Clinical and Radiographic Evaluation of Antioxidants as an Adjunctive Therapy with Modified Widman Flap in Management of Patients with Chronic Periodontitis

Thesis

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بسم الله الرحمن الرحيم
"وما أوتيتم من العلم إلا قليلا"
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Dedication

I would love to dedicate my effort in this work to my generous gift from God; my "Parents", who guided me through my whole life with love & support, my dear sister and brother for every moment they tried hardly to give me out of their precious time.

CONTENTS

LIST OF ABBREVIATIONS

α 1-Pi	:	α1-antitrypsin
α-tocopherol	:	Vitamin E
AA	:	Ascorbic acid
AC	:	Alveolar crests
AO	:	Antioxidant
ascorbic acid	:	Vitamin C
bNOS	:	Nitric oxide synthase – brain enzyme
CAL	:	Clinical attachment loss
CAL	:	Clinical attachment level
CCD	:	Charge-coupled device
CMOS	:	Complimentary metal oxide semiconductor
CTGF	:	Connective tissue growth factor
DDR	:	Direct Digital radiography
ECM	:	Extracellular matrix
EIC	:	Elastase –inhibitory capacity
eNOS	:	Nitric oxide synthase – endothelial cell enzyme
GCF	:	Gingival crevicular fluid
GI	:	Gingival index
GSH	:	Reduced glutathione
H2O2	:	Hydrogen peroxide
HOCl	:	Hypochlorous acid
IKB	:	Inhibited form of nuclear factor KB
iNOS	:	Inducible nitric oxide synthase
IU	:	International unit
LPS	:	Lipopolysacharide
MB	:	Mesiobuccal
ML	:	Mesiolingual
MMP	:	Matrix metalloproteinase
MPO	:	Myeloperoxidase
MWF	:	Modified Widman flap

NF-ϰB	:	Nuclear factor ϰB
NO-	:	Nitric oxide radical species
NOS	:	Nitric oxide synthases
'O2	:	Singlet oxygen
O2-	:	Superoxide radical
OCL	:	Osteoclast
ODFR	:	Oxygen derived free radicals
OH-	:	Hydroxyl radical
ONOO~	:	Peroxynitrite ion
PD	:	Pocket depth
PGE2	:	Prostaglandin E2
PI	:	Plaque index
PKC	:	Protein kinase C
PMNs	:	Polymorphonuclear leukocytes
RANKL	:	Receptor activator of nuclear factor kB ligand
RNS	:	Reactive nitrogen species
ROS	:	Reactive Oxygen Species
RVG	:	Radio Visio Graphy system
SD	:	Standard deviation
SOD enzymes	:	Superoxide dismutase enzyme
TAOC	:	Total antioxidant capacity
TGF-β	:	Transforming Growth Factor -β
TNF-a	:	Tumor necrosis factor-alpha
UV	:	Ultra violet light

INTRODUCTION AND REVIEW OF LITERATURE

The supportive tissues surrounding the teeth are collectively termed the periodontium and comprise the root cementum, the periodontal ligament, the gingival tissue and the alveolar bone. Together, the components of the periodontium provide attachment and stability to the teeth as well as mobility and mechanical force adaptation. Moreover, it offers nutrition and regenerative capacities *(Palmqvist, 2006)*.

According to the American Academy Of Periodontology , periodontal diseases including gingivitis and periodontitis are serious infections that if left untreated can lead to tooth loss . The periodontal diseases were classified into gingival disease associated with or without dental plaque , chronic periodontitis , aggressive periodontitis ,periodontitis associated with systemic diseases , necrotizing periodontal disease , abscess of periodontium , periodontitis with endodontic lesion and periodontitis with developmental or acquired deformities.
(Armitage ,2002).

Chronic periodontitis is considered to be the most common form of periodontitis. It is known as chronic inflammatory disease that caused by oral bacterial infection, resulting in progressive destruction of periodontal ligament and alveolar bone with either pocket formation or recession, or both (Page and Kornman,1997; Flemming ,1999 and Heitz-Mayfield ,2005).

