Oxidative Stress and the Periodontium

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Abstract

This review is intended to provide a critical, up-to-date summary of the field, with particular emphasis on its implications for the application of "anti-oxidant therapy" in periodontal disease. We have reviewed the mechanisms of actions, features, and sources of most common free radicals and reactive oxygen species, as well as analyzed the biological targets for oxidative damage. Based on the direct and indirect anti-oxidant host defenses in relation to the role of polymorph nuclear neutrophils in periodontitis, there is a current evidence for oxidative damage in chronic inflammatory periodontal disease, and the possible therapeutic effects of anti-oxidants in treating and/or preventing such pathology.

Keywords- reactive oxygen species, free radicals, anti-oxidants, polymorph nuclear neutrophils, periodontal disease

I. INTRODUCTION

Oxidative stress was defined by Sies 1985[1] as "A disturbance in the pro oxidant – antioxidant balance in favour of the former leading to potential damage". It is an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to repair the resulting damage. Gowri Pendyala 2008[2] stated that Oxygen is required for all living organisms for their survival but at the same time it is potentially toxic. Oxygen is the ultimate electron acceptor in mitochondrial electron transport chain where flow of electrons ultimately produces energy in the form of ATP. The leaked out electrons are exposed to oxygen leading to formation of free radicals which causes toxicity to tissues. The living organism has adapted itself to an existence under a continuous efflux of free radicals. Among different adaptive mechanisms the anti-oxidant defense mechanism is of major importance. The body always maintains the balance between oxidant production (ROS) and anti-oxidant reserve. If the level of ROS overcomes the antioxidant level it results in "OXIDATIVE STRESS". In periodontitis, neutrophils play a central role in the initial host inflammatory response to the periodontal pathogens. After stimulation by bacterial pathogens neutrophils produce free radicals which are responsible for the majority of host-tissue destruction in periodontitis. As a result oxidative stress is enhanced during periodontitis.

Oxidative homeostasis:

The body maintains balance between free radical (oxidants) production and antioxidant reserve. Oxidative stress is the derangement of normal physiological balance between the levels of Reactive oxygen species (ROS) and the endogenous antioxidant defenses of the host. ROS is a collective term for radical or non-radical forms of partially reduced oxygen.

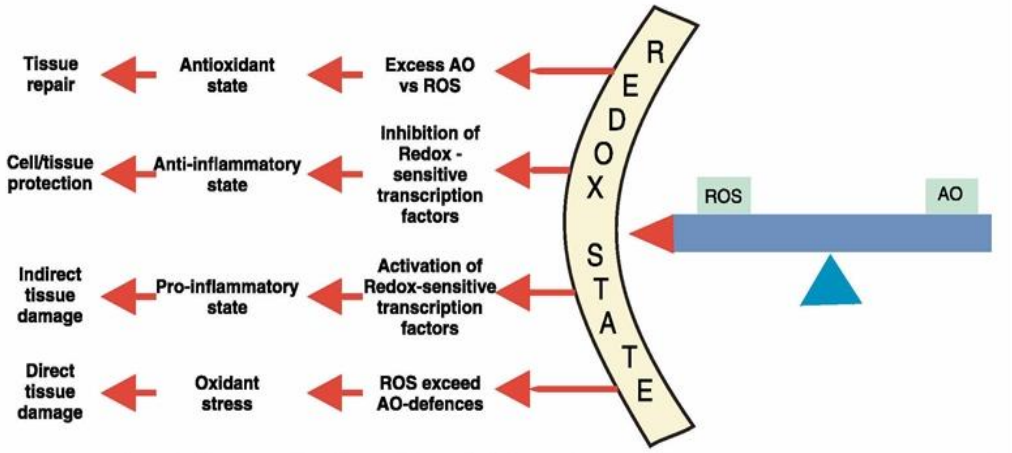

Fig. 1: The balance of activity between ROS and AO species.

II. CLASSIFICATION OF PRO OXIDANTS

Alok Sharma et al 2011[3] stated that pro oxidants are of two forms namely True Radicals and Reactive Oxygen Species (ROS). Free radicals are highly reactive and diverse species including not only oxygen species but also nitrogen and sulphur species, while the hydrogen radical (H•) is same as the hydrogen atom which is the simplest free radical containing only one proton and one electron. Reactive Oxygen Species (ROS) is a collective term which includes all oxygen derived free radicals, including those reactive intermediate oxygen species formed which are not true radicals but capable of radical formation in the intracellular and extracellular environment.

