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Post-treatment effects of subantimicrobial dose doxycycline on clinical parameters and gingival crevicular fluid transforming growth factor-β_1 in severe, generalized chronic periodontitis

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Gürkan A, Emingil G, Çınarcık S, Berdeli A. Post-treatment effects of subantimicrobial dose doxycycline on clinical parameters and gingival crevicular fluid transforming growth factor-β_1 in severe, generalized chronic periodontitis.

Abstract: *Objective:* Present study aimed to evaluate the effect of 3-month adjunctive subantimicrobial dose doxycycline (SDD) on clinical parameters and gingival crevicular fluid (GCF) transforming growth factor-beta1 (TGF-β_1) levels in chronic periodontitis patients over 12 months. *Methods:* Thirty-five patients with severe, generalized periodontitis participated in the present randomized, placebo-controlled study. Patients received scaling and root planing (SRP) plus 3 months adjunctive SDD or placebo. Clinical measurements and GCF sampling were performed at baseline, 3, 6, 9 and 12 months. Eleven periodontally healthy subjects served as controls for GCF TGF-β_1 analysis. *Results:* Clinical parameters of both SDD and placebo groups significantly improved during the study ($P < 0.0125$). SDD group exhibited significantly higher PD reduction at deep sites (baseline PD ≥7 mm) compared with placebo group at 6 months ($P < 0.05$). In SDD group significantly higher percentage of deep pockets resolved (PD reduction ≥3 mm from baseline) when compared with placebo group at 6 and 9 months (73.4% versus 49.7%; 79.9% versus 50.6%, respectively, $P < 0.05$). PD reduction ≥4 mm for deep pockets from baseline was also greater in SDD group than placebo at 6 months (53.4% versus 36.3%, $P < 0.05$). GCF TGF-β_1 levels of SDD group was significantly higher than baseline ($P < 0.0125$) and placebo group

($P < 0.017$) at 3 months. *Conclusions:* These results ensure further data for beneficial effects of adjunctive SDD therapy in the management of severe chronic periodontitis.

Key words: doxycycline; gingival crevicular fluid; non-surgical therapy; periodontitis; transforming growth factor-β

Introduction

Mechanical removal of bacterial biofilm and endotoxin by means of scaling and root planing (SRP) are effective treatment modalities in slowing or arresting periodontal diseases (1, 2). However, production of enzymes, cytokines and other mediators by host cells that exaggerate immune and inflammatory reactions accounts most of the connective tissue degradation (3). As conventional SRP treatment does not directly target the host response component of periodontal disease, novel treatment concept has changed towards regulation of exaggerated host response in conjunction with mechanical removal of microbial load. Current evidence suggests pharmacotherapeutic modulation of the destructive host response as a novel promising approach in the management of periodontal diseases (4, 5).

Extensive research on tetracyclines (TCs) and TC analogues has shown that these antibiotics exert host-modulatory properties besides being antimicrobial (6, 7). Accumulated evidence suggests that TCs are able to inhibit collagenolytic activity through the inhibition of matrix-metalloproteinases (MMPs) (7–11). Demonstration of TCs ability to sustain MMP inhibitory effect even at doses below those needed for antimicrobial efficacy (11) led to the use of subantimicrobial dose doxycycline (SDD) in the management of chronic periodontitis as an adjunct to non-surgical periodontal therapy. Previous randomized, placebo-controlled studies on chronic periodontitis patients have reported that non-surgical periodontal therapy supplemented with SDD yielded a significant reduction in probing depth (PD) and an increase in clinical attachment level (CAL) when compared with non-surgical periodontal therapy alone (12–19).

Transforming growth factor-β_1 (TGF-β_1) is a ubiquitous cytokine which has both pro-inflammatory and anti-inflammatory effects. TGF-β_1 is a key mediator not only in regulation, limitation and resolution of inflammation (20–22) but also in MMP regulation (23). Effects on cell proliferation and differentiation process, suggests a major role for this cytokine in wound healing, tissue remodelling and regeneration (24). TGF-β_1 has a pivotal role in regulation of collagen metabolism in both physiological and pathological conditions including periodontitis (25). Six-month results of 3-month adjunctive SDD therapy on clinical parameters and GCF TGF-β_1 levels in patients with severe and generalized chronic periodontitis were previously reported (19). The present study presents further findings of this former study which the follow-up period have extended to 12 months.

