Review Article

Oxidative stress in chronic periodontitis

Abstract

It is well documented that the primary etiological agent of periodontal disease is a polymicrobial complex, predominantly gram-negative anaerobic or facultative bacteria within the subgingival biofilm. These bacteria trigger the release of numbers of cytokines, leading to elevated numbers and activity of polymorphonucleocytes (PMNs). As a result of stimulation by bacterial antigens, PMNs produce the reactive oxygen species (ROS) superoxide via the respiratory burst as part of the host response to infection. The human body does contain an array of antioxidant defence mechanisms to remove harmful ROS as soon as they are formed and to prevent their deleterious effects. This review focuses predominantly on the role of ROS and antioxidant defence systems in the pathobiology of periodontitis, with a view to identify specific therapeutic targets for future host-modulating therapies.

Key words:
Antioxidants, oxidants, periodontitis, reactive oxygen species and vitamin C

Introduction

Over the past few years, strong evidence has emerged to implicate oxidative stress in pathogenesis of periodontal disease. It is a well-known fact that free radicals and reactive oxygen species (ROS) are essential to many normal biologic processes. At low concentration, these free radicals stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations it may result in tissue injury.[1]

Bacterial pathogens from subgingival dental plaque stimulate host cells to release proinflammatory cytokines. These cytokines recruit polymorphonucleocytes (PMNs) to the site of infection. PMNs play a major role in the etiology of periodontal disease by producing proteolytic enzymes, such as elastase and O_2 (molecular oxygen) by oxidative burst.[2] The human body incorporates a plethora of complex antioxidant defence system to remove harmful ROS as soon as they are formed and to prevent their deleterious effects.

The aim of this review is to focus on the origin of different types of free radicals and ROS, antioxidant system, and the role of ROS and antioxidant defence systems in the pathobiology of periodontitis. Medline and PubMed databases were searched under the following key terms: ROS, oxygen radicals, free radicals, antioxidants, and periodontitis. This search was limited to articles on human/animal studies which were published in English language. A total of 164 articles were identified, of which 25 were review articles. But, in our review article we did not include each and every article from the searched article. After reviewing the searched articles, we came across some scientifically proven facts which enlightened the pathogenesis of periodontitis in relation to oxidative stress and antioxidant defence system.

Reactive oxygen species, free radicals, and their origin

Free radicals have been defined as any species capable of independent existence that contain one or more unpaired electrons.[3] 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 functions, which not only include oxygen free radicals but also nitrogen and chlorine species. ROS is a term that has become more popular because it encompasses other reactive

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species which are not true radicals but are nevertheless capable of radical formation in the intra- and extra-cellular environments.

True free radicals include SuperoxideHydroxyl ($O_2^{\bullet-}$), Perhydroxyl ($HO_2^{\bullet-}$), Hydroperoxyl ($HOO^{\bullet}$), Alkoxyl ($RO^{\bullet}$), Aryloxyl ($ArO^{\bullet}$), Arylperoxyl ($ArOO^{\bullet}$), Peroxyl ($ROO^{\bullet}$), Acyloxyl ($RCOO^{\bullet}$), and Acylperoxyl ($RCOOO^{\bullet}$), whereas ROS includes Hydrogen peroxide (H_2O_2), Hypochlorous acid (HOCl), Singlet oxygen ($^1O_2$), and Ozone (O_3).

Antioxidant defence systems

The living organisms have adapted itself to an existence under a continuous efflux of free radicals. Among the different adaptive mechanisms, the antioxidant defence mechanisms are of major importance. Antioxidants are those substances which when present in lower concentration compared with those of oxidizable substrate will significantly delay or inhibit oxidation of that substrate. Antioxidants can be categorized on the basis of their mode of function, their location of action, solubility, and their origin/source. Few important classifications are described in Table 1.

Measuring reactive oxygen species and oxidative stress

Free radicals and other reactive species have extremely short half-life *in vivo* $(10)^{-6}$ to $(10)^{-9}$s and simply cannot be measured directly. *In vitro*, systems called spin traps are used to measure radical species, but there are currently no suitable spin traps/probes available for *in vivo* measurement of ROS production in the human. So, measurement of ROS is done by measuring the concentration of biomarkers of tissue destruction.

Biomarkers of lipid peroxidation

There are a large number of biomarkers of lipid peroxidation, most commonly used markers are as follows: Conjugated dienes, thiobarbituric acid reactive substances (notably malondialdehyde), isoprostanes, ethane/pentane, and hydrocarbons (volatile).

