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Full Length Research Paper

Short-term effects of intensive non-surgical periodontal therapy and low-dose doxycycline on serum levels of IL-6, TNF-α and lipid profile in advanced periodontitis

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Adjunctive use of sub-antimicrobial dose doxycycline (SDD) is a mode of host modulation therapy for periodontitis. However, the potential of SDD for moderating serum inflammatory mediators has not been investigated extensively. This study evaluated the effects of intensive scaling and root planning (ITSRP) ± SDD on the serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and lipid profile in advanced periodontitis. Forty systemically healthy patients were randomly assigned into two groups to take either a placebo or an SDD adjunctive to ITSRP in a double-blind controlled clinical trial. At baseline and after 4 weeks, we collected blood samples and recorded clinical indices. Statistically significant changes in TNF-α and IL-6 serum levels did not occur in either group compared to baseline. A significant increase in high-density lipoprotein (HDL) cholesterol levels and a decrease in white blood cell counts were observed in the SDD group. Between groups, significantly greater improvements in pocket depth, clinical attachment level, mean number of pockets ≥ 7 mm and IL-6 alteration occurred in the SDD group. This study demonstrated the effectiveness of combining ITSRP with SDD in gaining periodontal attachment, IL-6 alteration and HDL cholesterol levels over ITSRP alone. Adjunctive use of SDD is likely to have a protective role against vascular events by mediating patient HDL levels. Further investigation among a high-risk population is recommended.

Key words: Double-blind method, doxycycline, inflammatory mediators, cholesterol high-density lipoprotein (HDL), periodontitis, root planning, serum.

INTRODUCTION

With the growing evidence indicating the periodontal disease impact and interaction with a variety of systemic diseases or conditions, it is now becoming clear that one of the plausible mechanisms of this relationship is the altered level of circulating biomarkers of inflammation in periodontitis (Scannapieco et al., 2003). The susceptible host produces degrading enzymes, cytokines, as well as key mediators of inflammation, in response to periodontopathic bacteria, a process which leads to connective tissue breakdown in the dento-gingival unit (Golub et al., 1990). Based on available data, degradation of connective tissue elements is initially mediated by matrix metalloproteinases (MMPs), a family of zinc-dependent enzymes acting on extracellular matrix molecules such as collagen and involved in wound healing, angiogenesis, chronic inflammation, and tumor cell metastasis (Biljana et al., 2011). The level of MMPs has been shown to increase in diseased periodontal tissues and decrease after treatment (Golub et al., 1995; Cazalis et al., 2008). Currently, manipulating the host

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response in favor of controlling excessive local inflammatory reactions is considered a synergistic host modulation therapy (HMT) in conjunction with treatments targeting the microbial etiology of periodontal disease (Sgolastra et al., 2011).

As the only systemic host modulatory therapeutic now approved by the U.S. Food and Drug Administration, the tetracycline family especially doxycycline have the ability to downregulate the activity of MMPs. Sub-antimicrobial dose doxycycline (SDD) inhibits connective tissue breakdown through several synergistic mechanisms, other than direct inhibition, active MMPs cation chelation reactions, that is, downregulation of key inflammatory cytokines, interleukin-1 and 6 (IL-1 and 6), tumor necrosis factor-alpha (TNF-α), stimulating fibroblastic bone formation, reduction of osteoclastic bone resorption and prostaglandin E_2 (PGE_2) expression (Brown et al., 2004). Moreover, by virtue of its anti-inflammatory and anti-MMP properties, SDD was also shown to be effective in the management of systemic conditions such as coronary artery disease (CAD), and rheumatoid arthritis (O'Dell et al., 2006; Skoog et al., 2002). Given the higher levels of pro-inflammatory cytokines and inflammatory biomarkers in chronic periodontitis, it is crucial to control their plasma concentration, especially in those with other systemic inflammatory predispositions. For instance, it has been shown that plasma levels of TNF-α are significantly associated with coronary and carotid atherosclerosis (Blake and Ridker, 2002; Kleinbongarda et al., 2010), and also its elevated levels significantly predict the increased risk of recurrent coronary events (Ridker et al., 2000).

