Free Radicals and Oxidative Stress in Periodontal Disease

MARIUS BOARIU, ADRIAN MARINESCU, ALEXANDER KRALEV, CEZAR CLONDA, MEDA-LAVINIA NEGRUTIU, COSMIN SINESCU*
"Victor Babes" University of Medicine and Pharmacy, Faculty of Dental Medicine, 9 Revolutiei 1989 Blv., 300070, Timisoara, Romania

Within periodontal disease a lot of processes happen simultane ously. One of these processes is the oxidative stress. In order to understand this phenomenon it is important to know what the free radicals (FR) are, where they come from and how they act. There is numerous evidence pointing to the involvement of FR in periodontal disease. It has been reported in patients with rapidly progressive periodontitis, that the polymorphonuclear neutrophils (PMN) are functionally activated, produce high levels of O_2 and have a high response to the luminol-dependent (QL) chemiluminescent. Products of this oxidative damage such as advanced glycation end products and lipid peroxide proteins can lead to further ROS induced damage by their priming and chemotactic effect on neutrophils. In periodontal disease ROS causes bone resorption, connective tissue degradation and an increase in the activity of metallo proteinases matrix. Our study results showed that the total antioxidant capacity in serum in periodontitis patients was significantly lower when compared to healthy subjects.

Keywords: antioxidants, free radicals, periodontal disease, oxidative stress

A free radical may be defined as "any species capable of independent existence that contains one or more unpaired electrons". This spatial configuration generates a high instability.

Oxygen (O_2) exists in air and generates reactive oxygen species (ROS) (Table 1). Some of these ROS have the chemical characters of free radicals (FR). In the molecule of oxygen (O_2) the following FR or oxygen reactive species exists: anion superoxide ($O_2^{\cdot}$ É), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$). A fundamental characteristic of the reactions of free radicals is the capacity to participate in chain reactions, where a radical reaction consecutively generates another . In this sense, the body has an antioxidant system to counteract the generation of ROS, which maintains a homeostatic balance. However there are pro-oxidant factors that favour the generation of FR, causing an imbalance in favor of the latter, generating so-called oxidative stress (OS) [5].

ROS covers other reactive species that are not true radicals, but are however capable to react in intra and extracellular environment: peroxide of hydrogen, hypochlorous acid, oxygen, ozone. The living organism has adapted to an existence under a continuous output of radical free flow. Between the different antioxidant defense mechanism adaptation mechanism is of great importance. Antioxidants are "those substances that when are present in lower concentrations compared to the substrate of an oxidizable, significantly delay or inhibit the oxidation of the substrate" [3].

Antioxidant defense system is very dynamic and responsive to any disturbance that occurs in the body redox balance. Antioxidants can be regulated and neutralize the formation of radical free that can occur due to oxidative stress, such as the factor transcription factors Activator protein 1 and nuclear-kb are redox sensitive. Redox potential is a measure of the affinity of a substance for electrons [5].

The presence of inflammatory infiltrate is a constant feature in periodontal disease. It is known that these cells release lots of free radicals and so it is only normally to assume that these metabolites are involved in the pathogenesis of the disease. Also the presence of a dense inflammatory infiltrate in periodontal disease leads to the suspicion that the relationship of periodontal leukocyte-tissue has a double aspect. The role of these cells in the containment of the gingival bacteria and their products must be analyzed according to a balance with the destruction of tissue due to the release of the products from its action (FR and proteases). This is how a defensive mechanism can be made under the interaction of various factors to become harmful to periodontal tissues, and therefore involved in the pathogenesis of inflammatory periodontal disease.

There is numerous evidence pointing to the involvement of FR in periodontal disease. It has been reported in patients with rapidly progressive periodontitis, that the polymorphonuclear neutrophils (PMN) are functionally activated, produce high levels of O_2 and have a high

Radicals		Non-radicals	
Superoxide	$0_2^{\cdot}$	Singlet Oxygen	$^1 0_2$
Hydroxyl	$OH^{\cdot}$	Ozone	0_3
Hydroperoxyl	$HOO^{\cdot}$	Hypochlorous acid	HOCI
Alkoxyl	$RO^{\cdot}$	Hydrogen peroxide H	H_20_2
Aryloxyl	ArO□$^{\cdot}$		
Arylperoxyl	$ArOO^{\cdot}$		
Peroxyl	$ROO^{\cdot}$		
Acyloxyl	$RCOO^{\cdot}$		
Acylperoxyl	$RCOOO^{\cdot}$		

