Host Modulation Therapy for Periodontal Disease: Subantimicrobial-dose doxycycline, Medical as well as Dental Benefits

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INTRODUCTION AND BACKGROUND

A number of articles over the past two decades have reviewed the scientific and clinical rationale for incorporating a NOVEL NON-ANTIBACTERIAL formulation of doxycycline in the treatment of: (a) periodontal diseases (chronic periodontitis primarily, but also generalized aggressive periodontitis, and refractory periodontitis); (b) other oral inflammatory conditions such as pemphigoid; and (c) several medical conditions as well.[1-7] Doxycycline is a semi-synthetic tetracycline (TC) which has historically been used in short-term orally-administered regimens (10 days – 2 weeks) in traditional doses (100mg q.d. or b.i.d.) as a broad-spectrum antibiotic to treat infections. Doxycycline has advantages of superior half-life in the circulation (18 hours, requiring only one capsule every 12 hours, in contrast to traditional TC which requires 1 every 4 hours), and fewer side-effects (e.g., less photosensitivity) compared to other TCs such as minocycline.[5,7] In this regard, short-term regimens of systemically-administered antimicrobial-dose doxycycline (ADD) and other TCs have been used as an adjunct to scaling and root planing (SRP) in the non-surgical treatment of the periodontal patient. Higher doses, ADD and other TCs, reduce the bacterial "burden" in the periodontal pocket because of their ability to inhibit protein synthesis in periodontopathic bacteria such as *Porphyromonas gingivalis*. However, it is also known that within days after initiating treatment with antimicrobial doses of TCs, colonization with antibiotic-resistant bacteria occurs.[8-10] Furthermore, the short-term use of systemic TCs and other antibiotics as adjuncts to non-surgical periodontal therapy has not been shown to produce significant long-term clinical benefits, beyond SRP itself, in recent extensive statistical studies.[11] Yet another rationale for not using systemic antibiotics, such as ADD, as adjuncts to SRP has been the recognition, beginning about 4 decades ago,[12,13] that it is the host-response, not bacterial products, which is primarily responsible for the destruction of the connective tissue constituents (e.g., collagen fibers and proteoglycan ground substance) in the gingiva, periodontal ligament and alveolar bone during the development and progressive deepening of the periodontal pocket.[1,2,14]

However, it was the unexpected discovery that TCs, particularly doxycycline, can "benefit" the host-response which ultimately led to the development of a novel Non-antibacterial formulation of this drug now known as sub-antimicrobial-dose doxycycline (SDD, or Periostat®).[1,2,15] The research and clinical studies that resulted in governmental approval (USA/Food & drug Admin., Canada, Europe) of SDD as an adjunctive treatment for periodontal disease, and more recently the recognition of its systemic/medical benefits as well, are addressed below.

SDD, THE FIRST GOVERNMENT-APPROVED MATRIX METALLOPROTEINASE-INHIBITOR DRUG

About 30 years ago, Golub et al[15] discovered that TCs, including doxycycline, have the unexpected ability to inhibit host-derived tissue-destructive enzymes known as the matrix metalloproteinases (MMPs), and by mechanisms unrelated to the antibacterial/antibiotic properties of these drugs. The first enzyme in this category of proteinases, called collagenase, was described by J. Gross in 1962[16] and it was the only enzyme known at that time that was able to degrade the triple-helical collagen molecule under physiologic conditions of pH and temperature. Since then, over 25 different MMPs have been identified including three different collagenases (MMP-1, MMP-8 and MMP-13), two gelatinases (MMP-2 and MMP-9), and others such as MMP-12 (produced by macrophages and osteoclasts). These enzymes when present in pathologically-excessive levels are largely responsible for degrading various connective tissue constituents (such as the collagen fibers), and help mediate bone resorption, during various dental and medical diseases. However, low levels of these enzymes also have physiologic functions such as (but not limited to) normal connective tissue remodeling. This approach provides yet another advantage to SDD since it is a "mild" Host-Modulating drug[17] with decades of clinical trials and clinical usage demonstrating its safety as well as efficacy.