In spite of several clinical trials indicating clinical and biochemical efficacy of SDD yet, its short term effects on serum inflammatory biomarkers have been limitedly evaluated (Tüter et al., 2007). The aim of this study was to evaluate the short-term effects of intensive scaling and root planing (ITSRP), with or without SDD as an adjunct, on the serum levels of TNF-α, IL-6 and lipid fractions in advanced periodontitis.

MATERIALS AND METHODS

Study design

This study was a randomized, double-masked, placebo controlled, parallel group trial, conducted to evaluate the efficacy of short-term SDD (40 mg) when used in conjunction with intensive scaling and root planing on the serum level of IL-6, TNF-α and lipid fractions in patients with advanced, chronic periodontitis. This study was conducted between May 2010 and January 2011 in the Department of Periodontology in the School of Dentistry of the Guilan University of Medical Sciences (GUMS). In addition, we used a private periodontal clinic also located in Rasht, Iran as a separate site for selecting patients and performing some baseline examinations. The research project was approved by the Ethics Committee of GUMS and adhered to the Declaration of Helsinki guidelines. Upon entry, all the participants signed an informed consent. At baseline and reassessment time points, two examiners who received calibration training from an experienced examiner to reduce intra- and inter-examiner error (kappa > 0.75) and were blind to the study, measured periodontal clinical parameters, including clinical attachment level (CAL), probing pocket depth (PPD), and bleeding on probing (BOP), using a manual probe (Williams periodontal probe, Hu-Friedy, Chicago, IL, USA), at six sites per tooth. BOP was recorded using a bleeding point index (Lenox and Kopczyk, 1973), and the O'Leary plaque index was recorded and expressed as a percentage for each patient. Following the first round of blood sampling, all the eligible subjects underwent ITSRP, by a single and expert periodontist blind to the study protocol, in conjunction with a one-month drug therapy program starting at the baseline periodontal intervention, comprising 40 mg doxycycline or identical-appearing placebo capsules, based on a block randomization model. The non- commercially available SDD 40 mg and placebo (containing inactive filler; that is., starch) were provided by Razak Laboratories, Tehran, Iran. This study followed the protocol for ITSRP described by Quirynen et al, (1995), defined as full-mouth disinfection, consisting of high accuracy full-mouth deep scaling and roots planing (SRP) basically performed within 24 h, plus a local application of antiseptic. However, we prescribed a 0.2% chlorhexidine mouthwash once daily for 14 days and did not perform any intra-pocket disinfecting irrigation. A second visit was scheduled for all patients one week later to remove any possible residual calculus and to emphasize the need to follow the oral hygiene instructions. At the end of the study period totaling one month, all baseline evaluations were repeated upon the return of the subjects to their respective study center. Subjects were also questioned regarding drug compliance and any possible adverse reactions.

Study population and laboratory tests

In a previous study by D'Aiuto et al. (2005), 20 patients per treatment arm provided 80% power to discover a difference of 0.2 ng/L in IL-6 concentrations with a 95% confidence level. Accordingly, a total of 40 patients (17 male, 23 female, ≥ 23 years old) comprised our study population. Twenty patients were randomly assigned to the placebo group while the remaining 20 were assigned to the SDD group (placebo mean age = 49.83 ± 9.03; SDD mean age = 37.42 ± 12.6). Each patient had at least 20 standing teeth without any periapical lesions and was diagnosed as having severe untreated periodontitis manifested by attachment loss and probing depth (PD) ≥ 5 mm in at least 15% of the sites.

Classification of periodontitis was made according to a consensus report in 1999 by Lindhe et al. Exclusion criteria included: any relevant systemic illnesses pregnancy or breast-feeding; requirements for antibiotic prophylaxis; recent medication or periodontal therapy within 6 months of baseline; hypersensitivity to tetracyclines and or any gasterointestinal disorders. All the participants who were selected had a body mass index (BMI) between 20 to 27 $kg.m^2$. Levels of TNF-α, IL-6, and serum lipid fractions including measurements of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TRG), cholesterol (CHOL) and fasting blood sugar (FBS) amounts were obtained and white blood cell (WBC) counts were determined before and one month subsequently after periodontal intervention. Standard sandwich ELISA kits with a lower detection limit of 0.5 pg/ml were used for measuring serum levels of TNF-α and IL-6 (Immunotech SAS, Marseilles, France). Lipid fractions were measured using standard laboratrory procedures and controls (Myers et al., 1989).

