REVIEW ARTICLE

Oxidative Stress, Antioxidants, and Periodontitis: How are they Linked?

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ABSTRACT

Recent epidemiological studies reveal that more than two-third of the world's population suffers from one of the chronic forms of periodontal disease. Chronic periodontitis is a multifactorial infectoinflammatory disease caused by the interaction of microbial agents present in the biofilm associated with host susceptibility and environmental factors. There is significant evidence linking chronic periodontitis and oxidative stress. As the reactive oxygen species (ROS) and antioxidants (AOs) are in dynamic equilibrium, any disturbance in one would lead to changes in the other. This review focuses on the role of ROS, AOs, and oxidative stress in periodontitis.

Keywords: Antioxidants, Free radicals, Oxidative stress, Periodontitis.

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INTRODUCTION

Periodontitis is an inflammatory disease of the tooth-supporting tissues which occurs as a response to the lipopolysaccharides released by the Gram-negative anaerobes present in the plaque biofilm.[1] Although the primary etiological agent is specific, the greater part of periodontal destruction occurs as a result of an inappropriate host response to the periodonto– pathogenic microorganisms and their products.[2] As the periodontal diseases progress, there is a loss of attachment of the periodontal tissues to the tooth accompanied by a supporting bone loss which eventually results in tooth loss.

In recent years, reactive oxygen species (ROS) have gained more and more attention because of their central role in the progression of many inflammatory diseases. They are involved in normal cellular metabolism and continuously generated by the cells in most tissues. Another category of substances called antioxidants (AOs) exist in the cells and can effectively delay or inhibit ROS-induced oxidation. Under physiological conditions, ROS are effectively neutralized by AOs, which prevents ROS-mediated tissue damage. When inflammation happens, ROS production is drastically increased mainly due to cells of innate immune system, for example, neutrophils and macrophages during the process of phagocytosis through the metabolic pathway of the "respiratory burst."[3] Subsequently, high levels or activities of ROS cannot be balanced by the AO defense system, which leads to the oxidative stress and tissue damage.

Over the past several years, strong scientific evidence has emerged to associate oxidative stress in the pathogenesis of several chronic inflammatory diseases including periodontitis.[4-6] Professor Sies defined oxidative stress as "an imbalance between oxidants and AOs in favor of the oxidants, potentially leading to damage."[7,8]

Protection against these species is provided by AOs which essentially protects the cells by either removing or repairing the damage caused by them. AO are those substances which when present at lower concentrations, compared to oxidizable substrate, will significantly delay or inhibit oxidation of that substrate.[9] The extent of reduction in AO levels plays a significant role in disease pathogenesis. Minimal AO perturbations are readily corrected by the cell; however, larger depletion leads to either apoptosis or even necrosis. The latter consequently results in inflammation; either acute or chronic depending on how long the imbalance continues in oxidants level compared to AO. There seems to be an increasing volume of evidence to associate OS and periodontitis.[10]

The aim of this review is to highlight the role of ROS and AO defense systems in the pathobiology of periodontitis.

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Free Radicals (FRs) and ROS

Denham Harman in the early 1950s identified the role of FRs in aging following which the "mitochondrial theory of aging" evolved. This theory suggested that FRs were generated in the mitochondria and mutations of the mitochondrial DNA were implicated in the process of aging.[11] A significant number of radicals such as the superoxide FR anion or the hydroxyl radical (•OH) and another group of "non-radical reactive molecules" such as hydrogen peroxide (H_2O_2) and per-oxy nitrite were identified and their deleterious effects on the tissues described through the past several years.[12]

FRs have been defined as any species capable of independent existence that contain one or more unpaired electrons.[13] They are, by nature, highly reactive and diverse species, capable of extracting electrons and thereby oxidizing a variety of biomolecules vital to cell and tissue function, which not only include oxygen FRs but also nitrogen and chlorine species.