Materials and methods

Study population

Patients with untreated severe, generalized chronic periodontitis were recruited from the Department of Periodontology, School of Dentistry, Ege University, İzmir. Medical and dental histories were taken and patients received clinical and radiographic evaluation at prescreening visit. Inclusion criteria were being systemically healthy, having at least three natural teeth in each quadrant and at least a total of 14 teeth, diagnosed with severe, generalized chronic periodontitis (26), i.e. >30% sites with ≥5 mm clinical attachment loss and having at least two sites with a PD ≥ 6 mm in each quadrant that bled on probing. None of the patients had allergy or sensitivity to TCs and none had received antibiotics, non-steroidal anti-inflammatory drugs or periodontal therapy within 3 months of the prescreening visit. Women who were pregnant, breast-feeding or using oral contraceptives were excluded. Eleven (six males and five females) systemically and periodontally healthy subjects ranged in age from 34 to 56 (mean age: 40.90 ± 6.86 years) were selected as controls. None of the subjects in the healthy group exhibited PD > 3 mm, had bleeding on probing (BOP), clinical attachment loss or radiographic evidence of bone loss. None of them had a history of medication use in the past 3 months. Patients who fulfilled the inclusion criteria provided written informed consent and participated in the study.

Study design

The present study was a randomized, placebo controlled, double blind, parallel group clinical trial of 12-month duration.

The design of the study is outlined in Fig. 1. Clinical trial was organized into five stages including prescreening, screening, baseline, medication and evaluation. Prescreening examination was conducted to evaluate patient eligibility for inclusion into the study. Assessment of patient eligibility for the study and enrolment of patients into trial was determined by a single examiner. Patients eligible for the study returned to our clinic at screening visit for clinical measurements, 1 week after prescreening. The periodontal status of each patient was assessed by a single experienced examiner. The full mouth PD and CAL at six sites around each tooth were measured with a manual probe (Williams probe) using the cemento-enamel junction as the reference line. Third molar, partially erupted, endo-periodontally lesioned or crowned teeth and sites with subgingival restoration or caries and sites with restorations exceeding cemento-enamel junction were excluded. Teeth with poor prognosis were extracted just before screening visit and sites adjacent to fresh extraction sockets were also excluded. Papilla bleeding index (PBI) (27) and plaque index (PI) (28) for full mouth were also recorded. Clinical measurements were repeated at the last day of SDD or placebo therapy (3 months, recall 1) and 3, 6 and 9 months following the completion of the medication (6 months, recall 2; 9 months, recall 3 and 12 months, recall 4 respectively) by the same examiner who remained unaware to the treatment provided over the entire study period. Intra-examiner calibration exercise of the examiner indicated 97% reproducibility (κ = 0.939) within PD and CAL measurements 2–10 mm. Examined sites were subgrouped in accordance with baseline PD into three groups: group 1: shallow (0–3 mm), group 2: moderate (4–6 mm) and group 3: deep (≥7 mm). Change in PD and CAL values of subgrouped sites from baseline and the percentage of deep pockets (≥7 mm at baseline) exhibiting PD change more than 3 mm and 4 mm was assessed as primary efficacy measurements.

Non-surgical periodontal therapy was performed as previously described (21). Following completion of the first session patients were assigned to SDD or placebo groups via flip of a coin by an independent periodontist who kept the allocation information confident until the data collection and biochemical analysis were completed. SDD group received SRP plus SDD capsules b.i.d (containing doxycycline hyclate equivalent to 20 mg of doxycycline) whereas placebo group was given SRP plus placebo capsules (containing inactive filler; i.e. cornstarch) b.i.d for 3 months.

All patients were represented with a code and were supplied with 2-week doses of SDD or visually identical placebo capsules (28 capsules) in coded bottles and were instructed to take capsules once in the morning and once in the evening at approximately 12-h intervals, 1 h before meals and to return unused capsules. Patient compliance and any accompanying adverse effects were monitored at biweekly visits throughout the medication period. Patients who attended to these monitoring visits on exact day and ascertained to use all of the 28 capsules between two monitoring intervals via pill counts were assessed as 'compliant patients' and were supplied with additional 28 capsules. Adverse effects were also monitored during the evaluation period by phone interviews at 4-week intervals.