Periodontitis is a complex disease in which disease expression involves interaction of the biofilm with the host immunoinflammatory

response and subsequent alterations in bone and connective tissue homeostasis (Tatakis and Kumar,2005 ;Offenbacher et al.,2007 and Taubman et al.,2007).

It is widely known that the initiation and progression of periodontitis are dependent on the presence of microorganisms capable of causing disease. Among the characteristics that implicate an organism as etiologic agent are bacterial virulence factors, **such as:** capacity to colonize, the ability to produce substances that can directly initiate tissue damage and the ability to resist antibacterial host defense mechanisms. Once the major protective elements in the periodontium have been overwhelmed by bacterial virulence mechanisms, a number of host – mediated destructive processes are initiated. Polymorphonuclear leukocytes (PMNs) which normally provide protection, can themselves contribute to tissue destruction. There is increasing evidence that the bulk of tissue destruction is due to the mobilization of the host tissues via activation of monocytes, lymphocytes, fibroblasts ,and other host cells. Engaggement of these cellular elements by bacterial factors, specially bacterial lipopolysacharide (LPS), is thought to stimulate production of both catabolic cytokines (IL-1, IL-6, TNF-α) and inflammatory mediators including arachidonic acid metabolities such as prostaglandin E2 (PGE2). They in turn promote the release of tissue-derived enzymes, the matrix metalloproteinase, which are destructive to the extracellular matrix and bone **(Offenbacher,1996 and AAP,1999).**

There are many bacterial species residing in a biofilm on tooth surfaces referred to as dental plaque that have been closely associated with periodontitis. These include Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans , Bacteroides forsythus , non-

classified Spirochetes , Pre-votella intermedia , Campylobacter rectus , Eubacterium nodatum ,Prevotella nigrescens , Pepto-strepto-coccus micros , Fusobacterium nucleatum, and Eikenella Corrodens **(Haffajee and Socransky , 1994 and Darveau etal ,1997)** Of these , P. gingivalis shows extensive proliferation in diseased individuals (Haffajee and Socransky ,1994 ; Chapple et al ,1997 and Lamant and Jenkinson ,1998).

Periodontitis is a multifactorial disease with microbial dental plaque as the initiator of periodontal disease (Kinane , 1999). Althought the different forms of periodontitis are all caused by bacterial infection, a variety of local and systemic factors are associated with the risk of periodontal disease or the severity of the disease *(Brown et al ,1994 and Genco,1996)*. Systemic factors associated with progression of periodontal disease include smoking, diabetes mellitus, gender, osteoporosis and osteopenia, stress and inadequate coping (Genco, 1996).

It is known that nutrition is important for maintaining periodontal health (Hazen, 1968 and Enwonwu, 1995). However , the role of nutrients in modulating the pathogenesis of periodontal disease is not clear (Alfano ,1976 , Carlos and Walfe ,1989). By analyzing the systemic effects of certain nutrients, it can be hypothesized that periodontal treatment could be enhanced with the addition of these nutrients to periodontal therapy, providing a safe method to potentiate the clinical response following treatment (Neiva et al., 2003).

Also periodontitis is characterized by the presence of a variety of molecular species, among which are free radicals and the Reactive Oxygen Species (ROS) (Chapple , 1997; Betterridge ,2000 and Ceriello , 2000).

Role of Reactive Oxygen Species (ROS) in periodontitis :

A free radical may be defined as " any species capable of independent existence that contains one or more unpaired electrons" (Halliwell, 1991).They are highly reactive and diverse species, capable of extracting electrons and thereby oxidizing a variety of biomolecules vital to cell and tissue function (Chapple and Mattews, 2007)

ROS plays a critical role in the pathogenesis of a variety of diseases, in addition to providing an important function in normal metabolic reactions, including numerous diseases like AIDS , Cancer (Chung et al.,1999) ,atherosclerosis , chronic inflammatory conditions (Ceriello , 2000) ,aging process (Dulloo et al. , 1999), and periodontal disease (Fredriksson et al. , 1998 and Wei et al ,2004) .