After a series of experiments which identified the multiple mechanisms by which TCs inhibit pathologically-excessive MMPs (ie., reduction of synthesis, activation, and activity of these host-derived proteolytic enzymes), Golub et al.[1,2] designed two strategies of drug development to suppress connective tissue breakdown including bone loss during periodontitis and other dental and medical diseases using NON-antibacterial TCs. The 1st strategy involved systematically reducing the amount of doxycycline formulated in each capsule which, when administered to human subjects in clinical trials, produced blood levels of this drug that were too low (<1 µg/ml; typically 0.25 -0.8 µg/ml) to be effective as an antibiotic[2,18] (also see "package insert," Periostat ®). This formulation, once confirmed during clinical trials, contained 20mg doxycycline per capsule and was administered b.i.d.; this is in contrast to traditional ADD at 100mg b.i.d which produces "peak" blood levels of 2-5 µg/ml. This novel, "low-dose" formulation (better known as sub-antimicrobial-dose doxycycline, or SDD) reduced the side-effects of systemic antibiotic-dose TC therapy but retained the ability to suppress the tissue-destructive MMPs, to decrease inflammatory mediators (e.g., interleukin-1ß), and to reduce diagnostic biomarkers of bone resorption in the periodontal pocket.[2] One such formulation, Periostat®, was approved (as safe and effective) by the U.S. Food & Drug Administration in 1998 (subsequently also accepted by the American Dental Association), and a few years later in Canada and Europe, as the 1st-ever MMP-inhibitor drug and the 1st-ever systemically administered medication for periodontitis approved by any government regulatory agency. As discussed below, numerous clinical trials world-wide have demonstrated that SDD is safe and effective, as an adjunct to SRP, for treating periodontal disease. More recently, this dental formulation has shown clear evidence of efficacy in clinical trials on patients with medical disorders as well, including chronic inflammatory skin disease, pemphigoid, rheumatoid arthritis, type II diabetes, postmenopausal women with both periodontal and mild systemic bone loss (osteopenia), and even a rare and fatal lung disease, and these are discussed below.

Our 2nd strategy involved the chemical modification of the TC molecule to deliberately eliminate its antibacterial activity [ie., removal of a chemical side-chain on the TC molecule, the dimethylamino group at carbon-4, which is known to be necessary for the drug's antibacterial activity], but which retained or even enhanced its MMP-inhibitory properties—this allowed the drug to be used as a NON-antibiotic TC at both low and high oral doses.[1,2] As a result of these experiments, we identified another site on the TC molecule, this one responsible for its anti-MMP activity (the calcium and zinc-binding, ß-diketone moiety at carbon-11 and -12), and then developed a series of chemically-modified TCs (ie., the CMTs or COLs) which were therapeutically effective in animal models of various diseases.[1,2] One of these, CMT-3 (6-demethyl 6-deoxy 4-dedimethylamino TC) has been tested in patients with a type of cancer, Kaposi's sarcoma, and has shown evidence of

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efficacy as an antiangiogenesis agent.[19] The same compound significantly reduced mortality in a Yorkshire pig model of a 40% fatal lung disease, acute respiratory distress syndrome or ARDS. 20 However, a significant side-effect of CMT-3 (also known as COL-3) is increased sensitivity to sunburn. As a result, newer compounds are being developed by our group which are potent inhibitors of MMPs and reduce inflammatory mediators, but which are expected to be safer than CMT-3.

CLINICAL TRIALS: SAFETY AND EFFICACY

3.1 CHRONIC PERIODONTITIS.

Beginning more than 20 years ago, a number of publications have appeared which described extensive clinical trials demonstrating that SDD, adjunctive to mechanical debridement procedures, produces improvements not only in clinical measurements (probing depth, clinical attachment levels, bleeding-on-probing, radiographic measures of alveolar bone loss) but also significant reductions in diagnostic-biomarkers and mediators of inflammation (e.g., the cytokines, IL-1ß, and IL-6), collagen destruction (the MMPs, neutrophil collagenase or MMP-8, and the bone cell collagenase, MMP-13), and bone resorption (the bone type I collagen breakdown fragment, ICTP).[2,6,21,–23] As reviewed recently by Caton and Ryan, 6 even though a short-term (1 month) regimen of SDD reduces collagenase activity in the periodontal pocket, cessation of this treatment resulted in a "rapid rebound of collagenase activity to placebo levels". In contrast, a 3-month regimen produced a long-term benefit "without a rebound." More recent studies have confirmed the recommendation that SDD should be administered for longer periods of time (often 6 or 9 months, in some cases longer) to prevent a rapid rebound in collagen-destructive enzyme activity and to enhance clinical efficacy.[2,6,21,23] Also of extreme importance, in the series of clinical trials described in these reviews, the improvements in periodontal disease occurred in the absence of overgrowth of antibiotic-resistant bacteria, not only in the periodontal pockets but also in other anatomical locations including the skin, gut and vagina. Moreover, the clinical improvements resulting from SDD therapy occurred without any increase in adverse events (including no microbial complications) compared to the control subjects administered placebo capsules even when the drug was administered for up to 2-years duration.[6,7,23] These, and other clinical trials, resulted in the following quote from the package insert, that SDD is ineffective as an antibiotic: "The dosage of doxycycline achieved with this product during administration is well below the concentration required to inhibit microorganisms commonly associated with adult periodontitis. Clinical studies with this product demonstrated no effect on total anaerobic and facultative bacteria in plaque samples from patients administered this dose regimen for 9 to 18 months. This product should not be used for reducing the numbers of or eliminating those microorganisms associated with periodontitis."

