidants

Introduction

Trauma, stress, genetic determination, hypersensitivity, nutrition, immune disturbance and hormonal imbalance can disturb the oxidant/antioxidant balance of organism and can accelerate the formation of free radicals [3,25,36]. Oxidative stress occurs when the intracellular concentrations of reactive oxygen radicals (O_2, H_2O_2 and H_2O_2) increase over the physiologic values. The cytotoxic effects of free radicals are detrimental for cells, and lead to cell damage through its effects on peroxidation of double-chain fatty acids, protein and DNA, as well as, increase oxidative stress [25,36]. These effects are possibly related to the development of cardiovascular diseases, neurodegenerative diseases, cancer, and aging processes [2,6,15,18]. Accordingly, mammalian cells have developed antioxidant defense systems to prevent oxidative damage. The antioxidant balance of an individual is influenced by diet, sports, stress [6]. 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 [34]. Antioxidant enzymes such as superoxide dismutase and glutathione peroxidase provide protection within cells whilst low-molecular-weight scavenging antioxidants are present in extracellular fluid. These include ascorbic acid, α-tocopherol and β-carotene. In addition, dietary-derived components such as uric acid, non-protein thiols and glutathione [10,34]. Saliva composition represents plasma composition, and accordingly its analysis is warranted. Saliva analysis is easy, non-invasive, cheap and patient-friendly, and may replace plasma analysis of antioxidants, which in turn may be of importance in monitoring the severity of the condition and the success of its treatment [27]. Whole saliva is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluid released from salivary glands [30,34]. Saliva possesses a wide range of antioxidants including uric acid, vitamin C, reduced glutathione, oxidized glutathione, and others [10]. Such antioxidants work in concert, and total antioxidant capacity may be the most relevant parameter for assessing the defense capabilities [10,30]. Stimulated saliva contains a lower concentration of antioxidants, but when flow rates are taken into account, antioxidant capacity is higher than in unstimulated saliva [10]. A growing body of evidence implicates oxidative stress in the pathology of chronic periodontitis. Several studies demonstrated increased levels of biomarkers for tissue damage, by reactive oxygen species (ROS) in periodontitis patients [20,29,30,35]. In response to oxidative stress, antioxidant enzymes appear up-regulated in inflamed periodontal tissues and in gingival cervical fluid (GCF), and extracellular antioxidant scavengers are depleted both individually as well as in terms of total antioxidant activity [29,30]. Several reactive oxygen species (ROS) and lipid peroxidation products are produced in physiological amounts in the human body, but it is known, that overproduction of ROS occurs especially in chronic inflammation [14].

Methods for saliva collection

There are numerous methods available for saliva collection, including harvesting whole saliva, individual gland saliva and stimulated or unstimulated saliva. Whole unstimulated saliva represents the major intra-oral condition regarding saliva state and composition [22,30,35]. Stimulation of saliva may increase the flow of Gingival Cervical Fluid (GCF), which may result in a false increase in the concentration of antioxidants in saliva [22,30,35]. Unstimulated whole saliva samples are preferred in determination of antioxidant defense parameters – the total antioxidant capacity is higher in unstimulated saliva [22,30,35].

Saliva samples should be collected after patients had received mouth routine check-up. With the patients seated, the saliva should be collected over a 5-minute period with instructions to allow saliva to pool in the bottom of the mouth and drain to a collection tube when necessary. Subjects should be asked not to swallow any saliva for the duration of the collection to allow the calculation of salivary flow rates [35].

Salivary flow rates could vary in relation to circadian cycle, individual hydration, food stimulation, oral hygiene, etc. Normalization to total proteins is used as alternative to salivary flow to look for differences in the ratio of biochemical analytes present in saliva [24].

