Assessment of Superoxide Dismutase Activity in Chronic and Aggressive Periodontitis

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Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host immune-inflammatory response and subsequent alterations in bone and connective tissue homeostasis ***(Kornman, 2008),*** ending with periodontal ligament, alveolar bone and root cementum destruction which leads finally to tooth loss ***(Dereka et al., 2006).***

Following intensive discussions based on a comprehensive review of literature a decision was made on a new classification of the diseases. The resulting classification of the different forms of periodontitis was simplified to describe the three general clinical manifestations of periodontitis: chronic periodontitis, aggressive periodontitis and periodontitis as a manifestation of systemic diseases ***(Armitage, 1999).***

Chronic periodontitis is associated with the accumulation of plaque and calculus and generally has a slow to moderate rate of disease progression, but periods of more rapid destruction may be observed ***(Novak and Novak, 2006).*** Aggressive periodontitis comprises a group of rare, often severe types of periodontal diseases that affect mainly young patients, occur in localized and generalized forms, have a rapid attachment loss and bone resorption, and are marked by familial aggregation ***(Carvalho et al., 2009).***

Studies have demonstrated that periodontal disease affects between 10% and 15% of the world population, representing the greatest cause of tooth loss. There is

a strong evidence that this disease affects a specific, predisposed group of the population that presents an exacerbated inflammatory/immune response to the periodontopathogenic bacteria that accumulate on the teeth and around the gingival tissue, which in turn may lead to tissue damage **(*Borges et al., 2007).***

Infection by Gram negative microorganism is now well recognized as the primary aetiologic determinant of periodontal disease ***(Van Dyke, 2008).*** There are more than 300 distinct species of bacteria present in the gingival area of the mouth, most of which exist in a commensal relationship with the host. However, three species have been identified as being ubiquitous in periodontal plaque formation. These bacteria, known as periodontal pathogens, are *Aggregatibacter actinomycetemcomitans, Tannerella forsythia and Porphyromonas gingivalis* ***(Sculley and Langley-Evans, 2002).***

Once the bacterial components or their products interact with the epithelium and penetrate into the underlying connective tissue, the small blood vessels immediately below the junctional epithelium become dilated with increase in capillary permeability. Consequently, migration of neutrophils from the blood vessels through the junctional epithelium and into the gingival sulcus is markedly increased. Meanwhile, the perivascular collagen and other components of the extracellular matrix surrounding the blood vessels are destroyed ***(Page and Kornman, 1997 ; Page et al., 1997)***.

Although the acute inflammatory response that is resolved in a timely manner prevents tissue injury, inadequate resolution and failure to return tissues to

homeostasis results in neutrophil mediated destruction and chronic inflammation ***(Van Dyke, 2008).***

This results in changes of junctional epithelium position, loss of connective tissue attachment, loss of alveolar bone and finally irreversible changes in cementum morphology and biochemical composition ***(MCculloch, 1993)***. Consequently, periodontitis is considered the major etiological factor that causes tooth loss in adults ***(Andriamanalijaona et al., 2006)***.

Although the primary aetiologic basis for periodontal disease seem to be bacterial, the excessive host inflammatory response and/or inadequate resolution of inflammation may be critical to the pathogensis of periodontitis. Host immune-inflammatory mechanisms are activated by bacterial products. Such activation of the host response induces the expression of antibodies as well as activating polymorphnuclear leukocytes (PMNs) in an attempt to control the microbial challenge in the gingival sulcus. In addition, cytokines and prostanoids, as well as matrix metalloproteinases activated through the host response, may stimulate damage to connective tissue and bone and shape the clinical presentation of disease ***(Kornman, 2008).***

Several mechanisms of periodontal tissue destruction have been proposed and few studies have suggested the involvement of reactive oxygen species (ROS) in periodontal tissue destruction ***(Del Maestro, 1994; Hall et al., 1995; Akalin et al., 2005).*** The strong evidence linking ROS to the pathological destruction of the connective tissue during periodontal disease rests on the

presence of neutrophil infiltration as the main event in the host response to bacterial invasion ***(Waddington et al., 2000).***

Neutrophils represent the first line of cellular host response against bacteria in the gingival sulcus. The antimicrobial activities of neutrophils include oxygen-dependent and oxygen-independent mechanisms. The oxygen-dependent pathway involves the production of reactive oxygen species which are molecules capable of initiating periodontal tissue destruction. The production of reactive oxygen species by neutrophils is primarily focused towards bacterial killing, but extracellular release of reactive oxygen species results in collateral damage of the surrounding tissues ***(Guentsch et al., 2009).***

Reactive Oxygen Species (ROS)