FRs are formed continuously within cells as a consequence of both enzymatic and non-enzymatic reactions during metabolism. When they react with biomolecules, they remove electrons and convert them into FR, which in turn react with other adjacent molecules and this chain reaction continues producing more FR.[9]

The fact is that FRs begets FRs, that is, generate FRs from normal compounds which continue as a chain reaction. Oxidative stress can arise when cell cannot adequately destroy the excess FRs formed. These FRs can damage cell membranes and lipoproteins by a process called as lipid peroxidation. Proteins may also be damaged by ROS/NOS, leading to structural changes and loss of enzyme activity.[14] FRs may cause DNA strand breaks which can cause cell mutation. The body has several mechanisms to counteract these attacks using DNA repair enzymes and or AOs.[15] If not regulated properly; oxidative stress can induce a variety of chronic and degenerative diseases.

ROS is a term that has become more popular because it encompasses other reactive species which are not true radicals but are nevertheless capable of radical formation in the intra and extracellular environments. ROS at high concentrations has adverse effects on cellular components, such as lipids, proteins, and DNA. ROS are continuously generated by most tissues as an integral part of normal cellular metabolism. ROS collectively describe oxygen FRs and other non-radical oxygen derivatives involved in oxygen radical production. These include superoxide O_2, hydroxyl OH, hydroperoxyl HOO, nitric oxide NO, alkoxy RO, singlet oxygen (O_2), ozone O_3, hypochlorous acid (HOCl), and H_2O_2.[9] ROS are actively involved in cell signaling and metabolic processes. ROS also play a role in pathogenic processes in a range of inflammatory disorders. Excessively produced ROS molecules are proficient enough of initializing the periodontal tissue destruction.[16] The various mechanisms of tissue destruction by ROS include lipid peroxidation, DNA damage, protein damage, oxidation of important enzymes, and the release of pro-inflammatory cytokines by monocytes and macrophages.[17,18]

How ROS causes Periodontitis?

Periodontal diseases affect 10–15% of the world population[19] and are one of the leading reasons of tooth loss. Periodontitis is basically an inflammatory disease, which is initiated by the subgingival biofilm and is modified by the individual's aberrant inflammatory/immune response. The polymorphonuclear (PMN) leukocytes are the prime inflammatory cells in gingiva and periodontal tissues.[20]

After initiation of the host response by pathogenic biofilm, neutrophils become the most common inflammatory cells gathering in periodontal tissue and gingival sulcus, and they are believed to be the predominant source of ROS in periodontitis (Chapple IL *et al*). Following the stimulation by pathogens, neutrophils produce O_2 through the metabolic pathway called "respiratory burst" catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during phagocytosis.[21] O_2 can be released into phagosomal and extracellular environment and then converted to different radical and non-radical derivatives, such as H_2O_2, HOCl, •OH, and O_2.

Studies by Guarnieri *et al.*[22] and Kimura *et al.*[23] demonstrated that PMN present in the gingival crevicular fluid (GCF) and blood of patients with adult periodontitis showed an enhanced production of O□, following stimulation, compared with PMN isolated from the GCF and blood of a control, "healthy" group. It was also observed that the circulating PMN of such patients within the adult periodontitis group produced low levels of O_2 spontaneously, while the control group appeared to exhibit no spontaneous O_2 production. The generation of excessive or chronic ROS by neutrophils and resultant tissue damage is more likely within the periodontal pocket due to the presence of low oxygen tension (1%) and pH of 7.0–7.5.47 The neutrophils become harmful as they release the FRs and proteases, causing the destruction of the periodontal tissues, rather than exhibiting a defensive role. In this way, a defensive mechanism, under the interaction of various factors, can prove to be harmful to the periodontal tissues and hence are involved in the pathogenesis of inflammatory periodontal disease.

In vitro studies show that not only neutrophils but also other phagocytes and cells of periodontal tissues, for example, monocytes, gingival fibroblasts, and periodontal ligament cells exhibit enhanced ROS production on stimulation by periodontal pathogens and/or their components.[24,25] However, their contribution to oxidative stress in periodontitis still remains to be elucidated by the future studies.

Periodontal ligament cells and gingival epithelial cells undergo cellular damage and cell lysis in the presence of ROS. Proteoglycans associated with the periodontal tissues are damaged resulting in glycosaminoglycans (GAG). Core proteins are selectively damaged by H2O2. OH• has the ability to degrade the proteoglycans and the hyaluronan. Moseley *et al.*[26] reported that exposure to ROS causes degradation of the GAG and proteoglycans associated with mineralized and non-mineralized periodontal tissues.