3.2 RAPIDLY PROGRESSING AND REFRACTORY PERIODONTITIS

It is increasingly recognized that SDD alone (as an adjunct to mechanical debridement), or even in combination with an NSAID (discussed later), is also effective in patients with severely destructive forms of periodontitis. In this regard, Novak et al[24] described a novel regimen of treatment in a double-blind placebo-controlled study on 30 patients with "severe, generalized, chronic periodontitis". Their approach was to treat all of these subjects with a four-times repeated full mouth regimen of subgingival debridement (once per week for four weeks) followed by a 6-month regimen of SDD or placebo capsules b.i.d. Although the control (placebo) group showed some improvement due to the repeated mechanical debridement, those on SDD improved much more dramatically, and the benefits persisted after the drug therapy had stopped.

Consistent with these outcomes, Tenenbaum and Goldberg[25] have recently focused on another category of periodontal disease called refractory periodontitis, a diagnosis based on the observation that these cases do not respond to traditional periodontal therapies such as repeated regimens of SRP. Their approach has been to supplement repeated mechanical debridement procedures with a combination of SDD plus a low-dose of a potent non-steroidal anti-inflammatory drug, the NSAID flurbiprofen.

Their rationale was based on earlier reports that a non-antibacterial TC, used in combination with an NSAID such as flurbiprofen, produced a synergistic reduction in inflammation

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and decreased bone and cartilage destruction in the joints of a rat model of rheumatoid arthritis.[26,27] An additional rationale involved a clinical study on patients with severe periodontal disease who were treated, prior to flap surgery, with a combination of SDD and low-dose flurbiprofen which resulted in a dramatic synergistic suppression of tissue-destructive MMPs (collagenase and gelatinase) in the gingival tissues which were excised for therapeutic purposes.[28] As controls, the other two groups of patients were treated either with flurbiprofen alone (which had NO effect on these tissue-destructive proteinases) or with SDD alone (which did produce an expected reduction in these enzymes) in response to this short-term (3-week) pre-surgical experimental therapy. Goldberg and Tenenbaum, a decade ago, established a special clinic (University of Toronto) which treats refractory periodontitis patients, who are referred by local dentists and periodontists, due to lack of response to traditional non-surgical and surgical therapies. As a result of the studies described above[26-28] they have begun to treat these patients with the novel combination protocol, adjunctive to SRP and oral hygiene instruction, and are beginning to report a positive response in many of these patients.[25] However, additional studies are required to more objectively assess the efficacy of this approach.

SDD STRATEGIES FOR MEDICAL DISORDERS

Increasingly, over the past decade, this novel non-antimicrobial dental formulation of doxycycline, has been tested in patients with medical disorders in which excessive MMPs and inflammatory mediators play a role. Accordingly, this last section summarizes the potential use of SDD in the following: dermatologic disorders, pemphigoid, rheumatoid arthritis, a rare & fatal lung disease, type II diabetes, post menopausal alveolar and skeletal bone loss, and cardiovascular disease.

4.1 DERMATOLOGY

Double-blinded placebo-controlled clinical trials have been carried out at several medical institutions in the U.S.A. and have demonstrated that SDD significantly reduces the severity of inflammatory lesions in patients with acne and rosacea; the latter is characterized by erythema patches on the skin of the face, as well as pustules and papules, and telangiectasia–- swollen and "spider-like" veins on the nose and cheeks. In fact, a novel sustained-release version of SDD has been approved by the U.S./FDA for the treatment of rosacea.[5,7]

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Consistent with these observations, in a major clinical trial on post-menopausal women, a 2-year regimen of SDD was found to be safe (based on a statistical analysis of adverse events) and to reduce inflammatory skin lesions (e.g., acne, rosacea, hives) compared to those subjects on placebo capsules during this prolonged time period.[23] It should be noted , however, that the major objectives of this NIH-supported study were to determine the efficacy of SDD on periodontal disease, bone loss, and cardiovascular risk in this vulnerable group of subjects.[29] (see section 4.6, below).

4.2 BENIGN MUCOUS MEMBRANE PEMPHIGOID

A pilot study carried out by Cohen et al[30] indicated that patients with pemphigoid, who were treated with SDD for 3-4 months, showed a reduction in the blisters and ulcers in the oral mucosa which characterize this disorder. The clinical response of this autoimmune disease may be explained, at least in part, by mechanisms identified by Liu et al.[31] In brief, autoantibodies to the hemidesmosomal protein, collagen XVII, induce the production of MMP-9 by epithelial cells in the mucosa which degrades an endogenous inhibitor called α1-antitrypsin. The resulting local deficiency of this proteinase-inhibitor promotes excessive activity of elastase (a serine-proteinase) which then degrades the type XVII collagen facilitating the separation of the epithelium from the underlying connective tissue, a cascade which appears to be suppressed by the known ability of SDD to inhibit MMP-9. Several oral pathologists in the U.S.A., including Dr. D. Trochesette at Stony Brook University, have described efficacy in their patients using SDD therapy (personal communication).