Biomarkers of deoxyribonucleic acid damage

Products of hydroxyl radical attack on DNA bases (purines and pyrimidines) and carbohydrate moieties (deoxyribose) can be measured by various methods (high-pressure liquid chromatography: Gas or liquid), liquid chromatography, or antibody methods.[4] No individual reaction product should be used as the sole index of DNA damage,[5] but despite this 8-hydroxydeoxyguanosine is frequently used biomarker for DNA damage.

Biomarker for protein damage

The mechanism of ROS-mediated protein damage is highly complex. Free radicals, generated as a result of host-microbial interaction, may attack C = C bonds of proteins and produce carbon-centered radical intermediates. These intermediate products are responsible for protein folding or unfolding, protein fragmentation, protein degradation, formation of protein radicals, and stable end products such as carbonyl compounds such as oxo-acids or aldehydes (e.g., alanine to acetaldehyde).[6]

The carbonyl assay measures protein carbonyl groups formed as relatively stable end products of protein oxidation by ROS. But carbonyls are not specific biomarkers of ROS damage.[5] Acrolein, a protein-bound aldehyde, has been widely used these days to measure oxidative damage.

Measuring antioxidant status

The body's antioxidant systems are highly integrated and complex. The study of individual systems and species greatly improves our understanding of their role in human diseases. It ignores their cooperative activities and may present a picture that does not accurately represent the *in vivo* situation. As a result of their cellular and extracellular ubiquity and rapid rates of sacrificial oxidation, the free radical scavengers confer substantial protection on vital macromolecules.[7]

Total antioxidant capacity has therefore been developed to reduce the costly and time-consuming task of measuring

Table 1: Classification of antioxidants

Mode of action	Example
Preventative antioxidants	Antioxidants enzymes: Superoxide dismutase enzymes (1, 2, and 3), catalase, glutathione peroxidase, DNA repair enzymes, e.g., poly(ADP-ribose) polymerase, others Metal ion sequestrators: Albumin, lactoferrin, transferrin, haptoglobin, ceruloplasmin, hemopexin, carotenoids, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, polyphenolicflavonoids
Scavenging (chain breaking) antioxidants	Ascorbate (vitamin C), carotenoids (including retinol—vitamin A), uric acid, α-tocopherol (vitamin E), polyphenols (flavenoids), bilirubin, albumin, ubiquinone (reduced form), reduced glutathione and other thiols (free or protein bound)
Based on location	
Intracellular Extracellular	Superoxide dismutase enzymes 1 and 2, catalase, glutathione peroxidase, DNA repair enzymes, e.g., poly(ADP-ribose) polymerase, others, reduced glutathione, ubiquinone (reduced form)
Membrane associated	Superoxide dismutase enzyme 3, selenium-glutathione peroxidase, reduced glutathione, lactoferrin, transferrin, haptoglobin, ceruloplasmin, albumin, ascorbate, carotenoids, uric acid α-tocopherol

individual antioxidant species. Moreover, it may also account for the influence of antioxidant substances that are as yet undiscovered or are technically difficult to assay.

Pathophysiology of periodontal tissue destruction

Periodontal pathogens from subgingival dental plaque are main etiological agents for the initiation of inflammatory changes in the periodontal tissue. Lipopolysaccharides and DNA from these bacteria cause the activation of both activating protein-1(AP-1) and nuclear factor-κB (NF-κB) pathways in the gingival fibroblast via CD14 and Toll like Receptor-4 (TLR-4) and production of inflammatory cytokines. The cell component of bacteria and inflammatory cytokines causes recruitment and activation of hyperresponsive PMNs and thus speeds up the production of ROS. On the other hand, activation of NF-κB and AP-1 causes activation of osteoclasts and increases the concentration of matrix metalloproteinases, which ultimately results in tissue damage. Periodontal tissue destruction leads to overproduction of lipid peroxides, inflammatory mediator, and oxidized proteins. These products further activate macrophages, neutrophils, and fibroblasts to generate more ROS. In short, we can say that in the presence of periodontal pathogens, ROS and tissue destruction form a vicious circle, which has been explained in Figure 1.

Reviewing the Literature: Reactive Oxygen Species in Chronic Periodontitis

To date, only thiobarbituric acid reactive substances and malondialdehyde have been investigated in chronic periodontitis. All the published studies have suggested that patients with chronic periodontitis have higher levels of lipid peroxidation than periodontally healthy controls.