Using Statistical Package for the Social Sciences (SPSS) package version 18 (IBM Corporation, NY, USA), after determining the normality of data distribution via a Kolmogorov-Smirnov test, the statistical significance of the differences in periodontal parameters as well as blood chemistry variables between pre- and post-treatment within and between test and control groups were compared using a paired *t*-test and an independent *t*-test, respectively. Pearson correlation analysis was used to analyze correlations

Table 1. Demographic characteristics of subjects in each group.

Variable	Placebo (n = 20)	SDD[1] (n = 20)	P
Age(mean ±SD)	49.83±9.03	37.42±12.6	0.02
Gender (%, male/female)	53/47	65/35	0.48
Smoking ± (%)	12/88	0/100	0.14
Educational level (%) BD/Diploma/AD [2]	53/35/12	18/53/22	0.08
Job ± (%)	65/35	59/41	0.72

[1]SDD: Sub-antimicrobial dose doxycycline [2]Three educational categories included: Below High School Diploma (BD), Diploma (that is, recognized as a high school graduate), and Above High School Diploma (AD).

between post-treatment changes in each clinical parameter and the cytokines, lipid fractions and white blood cell (WBC) counts. P values < 0.05 were assumed to be statistically significant. Demographic and socioeconomic characteristics of patients in test and control groups were compared using a χ^2 test.

RESULTS

All the assigned subjects completed the study and no adverse drug reactions were reported. The general characteristics of the test and control subjects showed no statistical differences except for age, meaning that we treated younger patients in the test group (Table 1). Pre- and post-treatment comparisons of clinical and laboratory parameters within and between test and control groups are summarized in Tables 2 and 3. At the baseline or pre-treatment time point, there were significant differences between the SDD and placebo groups for three clinical parameters; BOP, PPD and CAL, which indicates that although patients were all suffering from advanced periodontitis, the SDD group had significantly more advanced disease either as a matter of distribution, attachment loss, or both. Significant improvement in all clinical parameters was achieved in each group at the post-treatment time period. However, post-treatment comparison between groups shows a significant difference for mean changes (improvement) of PPD, CAL, and the percentage of pockets ≥ 7 mm, in favor of the SDD group, (Table 2). Of the laboratory parameters at baseline, the only difference was that the SDD group had significantly higher WBC counts than the placebo group, which was significantly decreased at reassessment. Following treatment, the alteration of IL-6 levels between the two groups showed a statistically significant difference favoring the SDD group ($P < 0.05$). Our Pearson correlation analysis did not show any significant association between the alteration of TNF-α and IL-6 levels with mean improvement in any of the clinical parameters.

DISCUSSION

We investigated the short term effects of SDD-40 mg plus intensive SRP on the serum levels of TNF-α, IL-6, and lipid fractions as the primary outcome, and on the clinical parameters of advanced periodontitis, as the secondary outcome in this randomized, placebo-controlled study. In spite of a high compliance rate (> 87%) reported by Pershaw et al. (2004) in their clinical trial with the 20 mg formulation of SDD, for the sake of adhering to the principle of simplicity, we designed a once-daily regimen of SDD-40 mg which has a consistent therapeutic efficacy equal to the twice-daily 20 mg dosage (Pershaw et al., 2008). Moreover, it has become clearly evident in the medical community that the most significant factor associated with compliance is the daily dose frequency, and this relationship is a linear one (Kardas, 2007; Claxton et al., 2001).

Although all the participants were categorized as having advanced periodontitis and despite the random assignment of patients for each group, individuals in the test group had more advanced disease and their mean age was significantly lower than the controls. However, our results may be slightly affected by this age difference since the existing literature indicates a limited, if any, influence of the patient's age on the healing process or the individual's treatment response to periodontal non-surgical therapy (Lindhe et al., 1985; Trombelli et al., 2010). Nevertheless, the only baseline difference in laboratory parameters observed between the groups were significantly higher WBC counts in the SDD group.

