Evaluation of the effect of roxithromycin administration in conjunction with non-surgical periodontal therapy on the level of matrix metalloproteinase-1 in gingival crevicular fluid

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Dedication

To my father

For being my guide, support and strength

He is my unique encouragement for going along the way till the end

To my mother

For being a dependable source of comfort,

helped me in times of trouble and supported me whenever I called.

And to my husband who supported me with patience and long days of guidance

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LIST OF ABBREVIATIONS

CAL	Clinical attachment level
$CD4^+$	Cluster of differentiation 4
CHX	Chlorhexidine chip
CP	Chronic periodontitis
ECM	Extracellular matrix
EGF	Epidermal growth factor
EHOM	Engineered human oral mucosa
ELISA	Enzyme linked immunosorbent assay
GAgP	Generalized aggressive periodontitis
GCF	Gingival crevicular fluid
GI	Gingival index
HGF	Human gingival fibroblasts
HPDF	Human Periodontal ligament cells
Hpx	Hemopexin
I/E	Intracellular/extracellular ratio
ICAM	Intercellular adhesion molecules
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin

INF	Interferon
LAP	Localized aggressive periodontitis
LPS	Lipopolysaccharides
MCP-1	monocyte chemoattractant protein-1
MIC	Mean inhibitory concentration
MMPs	Matrix metalloproteinases
MT-MMPs	Membrane type matrix metalloproteinases
N-FMLP	N-formyl-methionyl-leucyl-phenylalanine
NSAID	Non steroidal anti-inflammatory drugs
PDGF	Platelet derived growth factor
Pg	Porphyromonas gingivalis
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PMN	Polymorphonuclear leucocytes
PPD	Probing Pocket depth
RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard Deviation
SDD	Subantimicrobial dose of doxycycline
SRP	Scaling and root planing
TGFα	Transforming Growth factor alpha
TGFβ	Transforming Growth factor beta

Th1	T helper 1
Th2	T helper 2
TIMPs	Tissue inhibitors of matrix metalloproteinase
TNF-α	Tumor necrosis factor- alpha
TNF-β	Tumor necrosis factor-beta
VCAM-1	Vascular cell adhesion molecule-1
α_2 M	Alpha-2 macroglobulin

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INTRODUCTION

AND REVIEW OF LITERATURE

INTRODUCTION AND REVIEW OF LITERATURE

Chronic periodontal diseases encompass a number of conditions characterized by destructive inflammatory process affecting the supporting tissues of the tooth. Although bacteria cause plaque-induced inflammation, progression and clinical characteristics of these diseases are influenced by both acquired and genetically derived factors that can modify host susceptibility to infection ***(Salvi and Lang, 2005)***.

Chronic periodontitis is defined as inflammation of the gingiva extending into the adjacent attachment apparatus. The disease is characterized by loss of clinical attachment due to destruction of the periodontal ligament and loss of the adjacent supporting bone. Although chronic periodontitis is the most common form of destructive periodontal disease in adults, it can occur over a wide range of ages. It usually has slow to moderate rates of progression but may have periods of rapid progression. Chronic periodontitis may be localized involving one area of a tooth's attachment or more generalized. A patient may have simultaneously areas of health and chronic periodontitis with slight to moderate or advanced destruction ***(AAP, 2000a)***.

The vast majority of periodontal diseases are caused by microorganisms that reside at or below the gingival margin. The search for pathogens of periodontal diseases has been underway for more than 100 years and continues to date. The world workshop in periodontics in 1996, designated *Aggrigatibacter actinomycetem comitans*, *Porphyromonas gingivalis and Tannerella forsythia* as periodontal pathogens ***(Zambon, 1996)***. This determination was based on weight of evidence evaluation that examined such factors as association with

disease status, clinical improvement accompanying pathogen reduction after periodontal therapy, studies of virulence factors and the effect of species in experimental animal model systems.

The world workshop also formulated a list of putative periodontal pathogens for which the weight of evidence was less conclusive. These included; *Prevotella intermedia, Prevotella nigrescens, Fusobacterium nucleatum, Campylobacter rectus, Eikenella corrodens, Peptostreptococcus micros, Selenomonas Species, Eubacterium species* and *Spirochetes* including *Treponema denticola* ***(Zambon, 1996)***.

Bacterial plaque biofilms have been shown to be the primary etiological factor in the initiation of gingival inflammation and the subsequent destruction of periodontal tissues ***(Haffajie and Socransky, 1994)***. The progression of the disease process and the majority of soft and hard tissue destruction associated with periodontal disease is the result of the host's immuno-inflammatory response to the bacterial challenge. The underlying biological mechanisms of the response are characterized by the production of host derived inflammatory mediators including cytokines and bioactive lipids by neutrophils, monocytes, lymphoctes and fibroblasts. Acquired and environmental risk factors such as diabetes mellitus, cigarette smoking and stress as well as generalized transmitted traits such as interleukin-1 (IL-1) gene polymorphism may accentuate the host inflammatory response to the bacterial challenge and eventually the susceptibility to the disease ***(Korman and di Giovine, 1998; Salvi et al., 1998 and Kinane and Chestnutt, 2000 and Albander 2002)***.

The bacteria in the plaque biofilm produce large quantities of metabolites that can interact with junctional epithelium and penetrate into the underlying connective tissue. These may include fatty acids (butyric

and propionic) that are toxic to the tissues, peptides of the N-formyl-methionyl-leucyl-phenylalanine (N-FMLP) that are potent chemoattractants for leukocytes and lipopolysaccharides (LPS) of gram-negative bacteria. These bacterial products and components stimulate junctional epithelial cells to synthesize inflammatory mediators as interleukin-1α (IL-1α), tumor necrosis factor α (TNFα), prostaglandin E_2 (PGE_2) and matrix metalloproteinases ***(Wilson et al., 1996 and Madianos et al., 2005).***

Following the generation of proinflammatory stimuli within the junctional epithelium and early in local inflammatory response, subepithelial postcapillary venules are activated and display an increase in vascular permeability, with the expression of leukocyte adhesion molecules and the release of specific leukocyte activating agents ***(Osborn 1990 and Moughal et al., 1992)***. The effect of these phenomena are thought to be an increased leakage of plasma components including acute phase proteins into the gingival crevicular fluid (GCF) and increased leukocyte extravasation leading to formation of perivascular connective tissue infiltrate ***(Kinane et al., 1991 and Adonogianaki et al., 1994)***.

The polymorphonuclear neutrophils (PMNs) exit the inflamed vessels of the microcirculation and migrate along a gradient of chemoattractant through the connective tissue and junctional epithelium to form a barrier between the subgingival microbial plaque and the gingival tissue. The movement of leukocytes from the vasculature to the tissue is regulated by different types of adhesion molecules that are expressed on endothelial cells and leukocytes ***(Moughal et al., 1992 and Tonetti et al., 1994)***.