Antioxidant assays

Salivary antioxidants can be classified in three groups, according to their function [4,23]. The first group is formed by preventive antioxidants, which are those which inhibit the production of free radicals, such as antioxidants superoxide dismutase (SOD), carotenoids, catalase, glutathione, peroxidase, transferrin, albumin and haptoglobin. Secondly there are 'sweeping' antioxidants, such as vitamin A and E, uric acid (UA), albumin and bilirubin, which eliminate free radicals in order to inhibit the starting and spreading of cell damage. The last group are enzymes such as proteases, transferase, lipases, etc, which repair the damage caused in the tissues [4,23]. Superoxide dismutase (SOD) and uric acid (UA) are responsible for 70% of the antioxidant potential of saliva [4]. SOD is capable of increasing in response to different inflammatory reactions such as tonsillitis, pulpitis, periodontitis and peri-implantitis [13]. Where one antioxidant is depleted another may be used to replace it, or to compensate for its function [35]. Antioxidant status can be estimated by measurements of advanced oxidation protein products (AOPP) – markers of oxidative damage to proteins, thiobarbituric acid reactive substances (TBARS) markers of lipoperoxidation, and advanced glycation end products (AGEs) – markers of carbonyl stress [21].

Salivary AOPP levels can be determined using a spectrophotometric method by Witko-Sarsat et al. [20]. A volume of 200µL of saliva is incubated with 20µL of glacial acetic acid. The absorbance is read immediately. Salivary AGEs are assessed using spectrofluorometric method according to Munich et al. [26]. Saliva samples are diluted 10-fold with PBS (phosphate buffered saline, pH 7.2), and the specific fluorescence of AGEs is expressed in arbitrary units.

Total antioxidant activity (TAA) can be determined in whole saliva using the ferric reducing ability of plasma (FRAP) assay adapted for a microplate reader [7]. Salivary ascorbate concentrations can be determined using the colometric method of Butts and Mulvihill [11]. Urate can be assayed using an enzymic conversion method (e.g. Sigma Diagnostics kit). Salivary albumin can be determined using the Bromocresol Green method [5]. Salivary peroxidase activity can be measured according to thionitrobenzoic acid assay [31].

The total antioxidant capacity of saliva can be also evaluated using the spectrophotometric assay. The method is based on the principle that, when a standardized solution of Fe-EDTA (Iron- Ethylenediaminetetraacetic acid) complex reacts with hydrogen peroxide by a Fenton-type reaction, it leads to the formation of hydroxyl radicals (OH) [37]. These ROS degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Thiobarbituric acid reacting substances (TBARS) are a marker of lipid peroxidation widely used in experimental research as well as in clinical studies [37]. Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color is defined as the antioxidant capacity [30].

Unlike other fluid compartments such as plasma, saliva is not a static fluid and its composition changes rapidly. Absolute antioxidant concentrations may thus be misleading and its more important to consider the rate of delivery of antioxidant components into the oral cavity, when considering the exposure of the gums and teeth [4]. Antioxidant flow rate is

obviously affected by the amount of saliva collected during sampling. Contamination of saliva samples by bleeding from the gums is also an important issue [23]. Saliva sampling should take place after dental examination. Leakage of antioxidants from the plasma component into saliva may have impact upon the results.

Comparison of salivary antioxidant status in male and female subjects revealed a significant gender-related difference in saliva composition. TAA was significantly lower in women than in men, whether considered as an absolute concentration or as an antioxidant flow rate. The difference in TAA flow rate was related to reduced flow of both ascorbate and urate in the women [35]. Recent study by Hershkovich on 80 patients (45 men and 35 women, 20–80 years old), found a significantly reduced total value of salivary antioxidant capacity in elderly persons, increased oxidative stress, and increased salivary concentrations and total values of reactive nitrogen species, all contributing to increased DNA oxidation of oral epithelial cells, which may explain the higher prevalence of oral cancer in the elderly population [19].

Surprisingly also aerobic exercise change oxidative/antioxidative balance in human saliva. Aerobic exercise cause an increase in both salivary uric acid and total antioxidant activity, and a decrease in salivary lipid hydroperoxidase. The fact above suggests that aerobic exercise seems to inhibit lipid hydroperoxide generation, a marker of oxidative stress in human saliva [17].