Superoxide anions and OH• were able to cleave collagen into small peptides at proline and hydroxyproline residues, liberating hydroxyproline containing peptides. In addition, modification of collagen and serum proteins indirectly by ROS, through interaction with lipid peroxidation products such as malondialdehyde (MDA), can significantly alter fibroblast functions such as adhesion, proliferation, and longevity.[27]

Osteoclasts have shown to be activated by ROS to enhance bone resorption. RANKL which is a key factor stimulating the differentiation and activation of osteoclasts, generates ROS in bone marrow monocyte-macrophage lineage cells through the involvement of TRAF6 (tumor necrosis factor receptor-associated factor 6), Rac1, and NADPH oxidase 1 (Nox1).[28,29] Studies suggest that periodontitis associated with diabetic patients have ROS as an underlying mechanism of tissue destruction, due to the oxidation of tissue components and the formation of AGE during hyperglycemia.[30] These AGEs have also been associated with activation of ROS production by macrophages.

Mechanism of Periodontal Tissue Damage by ROS

ROS are very active and their lifetime is extremely short. They can cause direct damage to the tissues resulting in a variety of metabolites of lipid peroxidation, DNA damage, and protein damage, which are usually used to evaluate the destruction of tissue by ROS.

Lipid peroxidation

Lipid peroxidation is one of the most important reactions of FR species. Lipid peroxidation by FRs results in the changes of structural integrity and function of cell membranes. Several products of lipid peroxidation such as MDA, 4-hydroxy-nonenal (HNE), and isoprostane have been used to evaluate both local and systemic oxidative damages associated with periodontitis.

MDA

MDA is a well-established lipid peroxidation product to evaluated oxidative stress, and it is also the most investigated lipid peroxidation product in periodontitis.[31] Thiobarbituric acid reacting substances (TBARS) is a conventional method to detect MDA based on the reaction with thiobarbituric acid and measured by spectrophotometric assay.[32] Studies have shown that shown that periodontitis is associated with higher levels of TBARS in blood plasma and erythrocytes systemically as well as in GCF and gingival tissue locally.[33] Liquid chromatography and mass spectroscopy are more reliable and specific methods for the detection of MDA.[34] These methods were used to study MDA levels in serum, GCF, and saliva of periodontitis patients.[35,36] Significantly higher levels of MDA were found in GCF and gingival tissue of periodontitis patients compared to periodontal healthy controls.[37]

Moreover, a study by Ghallab *et al.* demonstrated that levels of MDA in GCF could discriminate between general AgP, CP, and periodontally healthy controls.[38] Meanwhile, studies including patients with diabetes mellitus, hyperlipidemia, and acute coronary syndrome indicated that periodontitis could also contribute to higher circulating level of MDA among people with these systemic diseases.[39,40]

HNE

HNE is another major aldehydes end product associated with lipid peroxidation,[41] but data on this biomarker in periodontitis are limited to date. A study by Hendek *et al.* investigated the impact of periodontitis, smoking, and periodontal treatment on HNE levels in GCF, saliva, and serum, and found significant different GCF levels of HNE between smokers with periodontitis and periodontally healthy non-smokers.[42] In contrast to this study, Önder *et al.* showed that the levels of HNE are increased by periodontitis only in serum but not in saliva.[43]

Isoprostane

Isoprostane is a product of arachidonic acid peroxidation and is often measured in urine, serum, or plasma as a reliable marker of oxidative stress.[44] There are few studies investigating isoprostane levels in periodontitis.[45,46] Another study by Pradeep *et al.* showed that 8-isoprostane levels in GCF increased progressively

from healthy controls to gingivitis and periodontitis and correlated with gingival index, probing depth, and clinical attachment level.[47]

Protein damage

The biology of ROS-mediated protein damage is highly complex and remains poorly understood. Dean *et al.* comprehensively reviewed the field and pointed out that some oxidized proteins are poorly handled by cells so they accumulate during aging and in chronic conditions such as diabetes.[48]

