**Reactive Oxygen Species and Antioxidants in Periodontics: A Review**

**Alok Sharma, Swati Sharma**

**Abstract**

Various forms of antioxidants have been introduced as an approach to fight dental diseases and improve general gingival health. This article focuses on the classification of antioxidants and the link between oxidative stress and periodontal disease.

**Key Words:** Oxidative Stress; Antioxidants; Periodontitis

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Periodontitis is a term used to describe an inflammatory process, initiated by the plaque biofilm, that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. (1) Primary etiologic agents for gingival and periodontal diseases have been shown to be predominantly gram negative anaerobic or facultative anaerobic bacteria within the sub gingival biofilm. (2) However the majority of periodontal tissue destruction is caused by an exaggerated host response to those organisms and their products. (3)

The neutrophils play a pivotal role in host defense and are the first line of defense against this infectious periodontal disease. (4) Neutrophils have several selective mechanisms for controlling bacterial invasion, including both intracellular and extracellular oxidative and non-oxidative killing mechanisms. (5) The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS). (5) Free radicals are a family of highly reactive and diverse species, capable of extracting electrons and thereby oxidizing a variety of bio molecules vital to cell and tissue function, which not only includes oxygen free radicals, but also nitrogen and chlorine species. (4)

In recent years, the term "reactive oxygen species" (ROS) has been adopted to include molecules such as hydrogen peroxide (H₂O₂), hypochlorous acid (HOCI) and singlet oxygen (¹O₂), which though, not radical in nature, are capable of radical transformation in the extra and intracellular environments. (6)

Whilst most ROS have extremely short half-lives, they can cause substantial tissue damage by initiating free radical chain reactions. Therefore the body contains a number of protective Anti-Oxidant (AO) mechanisms, whose specific role is to remove harmful oxidants (ROS), as soon as they form, or to repair the damage caused by ROS in vivo. (6)

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when that equilibrium shifts in favour of ROS, either by reduction in anti-oxidant defenses or an increase in ROS production or activity, oxidative stress results. This imbalance between the ROS-AO has been implicated as one of the progressive or pathogenic factors for periodontal disease. In this monograph, we discuss the current status of ROS and AO systems and their influence on the periodontal disease. (6)

**Reactive oxygen species:** The various true radicals and reactive oxygen species (ROS) and their symbols have been summarized in Table 1.

<table>
<thead>
<tr>
<th>RADICALS</th>
<th>NON-RADICALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O₂</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>OH</td>
</tr>
<tr>
<td>Hydroperoxyl</td>
<td>HOO</td>
</tr>
<tr>
<td>HOCl</td>
<td>Hypochlorous acid</td>
</tr>
<tr>
<td>Alkoxyl</td>
<td>RO</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydroperoxide</td>
</tr>
<tr>
<td>Aryloxyl</td>
<td>ArO</td>
</tr>
<tr>
<td>Arylperoxyl</td>
<td>ArOO</td>
</tr>
<tr>
<td>Peroxyl</td>
<td>ROO</td>
</tr>
<tr>
<td>Acyloxyl</td>
<td>RCOO</td>
</tr>
<tr>
<td>Acylperoxyl</td>
<td>RCOOO</td>
</tr>
</tbody>
</table>

**Exogenous source** are heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise and therapeutic drugs. (7)

**Endogenous source** (7) which result in formation of ROS include:-

a. Bi-products of metabolic pathways:- During cell metabolism, electrons leak from their transporters at a constant rate during the process of glycolysis, reducing oxygen to superoxide anion.

b. ROS can also be generated by phagocytes and cells of the connective tissue. The process comprises the “respiratory burst” and is stimulated by a variety of mitogens or antigens. (8)

**Mechanisms of Tissue Damage**

The reactive oxygen species may cause damage to various cellular and extracellular tissues by targeting the following substances:
1. Protein damage: It results in fragmentation and polymerization reactions of various protein molecules leading to the formation of protein radicals and protein-bound ROS.(8)
2. Lipid peroxidation: It is one of the most important reactions of free radical species. Hydroxyl and peroxynitrite anion are most effective in activating this process.(9)
3. DNA damage: The mechanism of DNA damage by peroxynitrite and hydroxyl radical include; Strand breaks, Base pair mutations, Deletions, Insertions, Nicking and Sequence amplifications.(10)

Evidence for the Presence and Role of ROS in Periodontal Tissue Damage: Halliwell proposed four criteria, similar to that proposed by Robert Koch in 1884, to establish causal relationship between an organism and disease.(7)
1. ROS or the oxidative damage caused must be present at the 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.

In patients with early onset periodontitis, low superoxide production is seen after fMLP stimulus of blood neutrophils. Even in patients with adult periodontitis, increased generation of free oxygen radicals and increased ratio between released elastase and lactoferrin is seen in peripheral neutrophils thus causing tissue destruction.(7)

Enhanced ROS generation by peripheral neutrophils from patients with both chronic and aggressive disease has been shown to be stimulated with opsonized bacteria associated periodontal disease (Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans). This finding suggests that the hyper active phenotype of peripheral neutrophils could have local tissue damaging consequences.(11)

Myeloperoxidase is released into the phagosome and extracellularly during phagocytosis and activation of neutrophils when it is important for generating hypochlorous acid and other ROS. Raised levels of all three forms of myeloperoxidases in gingival crevicular fluid from diseased sites have been reported in gingivitis, chronic periodontitis(12), rapidly progressive periodontitis, localized juvenile periodontitis and aggressive periodontitis. These studies have demonstrated a relationship between gingival crevicular fluid level of myeloperoxidases and clinical measures of the disease and that the levels reduce after treatment.(13)