Table - 1 Reactive Oxygen Species (Battino et al 1999)[4]

True radicals	*ROS*
Superoxide	*Hydrogen peroxide*
Hydroxyl	*Hypochlorous acid*
Perhydroxyl	
Hydroperoxyl	*Singlet oxygen*
Alkoxyl	*Ozone*
Aryloxyl	
Arylperoxyl	
Peroxyl	
Acyloxyl	
Acylperoxyl	

A. Source and Function of ROS and Oxygen True Radicals:

1) Exogenous Sources:

Halliwell B 1992[5] stated that ROS may be produced from heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise. Canakci et al 2005[6] concluded that over dose of the analgesic paracetamol (therapeutic drug) may also produce true radicals which cause tissue damage.

2) Endogenous Sources:

Bi-products of metabolic pathways-electron leakage from mitochondrial electron transport systems forming superoxide and functional generation of ROS by host defense cells (phagocytes) and cells of the connective tissues (osteoclasts and fibroblasts).

B. Anti-Oxidant Defense Systems (Chappel& Halliwel B 2007[7]):

1) Based On Mode Of Action:

- Preventive antioxidants: Superoxide dismutase1, 2, 3, glutathione peroxidase, DNA repair enzymes, Albumin, Lactoferrin, Transferrin, Carotenoids, Uric acid.
- Scavenging antioxidants: Vitamin C, Carotenoids, Vitamin-A, Bilirubin, Albumin,
- α-Tocopherol, Polyphenols.

2) Based On Location:

- Intracellular: Superoxide dismutase1, 2, glutathione peroxidase, DNA repair enzymes
- Extracellular: Selenium, Glutathione peroxidase, Ascorbate, Carotenoids, Lactoferrin, Transferrin, Hepatoglobin, Ceruloplasmin, Uric acid.
- Membrane associated: α-Tocopherol.

3) Based On Solubility:

- Water soluble: Hepatoglobin, Ceruloplasmin, Albumin, Vitamin-C, Transferrin, Flavenoids.
- Lipid soluble: α-Tocopherol, Carotenoids, Bilirubin.

4) Based On Structures They Protect:

- DNA protective antioxidants: Superoxide dismutase1, 2, Glutathione peroxidase, DNA repair enzymes, reduced glutathione.
- Protein protective antioxidants: Sequestration of transition metals by preventive antioxidants.
- Lipid protective antioxidants: α-Tocopherol, Ascorbate, Carotenoids, Glutathione peroxidase.

5) Based On Their Origin:

- Exogenous antioxidants: Carotenoids, Ascorbic acid, Tocopherol, Flavenoids, Folic acid.

- Endogenous antioxidants: Catalase, Superoxide dismutase (SOD), Glutathione peroxidase, Ceruloplasmin, Transferrin, Ferritin.
- Synthetic antioxidants: N-acetyl cysteine, Penicillinamine, Tetracycline

III. ROLE OF REACTIVE OXYGEN SPECIES IN PERIODONTAL TISSUE DAMAGE

A. Halliwell's Postulates:

The four criteria proposed by Halliwell in 2000 required to fulfill before ROS can be concluded to be key mediators of tissue injury in a given disease similar to those proposed by Robert Koch in 1884.

1) ROS or the oxidative damage caused must be present at site of injury.
2) The time course of ROS formation or the oxidative damage caused should occur before or at the same time as tissue injury.
3) Direct application of ROS over a relevant time course to tissues at concentrations found in vivo should reproduce damage similar to that observed in the diseased tissue.
4) Removing or inhibiting ROS formation should decrease tissue damage to an extent related to their antioxidant action in vivo.

B. Direct Actions of ROS in Periodontal Destruction:

Canakci et al 2005[6] stated that excessive production of ROS principally by PMNLs in periodontal disease causes indiscriminate damage to cellular components like DNA molecules, lipid membranes and proteins, as well as extracellular matrix components of the periodontal tissues.

Waddington et al 2000, Canakci et al 2005[6] stated that ROS have also been demonstrated to be capable of degrading bone proteoglycans and collagen degradation, resulting in a reduction of collagen gelation, increased aggregation, cross-linking and collagen insolubility.

C. Indirect Actions of ROS in Periodontal Destruction:

ROS are capable of causing cell injury indirectly by enhancing pro-inflammatory gene expression, including cytokines (e.g. TNFα. IL-1), chemokines (e.g. IL-8) and cellular adhesion molecules. The transcription factors like NF-kβ and AP-1 are redox sensitive and ROS are thought to modulate their activity. ROS are also believed to increase apoptosis. Induction of apoptosis may be seen in response to DNA damage, which occurs through ROS, particularly NO•.

D. Factors Affecting the ROS Generation by Neutrophils at the Site of Disease:

- Plaque bacteria and their products.
- Opsonized bacteria associated with periodontal disease (F.nucleatum, Aggregatibacter actinomycetemcomitans).
- Hyper reactive phenotype of PMNs.
- F.nucleatum induces significantly greater ROS generation than Phagocytosis of P.gingivalis or Aggregatibacter actinomycetemcomitans.
- Pro-inflammatory cytokines (TNF-α, GMCF, IL-8, IL-1).
- Growth factors (platelet activating factor).
- Lipopolysaccharides (LPS).
- Cytokines can modulate the respiratory burst activity of neutrophils and have a role in determining oxidative stress locally within the tissues.