4.3 RHEUMATOID ARTHRITIS

A number of reports have described the ability of TCs, by non-antibacterial mechanisms, to reduce the severity of rheumatoid arthritis (RA) in animal models and in clinical trials.[32] However, O'Dell and his colleagues at the University of Nebraska Medical Center, were the first to test the dental formulation, SDD, in patients with rheumatoid arthritis.[33] In this double-blind placebo-controlled study, patients

with early RA were administered SDD in combination with a potent anti-inflammatory drug, methotrexate, over a 2-year time period and this regimen was found to be 2-3 times more effective in reducing arthritis-severity scores than methotrexate plus placebo. No side-effects of this 2-year regimen, different from placebo treatment, of SDD were described.

4.4 LYMPHANGIOLEIOMYOMATOSIS

Perhaps the most dramatic (and briefest) of these reports on SDD in medicine was that published in the New Engl. J. Med. several years ago by the famed cancer researcher, Dr. Judah Folkman and his colleagues at the Harvard Medical School.[34] He described the use of SDD in the treatment of this rare & fatal lung disease which is characterized by elevated MMP-2 & MMP-9 levels in the urine of the patient as a diagnostic marker of the severity of this progressively-destructive disease. They reported that within weeks after initiating SDD therapy, the elevated levels of MMPs decreased in the urine, followed shortly thereafter (as doxycycline dose-escalation occurred) by physiologic and clinical improvements in lung function. The result? "The patient has had a clear improvement in the quality of life and has been taken off the waiting list for a lung transplant – an outcome that was predicted by the decreasing urinary levels of MMPs."[34]

4.5 TYPE II DIABETES

Considering the seminal study 3 decades ago,[15] which discovered that TCs can function as host-modulating drugs (ie., MMP-inhibitors) utilizing a rat model of diabetes, it is not surprising that a number of subsequent reports described a variety of beneficial effects of this unexpected non-antibacterial property of TCs in both type I and type II models of this increasingly widespread disease.[1,2] These observations contrasted with earlier clinical trials which proposed using antimicrobial (antibiotic) agents as adjuncts to SRP in the management of the increased severity of periodontal disease exhibited by diabetics.[35] With this background in mind, Engebretsen and Hey-Hadavi[36] compared the efficacy of SDD adjunctive to SRP, to SRP and placebo or SRP plus a 2-week regimen of antibiotic-dose doxycycline, in type II diabetic patients with periodontitis. Only those diabetics on the host-modulating regimen (SDD), adjunctive to mechanical debridement, showed a statistically and clinically significant reduction in circulating levels of HbA1c, "a surrogate measure for systemic control and treatment decision-making in clinical medicine." These findings, after they are confirmed and extended, could add an important dimension to the collaboration between the dental and medical practitioners managing the patient with this all-too-common clinical profile.

4.6 POST-MENOPAUSAL BONE LOSS

Numerous in vitro and in vivo / animal model studies over the past several decades have demonstrated that TCs, by NON-antimicrobial mechanisms, can reduce pathologic bone loss of relevance to oral (periodontitis) and systemic (estrogen-deficiency; insulin-deficiency) diseases.[1,2] The initial mechanism identified in studies using this treatment strategy was the ability of TCs to inhibit osteoclast-mediated bone resorption. Subsequent experiments demonstrated that these drugs can also "normalize" osteoblast activity and bone formation (see Payne and Golub[23] for a review); this recent publication also described the results of an extensive NIH-supported study on the benefits of long-term SDD therapy in a common clinical condition–-post-menopausal women exhibiting both periodontitis (alveolar bone loss) and early systemic bone loss or osteopenia. In brief, the women in the experimental group were administered a 2-year daily regimen of SDD adjunctive to periodontal maintenance therapy (the latter, every 3-4 months); the control subjects were treated identically except that they were administered look-alike placebo capsules over the 2-year protocol. The subjects in both groups received calcium and vitamin D supplements. Statistical analysis revealed that the long-term treatment with SDD was safe, and produced the following evidence of therapeutic efficacy both locally and systemically including:

(i) Decreased progression of periodontal breakdown, and reduced loss of alveolar bone in pockets with "baseline probing depths 5mm or greater;"

(ii) Reduced levels of local biochemical biomarkers and mediators of periodontal breakdown including decreased leukocyte type collagenase (MMP-8) and ICTP (a bone-collagen breakdown product and diagnostic marker of bone resorption); and

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(iii) Reduced levels of systemic biomarkers of bone resorption in the circulation indicated a reduced risk, in these postmenopausal women, of conversion of mild skeletal bone loss (osteopenia) into the more severe form of bone loss, osteoporosis.