When host cells are stimulated by bacterial pathogens, they release pro-inflammatory cytokines as part of the immune response. These include IL-1 α and - β , IL-6 , IL-8 and tumour necrosis factor .These cytokines recruit PMN to the site of infection (Lamont & Jenkinson, 1998 and Waddigton et al., 2000).

Patients with periodontal disease display increased PMN number and activity. It has been suggested that this proliferation results in a high degree of ROS (Reactive oxygen species) release, culminating in heightened oxidative damage to gingival tissue , periodontal ligament and alveolar bone (Sculley and Langely-Evans, 2002) .

Compared with healthy controls, patients with adult periodontitis generate higher levels of many ROS (Guarnieri et al , 1991). Significant ROS generation by neutrophils requires a minimum oxygen tension of about 1% and a pH of 7.0–7.5 (Gabig et al.,1979 and Allen et al.1997) . Both these conditions are found within periodontal pockets, indicating that chronic or excess ROS production is possible at this important site of periodontal tissue damage (Mettraux et al.,1984 and Eggert et al., 1991).

There is evidence that other cells normally resident within periodontal tissues may contribute to local oxidative stress (by ROS production) such as osteoclasts and fibroblasts. Fibroblast , which represent the largest population of cells in healthy gingiva and periodontal ligament, are able to spontaneously release detectable levels of ROS.(Murrell etal.,1990 and Skaleric etal.,2000).

Reactive oxygen species cause tissue damage by a variety of different mechanisms, **which include the following:** DNA damage, lipid peroxidation, protein damage (Bartold et al., 1984), oxidation of important enzymes (e.g. anti-proteases such as αl-antitrypsin) (Varani et al., 1985), stimulation of pro-inflammatory cytokine release by monocytes and macrophages (by activating nuclear factor KB (NF-KB) (Staal et al., 1990).

Recent animal studies demonstrated that osteoclastic differentiation and function are stimulated by oxidative stress, particularly that induced by hydroxyl radicals, superoxide anion and to lesser extent, hydrogen peroxide (Koh et al ,2005 ; Lean etal,2005 and Kim et al , 2006).

Reactive Oxygen Species (ROS) is a collective term which includes oxygen derived free radicals (ODFR), such as the superoxide radical (O2-), hydroxyl radical (OH-), nitric oxide radical (NO-) species, and the non radical derivatives of oxygen, such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and singlet oxygen (O). (Halliwell and Guttridge, 1990a) .

A) Superoxide anion:

Superoxide (O2.-) is formed chemically by addition of an extra electron to the oxygen molecule,this reaction may be brought about by accident, when electrons leak from their carriers within the respiratory chain of mitochondria and pass directly onto oxygen (Fridovich 1989, Imlay's and Fridovich 1991).However, the most important source of superoxide in the periodontal tissues is thought to be a functional one, when activated phagocytes (such as polymorphonuclear leukocytes or PMNLs, and macrophages) and to a lesser extent eosinophils and lymphocytes, produce superoxide as an antibacterial agent (Curnette and Babior 1987, Maly 1990). Fibroblasts have also been shown to produce superoxide (Murrell et al., 1990, Meier et al., 1990).

Superoxide is regarded as a weak reactive radical, relative to the hydroxyl radical, but it can attack a number of biological targets of periodontal relevance, and can spontaneously dismutate in aqueous solution to form hydrogen peroxide and singlet oxygen , which can in turn cause cell damage (Chapple, 1997). Key et al.,1994 and Steinbeck et al.,1994 found that superoxide has also been localized at the ruffled border space of osteoclasts, suggesting that it may be involved at the osteoclast-bone interface in bone matrix degradation .