At the re-evaluation time point, the SDD group showed greater reduction in mean PPD, CAL and pockets ≥ 7 mm. Such a finding is consistent with the similar clinical research investigations (Golub et al., 1998; 2001; Caton et al., 2001; Lee et al., 2004; Gürkan et al., 2005; Pershaw et al., 2008; Duarte et al., 2010). Considering the lipid profile changes, a one-month regimen of SDD resulted a significant increase in HDL levels in the test group.These findings are in accordance with Tüter et al. (2007). In their 6-week study among CAD and periodontitis patients, greater improvement was achieved in SDD over the placebo group in PPD and GI measurements as well as in HDL and apolipoprotein-A (APO-A) levels. Pussinen et al. (2007) reported that serum endotoxin concentration had a positive correlation with total cholesterol and antibody levels to *Porphyromonas*

Table 2. Comparison of clinical parameters for placebo and SDD groups, pre- and post-treatment, and comparison of mean alterations in each parameter post-treatment between groups.

Variable	Placebo (n=20; mean ± SD)		P*	SDD (n = 20; mean ± SD)		P*	Placebo Mean ± SD	SDD Mean ± SD	P†
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment		Pre/post alteration	Pre/post alteration	
Plaque index	52.24±18.81	15.8±7.26	0.001	62.86±26.75	17.75±8.0	0.001	45±22.48	36.5±18.5	0.23
Bleeding index	64.43±19.56	10.78±11	0.001	81.13±20.75	21.77±11.38	0.001	53.65±19.17	59.3±23	0.43
Probing depth (mm)	2.69±0.29	1.48±0.26	0.001	3.66±1.6	1.96±0.71	0.001	1.2±0.29	1.7±0.85	**0.03**
Clinical attachment level (mm)	3.52±0.39	2.32±0.4	0.001	4.41±1.48	2.71±0.88	0.001	1.19±0.32	1.7±0.8	**0.02**
Pockets 4-6 mm (%)	20.24±5.51	1.88±5.31	0.001	27.24±7.86	8.51±13.93	0.001	18.35±6.4	18.72±17.5	0.925
Pockets ≥7mm (%)	1.66±2.47	0.02±0.12	0.001	14.16±17.74	0.78±2.28	0.001	1.63±2.37	13.37±15.69	**0.001**

*Pre- and post-treatment changes within each group (Paired *t*-test). P<0.05 is considered significant. †Comparison between groups (Independent *t*-test).

Table 3. TNF-α, IL-6, and blood lipid levels in both groups before and after treatment and comparison of mean alterations between the two groups.

Variable	Placebo (n = 20; mean ± SD)			SDD (n = 20; mean ± SD)			Placebo Mean ± SD	SDD Mean ± SD	
	Pre-treatment	Post-treatment	P*	Pre-treatment	Post-treatment	P*	Pre/post alteration	Pre/post alteration	P†
TNF-α	5.21±5.48	4.99±4.65	0.598	2.54±2.12	2.35±1.65	0.752	--0.24±2.52	0.22±7.51	0.811
IL-6	2.22±1.59	2.42±1.32	0.598	1.86±2.12	1.40±1.66	0.655	8.63±14.86	-0.12±4.98	**0.032**
CHOL	185.4±27.55	190.9±29.6	0.246	175.12±45.92	180.2±30.8	0.656	-5.47±18.72	-5.17±47.02	0.098
TRG	170.8±132.9	143±81.69	0.092	123.4±56.82	190.94±29.68	0.264	27.88±64.15	-9.47±63.75	0.098
HDL	43.94±9.41	46.17±11.1	0.116	45.47±13.39	50.29±12.23	**0.001**	-2.23±5.55	-2.58±7.04	0.872
LDL	101.8±26.79	115.5±25.7	0.268	111.4±27.87	109.4±32.87	0.730	-5.41±19.43	1.94±22.81	0.319
WBC	6247±1211	5994±1067	0.458	7382±1387	6558±1516	0.001	252.9±1371	823±1059	0.184

*Pre- and post-treatment changes within each group (Paired *t*-test). P<0.05 is considered significant. †Comparison between groups (Independent *t*-test). TNF-α, tumor necrosis factor-alpha, IL-6, interleukin-6, CHOL, cholesterol; TRG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WBC, White blood cell count.