4.7 CARDIOVASCULAR DISEASE

For decades, it has been recognized that chronic periodontitis is associated with an increased risk for cardiovascular disease (CVD).[37-39] Among the rationales proposed for this association is data showing that patients with moderate-severe periodontal disease exhibit elevated levels of biomarkers of systemic inflammation such as high sensitivity C-reactive protein and interleukin-6, which are risk factors for CVD.[29,38,40] Other proposed mechanisms involve the possibility that microorganisms such as *Porphyromonas gingivalis* in the periodontal pocket, may be carried by the circulation (e.g., by "reverse trafficking" in specialized macrophages called dendritic cells) to cholesterol-rich atherosclerematous plaques lining coronary (and other) arteries where they can promote atherosclerosis and cardiac events. 41 However, a major issue with this "periodontopathogen hypothesis" in CVD is that only tetracyclines (not other classes of antibiotics such as the penicillins, macrolides such as erythromycin, cephalosporins, and even azithromycin) have been shown to be associated with a reduced risk for cardiac events.[42-45] In fact, in response to the major study by Meier et al[42] over a decade ago, Golub et al[43] proposed that the reduced incidence of acute myocardial infarction in patients who had been treated with tetracycline was a result of the unique ability of this drug, but not other antibiotics, to modulate the host response. Subsequent clinical trials, now described, supported this hypothesis by demonstrating that SDD administration, ranging from 6 months[40] to 2-years duration,[29] reduced biomarkers of systemic inflammation strongly associated with CVD including the tissue-destructive proteinase, MMP-9, the long-term proinflammatory cytokine, IL-6, and the acute-phase protein, C-reactive protein. Of extreme interest, the 30 patients enrolled in the 6-month clinical trial just described[40] were diagnosed with acute coronary syndromes which includes a history of myocardial infarction, atherosclerosis, and blood chemistry indicating cardiac damage. The dramatic reduction in MMP-9 (as well as reduced hs-CRP and IL-6) in the plasma samples of the patients administered a 6-month regimen of SDD, compared to the control group administered placebo capsules, indicated a reduced risk for a fatal heart attack based on an earlier extensive study on over 1,000 patients with severe CVD.[46]

Several additional non-antimicrobial cardioprotective mechanisms have been attributed to doxycycline and other TCs including their ability to: (i) inhibit MMP-2 activity within cardiac myocytes reducing the degradation of contractile proteins,[47] (ii) inhibit MMP-mediated degradation of elastin and collagen fibers in the walls of blood vessels which could reduce the severity of hypertension[48] and retard the expansion of aortic aneurysms,[49] and (iii) inhibit MMP-mediated atherosclerematous plaque destabilization thus reducing the risk for thrombosis and acute myocardial infarction.[40]

(5) EFFECT OF LONG-TERM DOSAGE OF SDD ON THE HUMAN MICROBIOME

Prior to the approval of SDD as a host-modulating agent by the FDA, there was concern by the medical community and regulatory agencies that long-term treatment with SDD could alter the microbial inhabitants of the human body. As a result, important microbiological studies were incorporated into many of the double-blind placebo-controlled human clinical studies discussed above. These microbiology studies were conducted in the laboratories of Dr. Clay Walker and Dr. John G. Thomas of the University of Florida and West Virginia University, respectively, over a period of a decade. The aims of these microbiological studies were to determine if long-term oral consumption of SDD resulted in changes of the microbial flora's susceptibility to doxycycline or other commonly used antimicrobials, changes in the proportions of the normal flora, or if the flora developed increased numbers of opportunistic pathogens such as *Candida* species and *Staphylococcus aureus*.[50-56] The flora was sampled from the subgingival plaque,[50-52,55-56] skin,[53] as well as faeces and the vagina.[54] The longest duration of SDD consumption lasted up to two years. The overall conclusions of these studies is that SDD consumption as 20 mg b.i.d. or as 40 mg q.d. does not have any noticeable affect on the human

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microbiome in comparison to participants who consumed a placebo. The microbial results of systemic oral SDD administration on the human microbiome are in marked contract to studies investigating the use of systemic ADD administration. ADD results in a statistically significant increase in the numbers of doxycycline-resistant bacteria in the normal microbial flora throughout the body.[57-60]

(6) CONCLUSIONS

Considering the following basic biologic facts: (i) that collagen is the major structural protein of all the connective tissues (calcified and uncalcified) throughout the body, and (ii) that MMPs (and inflammatory mediators that enhance their synthesis) are largely responsible for collagen-destruction and the destruction of other connective tissue constituents, it is not that surprising that SDD, as the first government-approved MMP-inhibitor drug, can improve a variety of dental and medical diseases. The proven efficacy of this MMP-inhibitor medication, as an adjunct to traditional periodontal therapy, highlights the importance of "Host-Modulation" management of inflammatory (and bone-destructive) periodontal disease and its therapeutic impact on the systemic health of the patient.