Gingival crevicular fluid (GCF) sampling

Gingival crevicular fluid sampling was done at baseline and was repeated at recalls 1, 2, 3 and 4 before recording the clinical parameters. GCF samples of chronic periodontitis patients

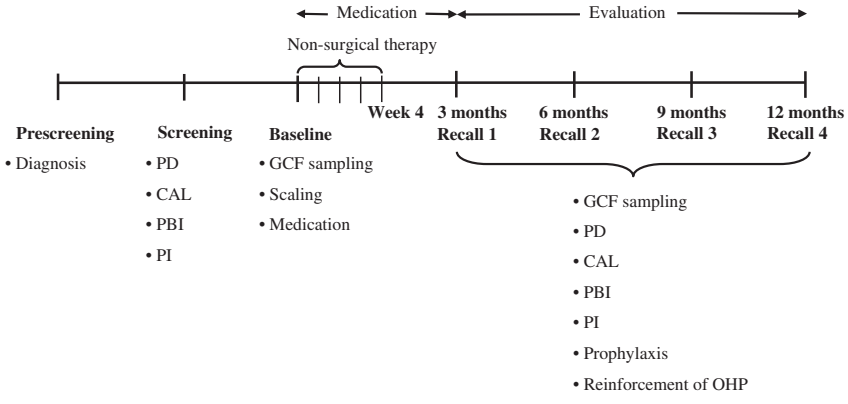

Fig. 1. Study design: following prescreening and screening visits non-surgical therapy started at baseline with full mouth supragingival scaling and followed by 4 week sessions of quadrant root planing and oral hygiene instruction. Adjunctive SDD or placebo administered for 3 months starting at baseline visit. Recall visits (3, 6, 9 and 12 months) consisted of GCF sampling, clinical measurements, prophylaxis and reinforcement of oral hygiene procedures. PD, probing depth; CAL, clinical attachment level; PBI, papilla bleeding index; PI, plaque index; GCF, gingival crevicular fluid; OHP, oral hygiene procedures.

were collected from mesiobuccal aspect of a single-rooted tooth in upper right quadrant exhibiting probing depth of 6–8 mm. In healthy group, GCF samples were collected from mesiobuccal aspect of a single-rooted tooth in upper right quadrant exhibiting probing depth up to 3 mm without BOP. Prior to GCF sampling, the supragingival plaque was removed from the sampling sites with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls. GCF was sampled with filter paper (Periopaper; ProFlow, Inc., Amityville, NY, USA). Paper strips were non-traumatically inserted into the crevice until mild resistance was felt and left there for 30 s. Strips contaminated with blood were discarded. The absorbed GCF volume of each strip was determined by an electronic device (Periotron 8000; ProFlow, Inc.) and placed into a coded sterile polypropylene tube and kept at −40°C until being analysed. The readings from the Periotron 8000 were converted to an actual volume (μl) by reference to the calibration curve.

GCF TGF-β_1 analysis

GCF TGF-β_1 levels were analysed by enzyme-linked immunosorbent assay (ELISA). GCF samples were eluted from the strips by placing them in 200 μl of phosphate buffered solution. To measure biologically active TGF-β_1, GCF samples were acidified using 20 μl 1 N HCl at room temperature for 15 min according to the manufacturer's instructions. Then, the samples were neutralized by adding 20 μl 1 N NaOH. The amount of TGF-β_1 was determined by using the relevant ELISA kit (Bendermed Systems Diagnostics, Vienna, Austria). Results were received as total TGF-β_1 (pg per sample) in the GCF sample. Calculation of the TGF-β_1 concentration in each GCF sample was performed by dividing the amount of TGF-β_1 by the GCF volume of the sample [TGF-β_1 concentration (pg μl^{-1}) = total TGF-β_1 (pg)/GCF volume (μl)].