Table 1
REACTIVE OXYGEN SPECIES

* *email: minosinescu@yahoo.com; Tel.: +40722280132*

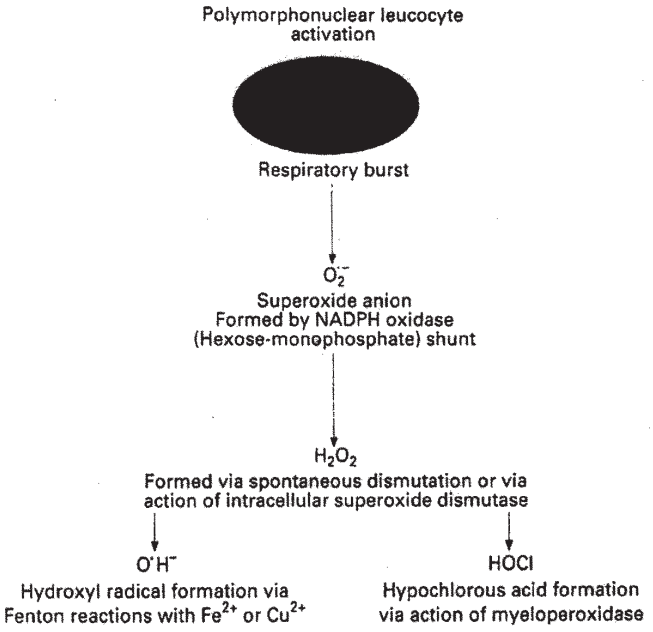

Fig. 1. Production of reactive oxygen species following activation of polymorphonuclear leucocytes

response to the luminol-dependent (QL) chemiluminescent. (fig. 1) There is an increase of the PMN oxidative response peripherals in patients with localized and generalized juvenile periodontitis, as well as in adult patients with periodontitis (AP). This increase is related to clinical periodontal status and is reversed by therapy.

It has also been compared the generation O_2 by the activated PMN in the gingival crevicular fluid (GCF) of patients with AP. The PMN activation with phorbolmyristateacetate causes a marked increase in the release of O_2 in patients with AP, while the antioxidant activity of the gum is similar to the controls. The effect of the PMN in crevicular fluid of patients is dependent on variations in the rate of formation of O_2 relative to the intrinsic antioxidant capacity of the gingival tissue (fig. 1).

In gingival epithelial cells in culture studies have shown the PMN may cause lysis of these through the action of the free myeloperoxidase (MPO), a leukocyte enzyme generating radicals. Its activity has been increased in the crevicular fluid of sites with gingivitis and periodontitis with respect to healthy sites.

There is a close relationship between free radical production by leukocytes and activation of proteases. These actions combined could have profound effects on the function and integrity of the gingival epithelium. Also there is a distinctive posibility that the general ethiological factors of periodontal disease could cause the breakup of lipid peroxidation systems, creating this way a low level antioxidant protection of periodontal tissue. In these circumstances, the local factors lead to the migration of neutrophils in gingiva and gingival fluid. The activation of these leukocytes in phagocytosis, causes the release of ROS, which leads to the outbreak of the lipid peroxidation of the soft tissues of the periodontium and activation of protease. This lipid peroxidation is the mechanism that triggers the development of morphofunctional changes in periodontium and its vessels, which results in destruction of collagen and bone resorption [4].

Most reactive oxygen species have extremely short half-lives (10-9 to 10-6 s),'7 but they can cause substantial tissue damage by initiating free radical chain reactions. There are exogenous sources of ROS such as cigarette smoke and ionising radiation. Endogenous production is, however, more pertinent to the pathogenesis of periodontal disease and arises either accidentally due to leakage of electrons from their carriers within the respiratory chainof mitochondria passing directly onto oxygen, '8"' or functionally through the generation of oxygen radicals by phagocytes. The latter process is thought to be implicated in the destruction of the connective tissues of the periodontium.

Because of the evidences that suggest a participation of the ROS in the pathogenesis of the periodontal disease, it has been sugested that the factors which promote a rupture of the antioxidant physiological system, contribute to the development of oxidative mechanisms that initiate the periodontitis. The main cause of lipid peroxidation in the periodontal disease seems to lie in ROS liberation by leukocytes in phagocytosis. These concept emphasize the utility of antioxidants in the prophylaxis and treatment of periodontal disease and therefore justify the search of new antioxidant preparations for this purpose. For example it was found that the cell death induced by *p. gingivalis* in the tissues is through the production of ROS [5].

In order to measure the total antioxidant capacity in the serum of chronic periodontal patients we started a clinical study. The objectives of the study is to investigate the total antioxidant capacity in serum of patients with periodontal disease and to compare it with the one present in serum of patients without periodontal disease.

Experimental part

Materials and method

The patients were split in 2 groups. Group 1 - control group 10 subjects with healthy periodontal conditions and group 2 - study group 10 subjects with clinically diagnosed periodontitis. The inclusion criteria in the study was; at least 5 mm of clinical attachement loss on periodontal probing, bleeding on probing, patients that have not been subject to any kind of periodontal treatment in the last 2 years. All measurements and samples have been taken before starting the periodontal therapy. The study exclusion criteria were: patients who are on antibiotic or anti inflammatory treatment, patients with a history of any systemic disease, patients with a history of smoking and tobacco consumption. The total antioxidant capacity of clinical samples was measured using spectrophotometric quantitation through formation of phosphomolybdenum complex. 6 mm of venous blood samples have been collected in aseptic conditions. Out of that 3 mL was collected in sterile heparinised tubes and rest of the blood was allowed to clot. The samples were centrifuged at 3000 rpm for 15 min and the supernatant serum was collected. A 0.1.mL sample solution containing reducing species (in water, methanol, ethanol, dimethylsulfoxide) was combined with 1mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and incubated at 95 degree for 90 min . After the samples were cooled to room temperature, the absorbance of each aqueous solution was measured at 695nm against a blank. The results showed that the total antioxidant capacity in serum in periodontitis patients was significantly lower when compared to healthy subjects. The group statistics showed a statistically significant difference between the antioxidant levels present in case and control groups.