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REFERENCES

1. Golub LM, Greenwald RA, Ramamurthy NS, McNamara TF & Rifkin BR: Tetracyclines inhibit connective tissue breakdown: New therapeutic implications for an old family of drugs. Crit. Revs Oral Biol. Med., 1991, 2:297-322.
2. Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J & Sorsa T: Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv. Dental Res., 1998, 12: 12-26.
3. Ryan M.E. and Golub L.M.: Modulation of matrix metalloproteinase activities in periodontitis as a treatment strategy. Periodontology 2000: 2000, 24: 226-238.
4. Barnett ML. Changing paradigms in periodontal therapy: host modulation with subantimicrobial doxycycline. Oral Health. Pp.53-65, October 2007.
5. Weinberg MA. New applications of doxycycline hyclate in medicine and dentistry. U.S. Pharmacist, April 2004.
6. Caton J and Ryan ME. Clinical studies on the management of periodontal diseases utilizing subantimicrobial dose doxycycline (SDD). Pharmacol. Res., 2011, 63: 114-120.
7. Monk E, Shalita A and Siegel DM. Clinical applications of non-antimicrobial tetracyclines in dermatology. Pharmacol. Res., 2011, 63: 130-145.
8. Heimdahl A and Nord CE. Influence of doxycycline on the normal human flora and colonization of the oral cavity and colon. Scand. J. Infect. Dis., 1983, 15 (3): 293-302.
9. Fiehn NE and Westergaard J. Doxycycline-resistant bacteria in periodontally diseased individuals after systemic doxycycline therapy and in healthy individuals. Oral Microbiolol. Immunol., 1990, 5 (4):219-222.
10. Rams TE, Babalola OO, Slots J. Subgingival occurrence of enteric rods, yeasts and staphylococci after systemic doxycycline therapy. Oral Microbiol. Immunol., 1990, 5: 166-168.
11. Cunha-Cruz J, Hujoel PP, Maupome G, Saver B. Systemic antibiotics and tooth loss in periodontal disease. J. Dent. Res., 2008, 87 (9): 871-876.
12. Fullmer H and Gibson WA. Collagenolytic activity in gingivae of man. Nature (London). 1966, 209: 728-729.
13. Taubman MA, Yoshie H, Ebersole JL etc. Host response in experimental periodontal disease. J. Dent. Res., 1984, 63 (3): 455-460.
14. Gu Y, Walker C, Ryan ME et al. Non-antibacterial tetracycline formulations: clinical applications in dentistry and medicine. J. Oral Microbiol., 2012, submitted for publication.
15. Golub LM, Lee HM, Lehrer G, et al.: Minocycline reduces gingival collagenolytic activity during diabetes: preliminary observations and a proposed new mechanism of action. J. Periodontal Res., 1983, 18: 516-526.
16. Gross J and LaPiere CM. Collagenolytic activity in amphibian tissues: a tissue culture assay. Pro. Natl.Acad. Sci. USA. 1962, 48: 1014-1021.
17. Sorsa T, Tjaderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL & Mantyla P: Matrix metalloproteinases: contribution to pathogenesis, diagnosis