Statistical analyses

The present study was planned to detect a 1.0 mm difference between treatment groups in PD reduction from baseline for deep sites and 0.6 mm inter-group difference for moderate sites with a significance level of 0.05 and 80% power. Statistical analyses was performed on data obtained from patients who completed the 12-month trial. Subjects were selected as the unit of analysis and mean clinical parameters were calculated at all time-points by averaging the data of all sites (whole mouth clinical parameters). In addition, sites were stratified into three subgroups according to baseline PD as group 1: 0–3 mm, group 2: 4–6 mm and group 3: ≥7 mm. Per-patient PD and CAL change from baseline at subgrouped sites and percentage of sites that exhibited PD change ≥3 mm and ≥4 mm from baseline with an initial PD ≥7 mm were averaged at each time-point for each corresponding tooth site in accordance with the baseline measurements. Clinical parameters and GCF volume of sampling sites, as well as GCF TGF-β_1 levels were also averaged at all time-points. Effect size was calculated with reference to the formula: effect size = (SDD group mean − placebo group mean)/placebo group SD. Number needed to treat (NNT) value was determined by the following formula: 1/(percentage in SDD group − percentage in placebo group).

Demographic variables were analysed using two-tailed Fisher's exact test. Intragroup comparisons of clinical periodontal parameters and PD and CAL change from baseline at subgrouped sites were assessed by Friedman test followed by Bonferroni-corrected Wilcoxon-signed ranks test to analyse significant changes over time. *P*-values <0.0125 were considered statistically significant. Significance of differences between SDD and placebo groups regarding clinical parameters was assessed with Mann–Whitney test with a significance level $P < 0.05$.

Intragroup comparisons of GCF volume and GCF TGF-β_1 levels of SDD and placebo groups were also carried out using Friedman test. When there were significant differences ($P < 0.05$) intragroup comparisons were performed by using Wilcoxon-signed ranks test. *P*-values <0.0125 were considered statistically significant. Intergroup comparisons of GCF volume and GCF TGF-β_1 levels of healthy, SDD and placebo groups were performed using Kruskal–Wallis test followed by Bonferroni corrected Mann–Whitney test. *P*-values <0.017 were considered statistically significant.

Results

Patient disposition and demographics

Flow of participants through each stage of the study is outlined in Fig. 2. Two patients ($n = 1$ in SDD group, $n = 1$ in placebo group) having inability to attend to visits discontinued therapy during quadrant root planing sessions. Noncompliance to study protocol aroused only between the baseline and recall 1 (during the medication period). These non-compliant patients ($n = 4$ in SDD group, $n = 3$ in placebo group) were excluded from the study and transferred to a regular maintenance schedule. Demographic and baseline clinical characteristics of patients completing the trial in SDD and placebo groups have been presented in Table 1. None of the patients

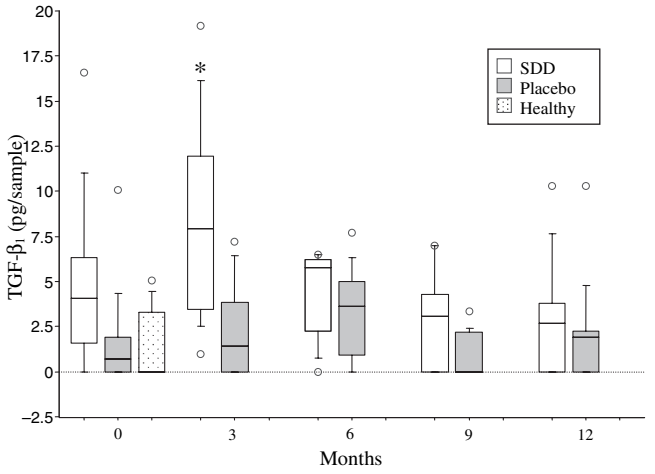

Fig. 2. Gingival crevicular fluid TGF-β_1 total amount (pg) of sampling sites in SDD, placebo and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as open circles. *Significantly different from baseline, 6, 9 and 12 months ($P < 0.0125$), healthy and placebo groups (Kruskal–Wallis test, $P < 0.05$; Mann–Whitney *U*-test, $P < 0.017$).

participated in the study complained of any adverse effects from the use of either SDD or placebo capsules.

Whole mouth clinical findings

There was not any significant differences between SDD and placebo groups regarding the baseline whole mouth mean PD, CAL, PBI and PI scores ($P > 0.05$). The whole mouth clinical parameters markedly improved between baseline and the re-examinations at 3, 6, 9 and 12 months in both groups ($P < 0.0125$). Whole mouth clinical parameters was similar in both groups at all time-points ($P > 0.05$) (Table 2).