E. Systemic and Local Presence of the Products of ROS in Periodontitis:

The majority of tissue destruction in periodontitis is considered to be the result of an inflammatory immune response to microbial plaque adjacent to the gingival margin and to involve prolonged release of neutrophil enzymes and ROS. Patients with chronic periodontitis have higher levels of lipid peroxidation than periodontally healthy controls. Of the three main markers of lipid peroxidation in common use (thiobarbituric acid reactive substances, malondialdehyde, isoprostane), Isoprostanes are considered the best available marker. To date, only thiobarbituric acid reactive substances and malondialdehyde have been investigated in chronic periodontitis.

F. Redox-Sensitive Signaling Pathways and Periodontal Disease:

1) Signaling Cascade Given By Forman H J Et Al 2004[8]

In an unstimulated state the protein intermediate is non-phosphorylated because of the greater activity of PTP(protein tyrosine phosphatase) v_s PTK(protein tyrosine kinase). Ligand binding causes receptor-associated NADPH oxidase (NOX) to generate hydrogen peroxide that inactivates PTP resulting in formation of the phosphorylated protein intermediate by PTK. This may also involve a second signal from the ligand-receptor interaction that activates PTK. Receptor-signaling cascades in normal cell function are controlled by local changes in redox balance and oxidation of GSH. The overall redox balance within the cell is maintained by glutathione and the ratio of GSH: GSSG. Oxidative stress as a result of excessive generation of ROS has the capacity to activate

redox sensitive signaling pathway in the absence of specific ligand-receptor interaction to cause irreversible damage to cellular proteins by overcoming the stabilizing effect of intracellular GSH.

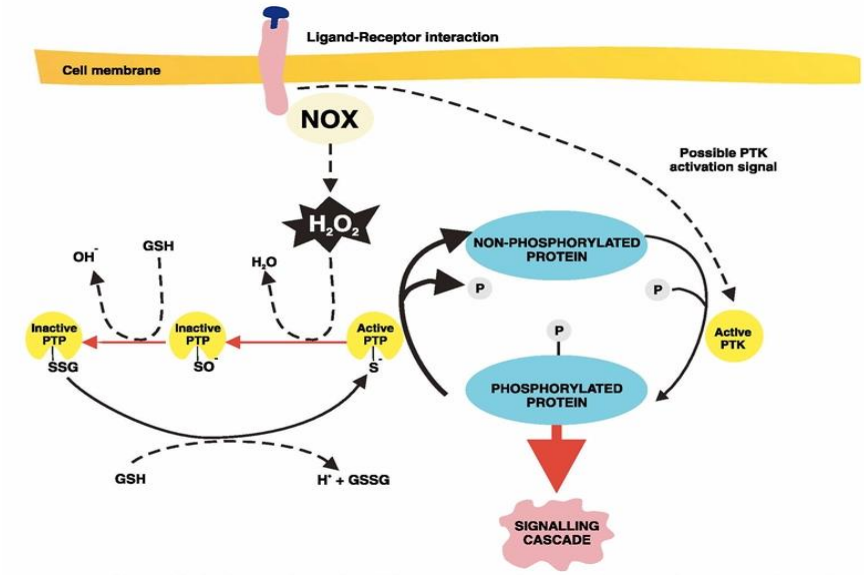

Fig 2: Signaling cascade

IV. AVAILABLE ANTIOXIDANTS FOR TREATMENT OF PERIODONTAL DESTRUCTION

A. Mouthwashes with Antioxidant Properties:

Host antioxidant defenses could benefit from mouthrinses used as adjunct to counteract plaque-associated bacteria. Battino M 2002[9] stated that mouthrinses with antioxidants as 'active ingredients' have direct effect on decreasing the oxidative damages suffered by the tissues during inflammation. Listerine has antioxidant activity because of methylsalicylate. Antioxidant activity (AA) of methylsalicylate is not surprising because salicylic acid and its derivatives readily react with $OH^{\cdot}$. Thymol has some antioxidant properties. Remarkable antioxidant activity was found for two more compounds of mouth rinses. Those are ZnCl2 and NaF. Two mechanisms are attributed to the antioxidant role of zinc. One is the protection of sulfhydryl groups against oxidation; the other involves the prevention of H_2O_2 and superoxide production by transition metals. The strong AA of NaF is very peculiar and could be explained only by the specific chemical properties of both fluoride ions and iron ions arranged in a hexa coordinated complex. NaF probably acts as a preventive antioxidant (by binding to the iron of myoglobin causing its functional exclusion from the assay mixture) rather than a scavenger antioxidant capable of inactivating ROS molecules.