and treatment of periodontal inflammation. Ann. Med., 2006, 38: 306-321.
18. McNamara TF, Golub LM, Yu Z, Ramamurthy NS. Reduced doxycycline blood levels in humans fail to promote resistant organisms. Int. Conference on Periodont. Disease: Pathogens and host immune responses, Osaka, Japan, Abstr. No. B54, 100, Sept. 25-27, 1990.
19. Dezube BJ, Krown SE, Lee JY et al. Randomized phase II trial of matrix metalloproteinase inhibitor COL-3 in AIDS-related Kaposi's sarcoma: an AIDS malignancy consortium study. J. Clin. Oncol., 2006, 24: 1389-1394.
20. Steinberg J, Halter J, Schiller H. Gatto L, Carney D, Lee HM, Golub LM and Nieman G. Chemically Modified tetracycline prevents the development of septic shock and acute respiratory distress syndrome in a clinically applicable porcine model. SHOCK 2005, 24, 348-356.
21. Golub LM, HM Lee, JA Stoner, RA Reinhardt, T Sorsa, AD Goren and JB Payne. Doxycycline effects on serum-bone biomarkers in post-menopausal women. J. Dent. Res., 2010, 89: 644-649.
22. Emingil G, Gurkan A, Attila G and KantarCi A. Subantimicrobial-dose doxycycline and cytokine-chemokine levels in gingival crevicular fluid. J. Periodontol., 2011, 82: 452-461.
23. Payne JB, LM Golub. Using tetracyclines to treat osteoporotic/osteopenic bone loss: From the basic science laboratory to the clinic. Pharmacological Res. , 2011, 63 (2), 121-129.
24. Novak MJ, Johns LP, Miller RC et al. Adjunctive benefits of subantimicrobial dose doxycycline in the management of severe, generalized, chronic periodontitis. J. Periodontol., 2002, 73: 762-769.
25. Goldberg MB, Golub LM, Lee HM, Tenenbaum HC. Assessing the Effects of Subantimicrobial-dose Doxycycline/flurbiprofen on refractory Periodontal Disease. J. Dent. Res., 2010, 89 (Special Issue), abstract #1176.
26. Greenwald RA, Moak SA, Ramamurthy NS & Golub LM: Tetracyclines suppress metalloproteinase activity in adjuvant arthritis and, in combination with flurbiprofen, ameliorate bone damage. J. Rheumatol., 1992, 19: 927-938.
27. Leung MK, Greenwald RA, Ramamurthy NS, Moak SA, Koszulinski R, Dieudonne D & Golub LM: Tenidap and Flurbiprofen enhance the uptake of matrix metalloproteinase inhibitor 4-dedimethylaminotetracycline in inflamed joints of adjuvant arthritic rats. J. Rheumatol., 1995, 22: 1726-31.
28. Lee HM, Ciancio SG, Tuter G, Ryan ME, and Golub LM: Sub-antimicrobial dose doxycycline efficacy as a matrix metalloproteinase inhibitor in chronic periodontitis patients is enhanced when combined with an NSAID. J. Periodontol., 2004, 75: 453-463.
29. Payne JB, LM. Golub, JA. Stoner, HM Lee, RA. Reinhardt, T Sorsa, and MJ Slepian: The effect of subantimicrobial-dose-doxycycline periodontal therapy on serum biomarkers of systemic inflammation. J. Amer. Dent. Assoc., 2011, 142: 262-273.
30. Cohen DM, Lee HM, Bhattacharyya I et al. Effective treatment of cicatricial pemphigoid using a novel combination therapy. J. Dent. Res., 2000, 79 (spec. Issue): 627.
31. Liu Z, Zhou X, Shapiro SD et al. The serpin, Alpha-1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. Cell 2000; 102 (5):647-655.
32. Greenwald RA. The road forward: the scientific basis for tetracycline treatment of arthritic disorders. Pharmacol. Res., 2011, 64: 610-613.
33. O'Dell JR, Elliott TR, Mallek JA, et al. Treatment of early sero-positive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. Arth. Rheum., 2006, 54: 621-627.
34. Moses MA, Harper J and Folkman J. Doxycycline treatment for lymphangioleiomyomatosis with urinary monitoring for MMPs. N. Engl. J. Med., 2006, 354: 2621-2622.
35. Grossi SG, Skupcinski FB, DeCaro T. et al. Treatment of Periodontal disease in diabetics reduced glycated hemoglobin. J. Periodontol., 1997, 68: 713-719.
36. Engebretsen SP and Hey-Hadavi J. Sub-antimicrobial doxycycline for periodontitis reduces hemoglobin A1c in subjects with type 2 diabetes: a pilot study. Pharmacolog. Res., 2011, 64 (6): 624-629.
37. Friedewald VE, Kornman KS, Beck JD et al. The American Journal of Cardiology and Journal of Periodontology Editors Consensus: Periodontitis and Atherosclerotic Cardiovascular Disease. Amer. J. Cardiol., 2009, 104: 59-68.
38. Joshipura KJ, Wand HC, Merchant AT and Rinm EB. Periodontal disease and biomarkers related to cardiovascular disease. J. Dent. Res. 2004; 83 (2): 151-155.
39. Craig RG, Yip JK, So MK et al. Relationship of destructive periodontal disease to the acute-phase response. J. Periodontol. 2003; 74 (7): 1007-1016.
40. Brown D.L., Desai K., Vakili B.A., Nouneh C., Lee H.M., and Golub L.M.: Clinical and biochemical results of the Metalloproteinase Inhibition with Subantimicrobial Doses of Doxycycline to Prevent Acute Coronary Syndromes (MIDAS) Pilot Trial. Arterioscler. Thrombosis. Vasc. Biol., 2004, 24: 733-738.
41. Zeituni AE, Carrion J, Cutler CW. Porphyromonas gingivalis dendritic cell interactions: consequences for coronary artery disease. J. Oral Microbiol. 2010, 2:5782 – DOI: 10:3402/jom.v2i0.5782.
42. Meier CR, Derby LE, Jick SS et al. Antibiotics and risk of subsequent first-time acute myocardial infarction. JAMA 1999, 281: 427-431.
43. Golub L.M, Greenwald R.A. and Thompson R.W.: Tetracycline use and risk of acute myocardial infarction (Letter). JAMA, 1999, 282: 1997-1998.
44. Grayston JJ, Kronmal RA, Jackson LA et al. Azitheromycin for the secondary prevention of coronary events. New Engl. J. Med., 2005, 352: 1637-1645.
45. Cannon CP, Braunwald E, McCabe CH et al. Antibiotic treatment of Chamydia pneumonia after acute coronary syndrome. N. Engl. J. Med., 2005, 352: 1646-1654.
46. Blankenberg S, Rupprecht HJ, Poirier O, et al., Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation, 2003, 107 (12): 1579-1585.
47. Cadete VJ, Sawicka J, Jaswal JS, Lopaschuk GD, Schulz R, et al., Ischemia/reperfusion-induced myosin light chain 1 phosphorylation increases its degradation by matrix metalloproteinase 2. FEBS Journal, 2012, 279: 2444-2454.