gingivalis, as well as a negative correlation with high-density lipoprotein (HDL) cholesterol in a case-cohort study among a Finnish population. In fact there are several studies in support of the hypothesis that endotoxin/lipopolysaccharides (LPS) of Gram-negative periodontopathic bacteria can activate innate and adaptive immune responses, leading to elevated levels of several pro-inflammatory cytokines among which TNF-α (followed by IL-1β) is the first member of the so-called "cytokine cascade" (Gemmell et al., 1997; Koopmans et al., 1994; Van der Poll and Saurwein, 1993). Simply stated, through the action of these two cytokines, elevation in the levels of free fatty acids, LDL and TRG occurs by different mechanisms such as hepatic lypogenesis, increased lypolysis within adipose tissue, reduced plasma clearance of LDL due to reduced enzymatic activity of lipoprotein lipase, and alterations in the synthesis or clearance balance of TRG (Feingold and Grunfeld, 1987; Lanza-Jacoby and Tabares, 1990). However, according to the 2002 guidelines of the National Cholesterol Education Program that contain its last and most recent definition of hyperlipidemia, lipid profiles of the participants in this study were within normal limits at baseline, except for the TRG levels in the placebo group which after treatment and at reassesment, its mean value reached below the defined cut-off point (NCEP, 2002). Of special importance is the significant increase in the mean value for HDL in the SDD group. According to Castelli et al. (1986), every 1 mg/dl

increment in HDL level is asso-ciated with 2-3% decrease in the risk of cardiovascular disease (CVD) independent of LDL or TRG levels.

Reports indicate an early and sharp increase in the circulating TNF-α and IL-6 levels within 2 h of commencing a single episode of scaling, which may be responsible for some anecdotal reports of pyrexia following treatment (Quirynen et al., 2000; Aguillon et al., 1996). In a previous 4-month follow-up study, we showed that intensive scaling and root planing in patients receiving simultaneous 0.1% chlorhexidine irrigation of the pockets significantly reduces serum levels of C-reactive protein (CRP) and WBC counts in advanced periodontitis patients compared to water irrigation (Radafshar et al., 2010). Since IL-6 which is also called messenger cytokine, triggers the production of CRP in liver, and in the present study daily use of 40 mg SDD better controlled IL-6 elevation in serum following intervention, it can be postulated that adjunctive use of SDD with non-surgical periodontal treatment would further enhance the ability to overcome the systemic inflammatory burden of the host. D'Aiuto et al. (2005) reported a significant decrease in CRP, IL-6 and total CHOL levels after intensive periodontal therapy with the use of mynocycline gel in periodontal pockets in the case group as opposed to their controls receiving standard supragingival scaling after 2 months. Ranges of IL-6 in the sera of periodontitis patients have been reported differently in related studies at baseline, ranging from 0.7 pg/ml as noted by Yamazaki et al. (2005) to 1.56 pg/ml as cited by Ide et al. (2003) who both measured IL-6 before and after treatment as well as higher levels up to 6.35 pg/ml among periodontal patients as indicated by Gani et al. (2009). Corresponding values for TNF-α were 1.81 pg/ml reported by Yamazaki et al. (2005), and 1.82 by Ide et al. (2003). However our report indicates baseline values ranging from 1.86 to 2.22 for IL-6, which showed a significant difference in mean changes from baseline between case and control subjects.

In contrast, Radvar and his co-investigators (2008) in an *in vitro* study showed that surgical/non-surgical treatment was not effective in reducing IL-6 production by peripheral blood monocytes. Although the values for TNF-α in our study was higher (ranging between 2.54 to 5.21), its decreasing amount did not demonstrate a significant difference at reassessment. Similarly non-surgical periodontal treatment by Ikezawa et al. (2008) showed no significant changes in serum TNF-α. The lack of additional benefit of SRP + SDD combination therapy versus SRP alone on this biomarker of systemic inflammation may be to some extent due to our limited study population and the relatively short duration of drug treatment in this study.

Conclusion

The short-term use of SDD in conjunction with intensive non-surgical periodontal treatment has superior benefits over mechanical treatment alone in reducing the level of IL-6 but not TNF-α, and induces significant improvement in HDL levels. Moreover, with the use of SDD, these changes are accompanied by a more promising periodontal treatment outcome in terms of PPD and CAL improvement. Further prospective investigations with a larger sample size are needed to address the role of periodontal therapy with/without adjunctive SDD in improving the general health conditions of patients having both chronic periodontitis and another systemic disease such as CAD, metabolic syndrome, and diabetes mellitus.

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The authors report no conflicts of interest related to this study.

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