The causes of the poisonous properties of oxygen were obscure prior to the publication of Gershman's free radical theory of oxygen toxicity in (1954), which states that the toxicity of oxygen is due to the partially reduced forms of oxygen (***Gerschman et al., 1954*).** The world of free radicals in biological systems was soon thereafter explored by Denham Harman who proposed the concept of free radicals playing a role in the ageing process ***(Harman, 1956)***. This work gradually triggered intense research into the field of free radicals in biological systems ***(Valko et al., 2007).***

A second epoch of the research of free radicals in biological systems was explored in (1969) when McCord and Fridovich discovered the enzyme superoxide dismutase (SOD) and thus provided convincing evidence about the importance of free radicals in living systems ***(McCord and Fridovich, 1969).***

A third era of free radicals in biological systems dates from (1977) when Mittal and Murad provided evidence that the hydroxyl radical ($\dot{}$OH) stimulates activation of guanylate cyclase and formation of the "second messenger" cyclic guanosine monophosphate (cGMP) ***(Mittal and Murad, 1977)***. Since then, a large body of evidence has been accumulated that living systems have not only adapted to a coexistence with free radicals but have developed various mechanisms for the advantageous use of free radicals in various physiological functions ***(Valko et al., 2007).***

ROS is a collective term which includes oxygen derived free radicals, such as the superoxide radical (O_2^-), hydroxyl radical (^-OH) and nitric oxide radical (NO^-) species, and non-radical derivatives of oxygen, such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), table (1) ***(Waddington et al., 2000).***

Free oxygen radicals can be defined as any chemical species capable of independent existence and contains one or more unpaired electrons. It is important that, when free radicals react with non-radicals, a new radical can occur, which may result in chain reactions of free radical formation ***(Canakci et al., 2005).***

Molecular oxygen (dioxygen) has a unique electronic configuration. The addition of one electron to dioxygen forms the superoxide anion radical (O_2^-). Superoxide anion is considered the "primary" ROS, and can further interact with other molecules to generate "secondary" ROS ***(Miller et al., 1990).***

Sources of Reactive Oxygen Species

ROS are formed in all living organisms as byproducts of normal metabolism (endogenous sources) as mitochondrial respiratory chain and PMN activation in inflammation or as a consequence of exposure to environmental compounds (exogenous sources) as ionizing radiation, uremic milieu, pollutants such as automobile emissions and chemical oxidants in air, water, and food chains also contribute to the oxidant challenge, as do behavioral activities such as smoking and using areca nut and lime ***(Canakci et al., 2005)***.

Table (1): True radical and reactive oxygen species (ROS) and their symbols ***(Chapple and Matthews, 2007)***

True radicals	Radical symbol	Non radical	symbol
Superoxide	O_2^-	Hydrogen peroxide	$H_2 O_2$
Hydroxyl	$^-$OH	Hypochlorous acid	HOCl
Perhydroxyl	HOO•	Singlet oxygen	$1O_2$
Hydroperoxyl	HO_2•	Ozone	O_3
Alkoxyl	RO•		
Aryloxyl	ArO•		
Arylperoxyl	ArOO•		
Peroxyl	ROO• -		
Acyloxyl	RCOO•		
Acylperoxyl	RCOOO•		

Mitochondria as a Source of ROS. Mitochondria are unique organelles as they are the main sites of oxygen metabolism, accounting for approximately 85 - 90% of the oxygen consumed by the cell ***(Canakci et al., 2005)***. Cell metabolism involves the consumption of oxygen and its utilization via glycolysis to form pyruvate within the mitochondria. The amino acid cycle follows and adenosin tri-phosphate (ATP) is generated ***(Chapple and Matthews, 2007).*** Mitochondria thereby produce ROS as a byproduct. During energy transduction, a small number of electrons leak to oxygen prematurely, so under physiological conditions, about 1–3% of the oxygen molecules in the mitochondria are converted into superoxide ***(Valko et al., 2007).***

The mechanisms by which ROS are generated *in vivo* have now been well characterized, table (2). The primary product of oxygen metabolism in the mitochondrial respiratory chain is superoxide radical (O_2^-). Formation of O_2^- occurs via the transfer of one electron to molecular oxygen. After producing this radical, it is scavenged by the mitochondrial enzyme manganese superoxide dismutase (Mn SOD) to produce hydrogen peroxide (H_2O_2) ***(Canakci et al., 2005)***. The hydrogen peroxide molecule does not contain an unpaired electron and thus is not a radical species. Under physiological conditions, the production of hydrogen peroxide is estimated to account for about ~2% of the total oxygen uptake by the organism ***(Valko et al., 2006).***

Despite the ROS-detoxifying mechanism, in the presence of reduced transition metals such as Fe^{2+}, hydrogen peroxide can produce the highly reactive hydroxyl radical which is the most reactive radical and can cause extensive damage to proteins, lipids, and especially DNA molecules **(*Canakci et al., 2005)***.