The currently reported data demonstrate a significant enhancement of the salivary antioxidant system in juvenile idiopathic arthritis patients. This was demonstrated by various analyzed parameters, including the uric acid molecule [9]. Although salivary gland involvement in rheumatoid arthritis has been known for a long time, it did not draw much attention [27]. Salivary antioxidants play an important role in the oral protective system in the gastrointestinal tract after saliva has been swallowed. If an altered salivary antioxidant profile in rheumatoid arthritis patients accompanies reduction in saliva output there may be further injurious effects. The destructive effect on the salivary glands demonstrated in rheumatoid arthritis patients may be rendered by a free radicals-related process, and saliva analysis may contribute to the better understanding of the mechanism [27]. Also patients with chronic renal failure and hemodialysis were found to increase oxidative stress burden in both serum and saliva [8].

The increased serum and salivary levels of antioxidants among diabetics were also reported. It may be explained on the basis that the existence or increased free radicals production may enhance the antioxidant defence system which counte-balances the pro-oxidant environment [1]. Reznick et al. have shown that oxidative stress exists in diabetic patients as evidenced by the increased total antioxidant capacity in the saliva and blood of the patients [32]. Hyperglycemia leads to excess ROS production which stimulates NADPH oxidase, principally in neutrophils [13]. Disruption of redox balance results in stimulation of cell-signaling pathways associated with inflammation, dysregulation of insulin signaling, and development of diabetic complications [13,30]. Still, relationship between the antioxidant status, glycemic control, and the risk for development of chronic complications in individuals

with diabetes are not completely clear. Oxidative stress leads to an up-regulation of pro-inflammatory pathways implicated in the pathogenesis of both diabetes and periodontitis [13].

Reznick et al also reported in in-vivo and in-vitro studies a sharp drop of oral peroxidase activity in smokers and non-smokers after smoking a single cigarette [31]. The finding of reduced oral peroxidase levels in smoking subjects may represent a contributory mechanism for initiation and progression of cigarette smoke-related oral diseases such as oral cancer. In 2010, Goku et al. evaluated the oxidant-antioxidant status of blood samples and tumor tissue in patients with oral squamous cell carcinoma and reported that antioxidant levels were significantly reduced in tissue samples from these patients compared with the control group [16]. Smoking is also the largest single risk factor for periodontal disease [12]. Associations were noted between periodontal disease status and salivary antioxidant status. Individuals with the lowest total antioxidant activity flow were 4,5 times more likely to have periodontal disease [35]. It is certain that lower antioxidant concentrations in the gingival crevicular fluid will contribute to increased damage to the gingivae and surrounding structures by activated neutrophils [35].

Highly immunosuppressive oncologic drugs prior to the bone marrow stem cells transplantation (BMT) also generate reactive oxygen species (ROS), which damage the DNA of the oral epithelium and submucosa [33]. This damage generates dysfunction in salivary glands and changes in saliva. The effect of oncologic drugs on the oral cavity increase SOD activity with the appearance of mucositis, which could be interpreted as a defensive mechanism of saliva against oxidative stress produced by chemotherapy [33].

Several recent reports have indicated high levels of reactive oxygen species, causing oxidative stress, in the pathogenesis of HIV infection [28]. Oxidative stress may lead to enhanced HIV replication in infected cells and may also aggravate the immunodeficiency by reduction of cellular immunity and possibly by increased death of lymphocytes [28]. Saliva can constitute a first line of defense against free radical mediated oxidative stress. The results of study by Padmanabhan et al. indicated that the total antioxidant capacity of saliva decreased in children with HIV infection [28].

Study by Kamodyova et al. has shown that vitamin C supplementation led to significantly decreased carbonyl stress and increased antioxidant status, so salivary carbonyl stress and antioxidant status are also influenced by diet and vitamin supplementation [21]. Vitamin C supplementation could be used for antioxidative therapy of oral diseases in which increased oxidative stress was reported [21].

Conclusion

Whole saliva may contain simply measured indicators of oxidative processes. This may provide a tool for the development and monitoring of new treatment strategies. A non-invasive determination of the salivary concentrations of antioxidants such as SOD and UR allows the evaluation of the defensive capacity of the oral mucosa. Still, there is a need for standardization of methods for saliva sampling and testing protocol.

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