Results and discussions

In the past the periodontal research was carried out mostly from a microbiologi point of view. It is true that the oral biofilm is the most important factor that produce the periodontal disease however it is just one of many factors involved in its pathogenicity. In the last years an increasing number of publications are suggesting that tthe direct effectors of the periodontal tissue distructions are component of the host immune system [8]. The aim of the present study was to evaluate the total antioxidant capacity in chronic periodontitis patient serum. The study showed a statistically significant difference between the antioxidant levels present in case and control groups. ROS are produced by all the human body cells however it is phagocytes that produce the high levels required to facilitate the killing and destruction of microbes. In periodontal disease ROS causes bone resorption, connective tissue degradation and an increase in the activity of metallo proteinases matrix. Chronic inflammatory conditions are associated with an increased oxidative stress with phagocytes being involved in disease pathogenesis because of generation of oxidative burst during phagocytosis and killing of microbes. The neutrophyles enhanced free radical generation can be stimulated by periodontal disease associated bacteria. Dental plaque bacteria and its products are source for the factors that could stimulate neutrophils that infiltrate the periodontal tissues. Diseased sites will be associated with an increased level of cytokines and chemokines produced by inflammatory cells and normal resident cell from the periodontal tissues. [3]

Conclusions

Oxidative stress plays an essential role in the occurrence and development of periodontal disease. It also lies at the heart of the periodontal tissue damage that results from host-microbial interactions, either as a direct result of excess ROS activity/antioxidant deficiency or indirectly as a result of the activation of redox-sensitive transcription factors and the creation of a pro-inflammatory state. ROS produced by phagocytes has initially been associated with the defense of body to infection as they are essential for efficient killing of microbes, however ROS generation at high levels can cause oxidative stress within tissues and result in direct damage to cells and extracellular matrix. Products of this oxidative damage such as advanced glycation end products and lipid peroxide proteins can lead to further ROS induced damage by their priming and chemotactic effect on neutrophils [3]. Nuclear factor kb and activator protein, the two redox sensitive transcription factors are of potential importance in the pathogenesis of periodontal disease [8]. Our study results showed that the total antioxidant capacity in serum in periodontitis patients was significantly lower when compared to healthy subjects. The results are consistent with other clinical studies findings presented in the literature.

References

1.BROCK GR, BUTTERWORTH CJ, MATTHEWS JB, CHAPPLE IL. Local and systemic antioxidant capacity in periodontitis and health. J Clin Periodontol., 31, 2004, p. 515.
2.BATTINO M, BULLON P, WILSON M, NEWMAN H. Oxidative Injury and Inflammatory Periodontal Diseases. The Challenge of Anti-Oxidants to Free Free Radicals and Reactive Oxygen Species, CROBM, 10, 1989, p. 458.
3.CHAPPLE IL, MATTHEWS JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontology 2000, 43, 2007, p. 160–232.
4.CHAPPEL D, SCULLEY V, DANGLEY SC. Salivary antioxidants and periodontal disease status. Am J Clin Nutr., 86, 2007, p. 180.
5.NAVA-VILLALBA M, GONZÁLEZ-PÉREZ G, LINAN-FERNÁNDEZ M, TORRES-CARMONA M. Oxidative Stress in Periodontal Disease and Oral Cancer, Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants. Dr. Jose Antonio Morales-Gonzalez (Ed.), 2013 ISBN: 978-953-51-1123-8.
6.BORGES IJr., ADDISON MACHADO MOREIRA E, FILHO DW, BITTENCOURT DE OLIVEIRA T, DA SILVA MBS, Fröde TS. Proinflammatory and Oxidative Stress Markers in Patients with Periodontal Disease, Mediators of Inflammation Volume 2007, Article ID 45794.
7.AZIZ AS, KALEKAR MG, BENJAMIN T, SURYAKAR AN, PRAKASHAN MM, BIJLE NA. Effect of Nonsurgical Periodontal Therapy on Some Oxidative Stress Markers in Patients with Chronic Periodontitis: A Biochemical Study, World Journal of Dentistry, 4, vol. 1, 2013, p. 17.
8.EKUNI D, BATTINO M, TOMOFUJI T, PUTNINS EE. Studies on periodontal disease, Springer Science + Business Media New York 2014.

Manuscript received: 3.03.2014