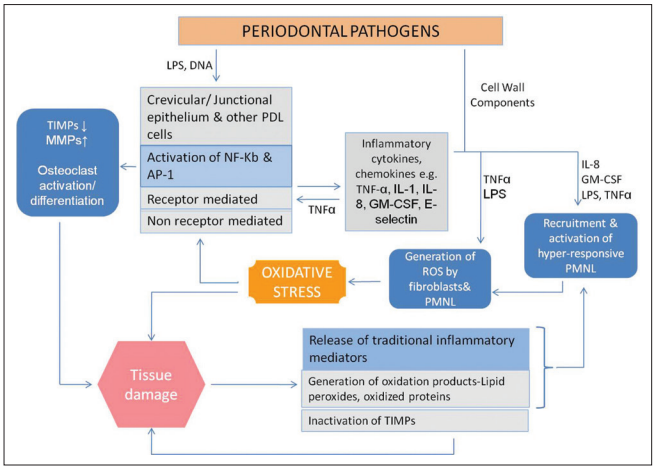

Figure 1: Simplified diagram illustrating a central role of ROS in generating chronic inflammation and tissue damage in response to periodontal pathogens. MMP - Matrix metalloproteinase; TIMP - Tissue inhibitor of matrix metalloproteinase; NF-κB - Nuclear factor kappa B; AP-1 - Activating protein-1; PDL - Periodontal ligament; TNF - Tumor necrosis factor; IL - Interleukin; GM-CSF - Granulocyte–macrophage colony-stimulating factor; LPS - lipopolysaccharide; ROS - Reactive oxygen species

Thiobarbituric acid reactive substances were raised in patients both systemically in plasma and erythrocytes[8] and locally in tissue homogenates.[8,9] Malondialdehyde was also found to be raised in gingival crevicular fluid and saliva of patients compared with controls.[10] Interestingly, the gingival crevicular fluid concentrations of malondialdehyde/4-hydroxyalkanal reported by Tsai *et al*.[10] were 200- to 400-fold higher than the respective saliva concentrations.

The majority of published data on oxidative damage to DNA has been reported by a Japanese group who investigated 8-hydroxydeoxyguanosine levels in saliva by enzyme-linked immunosorbent assay. These studies demonstrated that levels of 8-hydroxydeoxyguanosine in samples from subjects with chronic periodontitis were significantly higher than those from periodontally healthy controls.[11]

There are some studies available on evidences of protein oxidation in periodontal tissue. All these animal studies demonstrated an increased presence of inflammatory cells and nitrotyrosine-positive cells in periodontal tissues associated with ligated teeth compared with controls.[12,13]

Antioxidant Status in Periodontitis

Only few studies to our knowledge have investigated total antioxidant capacity in serum/plasma from periodontitis patients and controls. The results of these studies demonstrated significantly lower total antioxidant capacity in serum and plasma samples from periodontitis subjects.[14-16] Panjamurthy *et al*.[8] found lower plasma vitamin C, vitamin E, and GSH (reduced glutathione) in periodontitis patients even after adjusting for protein levels, whereas antioxidant enzyme levels were raised, the authors attributing this to a protective response to oxidative stress (thiobarbituric acid reactive substance levels were raised in periodontitis subjects).

Diab-Ladki *et al*.[17] found similar results to Sculley and Langley-Evans[18] in a small case–control study, the lower saliva total antioxidant capacity in periodontitis subjects being independent of salivary uric acid, ascorbate, and albumin levels, which did not differ between groups. Moore *et al*.[19] determined that the predominant antioxidant component of saliva was uric acid (>70% of antioxidant activity). Tsai *et al*. found that salivary glutathione concentrations were significantly reduced in periodontitis subjects relative to controls and that treatment increased glutathione concentrations.[10]

Guarnieri *et al*.[20] demonstrated spontaneous generation of superoxide in the gingival crevicular fluid of periodontitis

subjects, but found no differences in antioxidant scavenging capacity between cases and controls. Brock *et al*.[14] demonstrated a significantly lower total antioxidant capacity in periodontitis subjects relative to age- and sex-matched controls.

Conclusion

It has been now clear that the equilibrium between the free radicals/ROS and antioxidant is the main prerequisite for healthy periodontal tissue. The disturbance of this equilibrium either due to increased free radicals or decreased (or insufficiently increased) antioxidant might result in oxidative damage to periodontal tissue. This concept has led to search for appropriate "antioxidant therapy" and "host modulation therapy" for prevention and treatment of chronic periodontitis.

Further researches in this area may include a delivery system that would deliver antioxidant to specific cell types and/or calculation of optimum pharmaco-therapeutic dose of antioxidant for blocking inappropriate cell responses. Such studies will be very helpful in opening new vistas of research for understanding the treatment modalities for optimum generation of supporting tissues to tooth surface affected by chronic periodontitis.

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