48. Castro MM, Tanus-Santos JE, Gerlach RF. Matrix metalloproteinases: targets for doxycycline to prevent the vascular alterations of hypertension. Pharmacol. Res., 2011, 64 (6): 567-672.
49. Thompson RW, Baxter BT. MMP inhibition in abdominal aortic aneurysms. Rationale for a prospective randomized clinical trial. Ann. N. Y. Acad. Sci., 1999, 878: 159-178.
50. Thomas JG, Metheny RJ, Karakiozis JM, Wetzel JM, Crout RL. Long-term sub-antimicrobial doxycycline (Periostat) as an adjunctive management in adult periodontitis: effects on subgingival bacterial population dynamics. Adv. Dent. Res. 1998, 12(2):32-39.
51. Walker C, Thomas J, Nangó S, Lennon J, Wetzel J, Powala C. Long-term treatment with sub-antimicrobial dose doxycycline exerts no antibacterial effect on subgingival microfloral associated with adult periodontitis. J. Periodontol. 2000, 71(9):1465-1471.
52. Thomas J, Walker C, Bradshaw M. Long-term sub-antimicrobial does doxycycline does not lead to changes in antimicrobial susceptibility. J. Periodontol. 2000, 71(9):1472-1483.
53. Skidmore R, Kovach R, Walker C, Thomas J, Bradshaw M, Leyden J, Powala C, Ashley R. Effects of subantimicrobial-dose doxycycline in the treatment of moderate acne. Archives of Dermatology 2003:139(4):459-464.
54. Walker C, Preshaw PM, Novak J, Hefti AH, Bradshaw M, Powala C. Long-term treatment with sub-antimicrobial dose doxycycline has no effect on the intestinal flora. J. Clin Periodontol 2005:32(11):1163-1169.
55. Walker C, Puumala S, Golub LM, Stoner JA, Reinhardt RA, Lee H-M, Payne JB. Subantimicrobial dose doxycycline effects on osteopenic bone loss: Microbiology results. J. Periodontol. 2007:78(8):1590-1601.
56. Preshaw PM, Novak J, Mellonig J, Magnusson I, Polson A, Giannobile WV, Rowland RW, Thomas JG, Walker C, Sharkey D, Bradshaw MH. Modified-release subantimicrobial dose doxycycline enhances scaling and root planing in subjects with periodontal disease. J Periodontol. 2008, 79(3):440-452.
57. Heimdahl A, Nord CE. Influence of doxycycline on the normal human flora and colonization of oral cavity and colon. 1983, Scand. J. Infect. Dis. 15(3):293-302.
58. Myrvold HE, Larsson L, Brandberg A. Systemic prophylaxix with doxycycline in intestinal surgery on patients with high incidence of abnormal fecal flora. 1989, Acta Chir. Scand. 155(4-5):277-280.
59. Fiehn NE, Westergaard J. Doxycycline-resistant bacteria in periodontally diseased individuals after systemic doxycycline therapy and in healthy individuals. 1990, Oral Microbiol. Immunol. 5(4):219-222.
60. Feres M, Haffajee AD, Goncalves C, Allard KA, Som S, Smith C, Goodson JM, Socransky SS. Systemic doxycycline administration in the treatment of periodontal infections (II). Effect on antibiotic resistance of subgingival species. 1999, J. Clin. Periodontol. 26(12):784-792.