B. Toothpaste with Antioxidant Properties:

Battino et al 2005[10] conducted an in-vitro study with cell free antioxidant assay and comet assay for keratinocytes cell line to evaluate the antioxidant effect of toothpastes enriched alternatively with sodium ascorbyl phosphate, alpha-tocopherol acetate, pycnogenol, allantoin and methylsalicylate or a mixture of these. They concluded that only toothpastes containing sodium ascorbyl phosphate displayed clear antioxidant activity with values ranging between 50 - 80 mg of toothpaste/ml water. The antioxidant activities of $ZnCl_2$ and NaF were significant which is because of the ability of Zn^{+2} to protect Thiol groups and prevent H_2O_2 and superoxide formation by transition metals and the ability of fluoride ion to form complex divalent iron ions.

C. Effect Of Green Tea Catechin, A Local Drug Delivery System As An Adjunct To Scaling And Root Planing In Chronic Periodontitis Patients:

The most abundant polyphenolic compound epigallocatechin gallate (EGCG) contributes to the beneficial antioxidant effects of green tea like anti-inflammatory, antioxidant, anti-diabetic, antimutagenic and antiviral properties. Yun JH et al 2004[11] stated that Green tea catechin significantly reduced the expression of matrix metalloproteinase-9(MMP-9) in osteoblasts and also inhibits the formation of osteoclasts. Thus, it may prevent alveolar bone resorption that occurs in periodontal disease. Green tea extract can be used as mouthwash for treating oral and periodontal infections. Maroofian et al 2011[12] extracted green tea polyphenols in a safe and stable formula for using as a mouthwash and demonstrated that green tea extract in mouth wash had therapeutic effects and could improve gingival status of patients with gingivitis and periodontitis. Anti-microbial Polyphenols in green tea leaf extract can improve oral malodor by suppressing those bacteria which causes production of volatile sulfur compounds (VSC) by proteolytic degradation.

Green tea catechin strips are transparent rectangular shaped with size of 2mm in width 4mm in length and 0.3mm in thickness with catechin used from green tea powder and hydroxyl propyl cellulose as carrier. These strips were sterilized by gamma radiation of dose 5k Gy

Hirasawa et al 2002[13] conducted a study to evaluate the periodontal status improvement by green tea catechin using a local drug delivery system. They showed that green tea catechin has bactericidal activity against black pigmented organism at a minimum inhibitory concentration of 1 mg/ml. Therefore the concentration of green tea catechins used in the chips prepared was decided to be maintained at 1mg/ml. Praveen kudva et al 2012[14] conducted a clinical and microbiological study in 14 patients (30-55 yrs) of chronic periodontitis with pocket depth of 5-8mm. Selected sites were divided randomly into test group which received scaling and rootplaning (SRP) along with green tea catechin and control group which received SRP only. Finally they reported that green tea catechin local delivery along with scaling and rootplaning was more effective than scaling and rootplaning alone.

D. Role of Antioxidants as an Adjunct in Periodontal Therapy:

Nutritional counselling and supplementation may very well reduce inflammation and thereby enhancing the outcome of conventional periodontal therapy. Antioxidants are the nutrients in fruits and vegetables that can neutralize free radicals by donating an electron without becoming unstable themselves. Poor nutrients do not cause periodontal disease directly but the disease may be more severe in people with nutrient-poor diet because of compromised host response.

RG Shiva Manjunath 2011[15] stated that selenium has the strongest association with gum diseases. Low levels of vitamin A, vitamin C, α-carotene also increased the risk of gum disease significantly. Periodontal tissue depends on natural antioxidants to overcome this Oxidative stress and maintain homeostasis.

V. CONCLUSION

Oxidative stress lies at the heart of periodontal tissue damage that results from host microbial interactions, either as a direct result of excessive ROS activity, antioxidant deficiency and activation of transcription factors and the creation of pro inflammatory state. While a myriad of possible mechanisms leading to periodontal destruction exists, the influence of free radicals and antioxidants cannot be over looked undoubtedly.

Antioxidant defense system is very dynamic and responsive to any disturbance taking place in redox balance of body. Antioxidants can be up regulated and neutralizes free radical formation that could take place due to oxidative stress. Several avenues of enquiry now exist for the development of anti-oxidant based approaches to periodontal therapy which includes traditional routes of increasing the antioxidant capacity of periodontal tissues and newer routes based on modulation of transcription factors. These arrays of pathways provide opportunities to develop novel antioxidant therapies that target the free radicals and which function not only as antioxidants in the traditional sense but also as powerful anti-inflammatory agents.

Oxidative stress which enhances periodontal tissue damage can be controlled by antioxidant therapy. Hence, the deterioration of periodontal damage can be controlled by administering antioxidant therapy which also helps in the improvement of general health.

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