Protein carbonyl (PC) groups are relatively stable end products of protein oxidation generated by multiple forms of ROS. It is the most widely used biomarker for oxidative protein damage with earlier production and greater stability compared with lipid peroxidation products.[49] The association between periodontal status and PC groups has been investigated in GCF, saliva, and serum and higher levels of PC groups were associated with worse periodontal status, as well as significant correlation between the level of PC groups and clinical periodontal parameters was observed within periodontitis patients.[50]

DNA damage

ROS can react with DNA and cause damage to purine and pyrimidine bases or the deoxyribose backbone. 8-hydroxydeoxyguanosine (8-OHdG) is most often used biomarker of oxidative stress-induced DNA damage, although it may not precisely reflect the whole DNA damage resulting from oxidative stress.[4] Studies showed higher level of 8-OHdG in GCF and saliva of periodontitis patients compared with that of healthy controls as well as their significant association with clinical periodontal parameters.[51,52] Almerich *et al.* showed that the level of 8-OHdG is associated with the presence and/or quantity of bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Streptococcus anginosus*.[53]

AO Defense Systems

AOs are "those substances which when present in lower concentration are compared to those of oxidizable substrate, will significantly delay or inhibit oxidation of that substrate."[54] Under normal physiological conditions, there is a balance between ROS and AOs. Oxidative stress happens only when the AO defense system could not neutralize the elevated ROS production. AOs can be classified into two categories based on their mode of function. The first category comprises preventive AOs including enzymatic AOs such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione reductase, and DNA repair enzymes, as well as some metal ion sequestrators such as albumin. The second category comprises scavenging AOs or chain breaking AOs such as ascorbic acid (Vitamin C), carotenoids (including retinol-Vitamin A), uric acid, a-tocopherol (Vitamin E), reduced glutathione, and polyphenols (flavonoids).

Role of AOs in Periodontitis

The presence of SOD enzyme in periodontal ligament was demonstrated by Jacoby and Davis[55] using both biochemical and immunohistochemical techniques. The activity of SOD in periodontal ligament was found to be considerably less than that in red blood cells and also decreased with age.

SOD and CAT activities were measured in human gingival tissue, and these activities were found to be reduced with the increasing periodontal pocket depth.[56] Another study showed that the activities of AO enzymes SOD, CAT, and glutathione reductase in the saliva of periodontitis patients exhibited a significant negative correlation with periodontal parameters.[1]

Similarly to ROS production, numerous studies indicate that the changes in the activity of AOs in periodontitis are influenced by systemic conditions.[1,39,57]

Smoking is also associated with decreased levels of SOD in GCF and saliva in both periodontitis patients and healthy individuals.[58] Diabetes mellitus can increase the activity of SOD and gene expression of SOD1 in gingival tissue of periodontitis patients.[36,59] However, higher activities of SOD, CAT, and glutathione reductase were found in saliva and plasma of systemically and periodontally healthy individuals compared to those with CP and/or diabetes mellitus.[1] Activities of SOD were also found to be decreased by pregnancy among periodontitis patients.[34]

AOs present a strong defense function against ROS; therefore, numerous studies tried to examine the application of AOs in the treatment of periodontitis. It has been shown that supplemental periodontal treatments with AOs such as Vitamin E, taurine, and lycopene result in improved clinical periodontal parameters, higher activities of local and systemic AOs, and lower levels of local and systemic ROS compared with conventional periodontal treatment.[60,61]

CONCLUSION

The current literature evidence points toward a strong association between OS and periodontal disease. The balance between AO mechanisms and ROS is absolutely important for periodontal health. Increased ROS and inhibited AO mechanisms and/or decreased AO

capacity compromise the homeostasis in the periodontal tissues resulting in periodontal disease. Products of lipid peroxidation, protein damage, and DNA damage can be used as the biomarkers of oxidative stress associated with periodontitis. Local and systemic activities of AOs can also be influenced by periodontitis. Different AOs have been applied as supplements to the conventional periodontal treatment and optimistic results were obtained, which provides new possibilities in the periodontal therapy.

The present review focused on the presence of oxidative stress associated with periodontitis, especially on the relationship between the local and systemic biomarkers of oxidative stress and periodontitis, giving us an implication of pathogenesis of periodontitis through oxidative stress, close relationship between periodontitis and systemic conditions, and promising therapeutic strategies involving AOs.

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