Smoking, which is an important risk factor for periodontitis, induces oxidative stress in the body and causes an imbalance between reactive oxygen species (ROS) and antioxidants, such as superoxide dismutase (SOD). A progressive reduction in SOD levels has been seen from healthy non-smokers to light smokers to heavy smokers, thus highlighting the role of oxidative stress in causing periodontal disease in smokers.(14)

Effects of ROS on Periodontal Tissues and Components: The reactive oxygen species cause periodontal tissue damage by,
1. Ground substance degradation
2. Collagenolysis either directly or indirectly or as a result of oxidation of proteases
3. Stimulation of excessive pro-inflammatory cytokine release through NF-κB activation
4. PG-E2 production via lipid peroxidation and superoxide release, both of which have been linked with bone resorption
5. Since IL-1 & TNF-α positively regulate their own production, the additive effects of endotoxin-mediated cytokine production and that arising from respiratory burst of PMNLs in response to the same organisms, lead to periodontal inflammation and subsequent attachment loss.(7)

Thus role of reactive oxygen species on periodontal tissue can be summarized as shown in the figure 1. While most ROS have extremely short half-lives, they can cause substantial tissue damage by initiating free radical chain reactions. Therefore the body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants (ROS) as soon as they form or to repair damage caused by ROS in vivo.

Antioxidants are defined as “those substances which when present at low concentrations, compared to an oxidisable substrate, will significantly delay or inhibit oxidation of that substrate.”(15) The various antioxidants are discussed below.

Antioxidants can be categorized by several methods:

| Preventative | Enzymes- superoxide dismutase, catalase, glutathione peroxidase, DNA repair enzymes.
| Metal ion sequestrators- albumin, lactoferrin, transferrin, ceruloplasmin, uric acid, Polyphenolic flavonoids.
| Scavenging | Ascorbate, Carotenoids, uric acid, vitamin E, bilirubin, reduced glutathione & other thiols. |
II. According to their location:

<table>
<thead>
<tr>
<th>Location</th>
<th>Enzymes/Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular</td>
<td>Superoxide dismutase enzymes-1 &amp; 2, catalase, glutathione peroxidase, DNA repair enzymes &amp; reduced glutathione.</td>
</tr>
<tr>
<td>Extracellular</td>
<td>Superoxide dismutase enzyme-3, selenium glutathione peroxidase, reduced glutathione,</td>
</tr>
<tr>
<td>Membrane</td>
<td>α-tocopherol</td>
</tr>
</tbody>
</table>

III. According to their solubility:

<table>
<thead>
<tr>
<th>Solubility</th>
<th>Proteins/Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble</td>
<td>Haptoglobin, ceruloplasmin, albumin, ascorbate, uric acid, Polyphenolic flavonoids, reduced glutathione &amp; other thiols, cysteine &amp; transferring</td>
</tr>
<tr>
<td>Lipid soluble</td>
<td>α-tocopherol, Carotenoids, bilirubin, quinones</td>
</tr>
</tbody>
</table>

**Ascorbic:** Ascorbic acid is the only endogenous antioxidant in plasma that can completely protect against peroxidative damage induced by the oxidants released from activated neutrophils. The protection is provided by scavenging superoxide and peroxyl radicals and decrease the pro-inflammatory gene expression via effects on nuclear factor κB transcription factor.(16)

*α- Tocopherol* (Vitamin E) is generally regarded as the most important and effective lipid soluble anti-oxidant in vivo. It is vital in maintaining cell membrane integrity against lipid peroxidation by peroxyl radical scavenging. Its antioxidant behavior is the result of a single phenolic OH group, which when oxidized gives rise to vitamin E radical.(7)

**Carotenoids** provide protection by formation of singlet oxygen and peroxyl radical. Carotenoids are derived only from diet (green vegetables, tomatoes, fruits), lycopene predominates in plasma, with tomatoes being the main dietary source in humans (other sources include red grapefruits & watermelon).(7)

**Co-Enzyme Q10** exists in an oxidized form (ubiquinone or CoQ) and a reduced form (ubiquinol or CoQH2), both of which possess anti-oxidant activity. CoQ10 is also regarded as a pro-oxidant molecule in response to various pathophysiological events.(17)

**Polyphenols** (Polyphenolic flavonoids) are absorbed following dietary intake of vegetables, red wine and tea. The cellular mechanisms involved in FR/ROS protection is mainly due to their direct antioxidant properties (e.g. by sparing vitamin E or by regenerating vitamin C) or to their inhibitory activity towards lipoxygenase.(18)

**Glutathione** plays a major role in maintaining the intracellular redox balance and thus regulating signaling pathways which are affected by oxidative stress.(19)

**Lazaroids** are 21-aminosteroids. They are a newly identified family of compounds which are derived from glucocorticoids, but lack both glucocorticoid and mineralocorticoid activities. These compounds scavenge lipid peroxyl radicals and inhibit iron-dependent lipid peroxidation by a mechanism similar to that of vitamin E.(7)

**Conclusion**

The following points can be concluded at the current time with respect to reactive oxygen species and antioxidants in periodontics

1. Reactive oxygen species cause periodontal tissue destruction either by degrading the ground substance or by release of collagenases or by release of various inflammatory mediators.
2. Antioxidants remove these harmful oxidants (reactive oxygen species) as soon as they form or repair the damage caused by ROS in vivo.

3. A delicate balance exists between antioxidant defense and repair systems and pro-oxidant mechanism of tissue destruction which if tipped in favour of tissue damage, could lead to significant attachment loss.

4. This array of pathways in periodontal tissue destruction provides opportunities to develop novel antioxidant therapies.

5. However, definitive involvement of this ROS and reduced antioxidant activities has not yet been established and further studies are required.

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References


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