Table 1. **Demographic features, smoking habits and baseline clinical characteristics of patients completing the 12-month trial**

	SDD group (*n = 13*)	Placebo group (*n = 13*)
Age (mean years ± SD)	46.38 ± 7.63	46.77 ± 6.95
Age range	34–59	35–59
Male: female (*n*)	10:3	9:4
Smokers (*n*)	4	5
Baseline PD 4–6 mm *n* site (%)	748 (42.45%)	686 (41.32%)
Baseline PD ≥ 7 mm *n* site (%)	376 (21.34%)	314 (18.92%)
Baseline PD 4–6 mm (mean ± SD)	4.97 ± 0.28	4.97 ± 0.24
Baseline CAL 4–6 mm (mean ± SD)	6.16 ± 0.64	6.11 ± 1.09
Baseline PD ≥ 7 mm (mean ± SD)	7.67 ± 0.36	7.43 ± 0.30
Baseline CAL ≥ 7 mm (mean ± SD)	8.63 ± 1.04	8.12 ± 0.77

SDD, subantimicrobial dose doxycycline; PD, probing depth; CAL, clinical attachment level.

Clinical findings of subgrouped sites

No statistically significant changes were observed at sites with a baseline PD 0–3 mm in both groups ($P > 0.05$) (data not shown). Significant PD reductions were observed at sites with a baseline PD 4–6 mm and ≥7 mm and this reduction was maintained over the entire study period in both groups ($P < 0.0125$). Mean PD reduction for sites with a baseline PD 4–6 mm and ≥7 mm were higher in SRP plus SDD group than SRP plus placebo group at 3, 9 and 12 months; however, the differences did not reach to significance ($P > 0.05$). At 6 months SDD group had exhibited significantly higher PD reduction in deep sites compared with placebo group ($P < 0.05$) (Table 3).

Sites with moderate and deep pockets initially exhibited significant clinical attachment gain at all time intervals when compared with baseline ($P < 0.0125$). Sites with an initial PD 0–3 mm did not exhibit statistical significant CAL chances over the study period ($P > 0.0125$). Although the mean clinical attachment gain for sites with a baseline PD 4–6 mm and ≥7 mm were greater in SRP plus SDD group when compared with SRP plus placebo group at all time-points, differences were not statistically significant ($P > 0.05$) (Table 3).

Analysis of sites with a baseline PD ≥ 7 mm revealed that higher percentage of sites was reduced by at least 3 mm following adjunctive SDD therapy than following adjunctive placebo therapy at all time-points. These effects were significant at 6 months (73.4% versus 49.7%, $P < 0.05$; NNT = 4.2, 95% CI 2.70–5.71) and at 9 months (79.9% versus 50.6%, $P < 0.05$, NNT = 3.4, 95% CI 2.22–7.34). In addition percentage of initially deep pockets exhibiting ≥4 mm or more PD reduction from baseline were significantly higher in SDD group than placebo at 6 months (53.4% versus 36.3%, $P < 0.05$; NNT = 5.9, 95% CI 3.47–18.73). None of the deep pockets exhibited a PD increase more than or equal to 3 mm during the study period.

Clinical findings of sampling sites

At baseline, no significant differences were detected between SDD and placebo groups regarding the PD, CAL, PBI, PI and GCF values of sampling sites ($P > 0.05$). Sampling sites of both groups showed improvements in clinical parameters during the 12-month study period ($P < 0.0125$). Mean PD, CAL and PI values of sampling sites showed similar improvements between study groups at all time-points ($P > 0.05$). In contrast, SRP plus SDD therapy resulted in statistically significant reduction in PBI compared with SRP plus placebo therapy at 6 months ($P < 0.05$) (Table 4).

Table 2. **The whole mouth clinical parameters of subantimicrobial dose doxycycline (SDD) and placebo groups (mean ± SD)**

	Months				
	0	3	6	9	12
Probing depth (mm)					
SDD group	4.55 ± 0.74	3.11 ± 0.57*	2.98 ± 0.54*	3.03 ± 0.64*	3.04 ± 0.63*
Placebo group	4.33 ± 1.00	3.10 ± 0.33*	3.13 ± 0.56*	3.12 ± 0.55*	3.12 ± 0.62*
Clinical attachment level (mm)					
SDD group	5.67 ± 0.82	4.85 ± 0.81*	4.73 ± 0.89*	4.74 ± 0.82*	4.80 ± 0.80*
Placebo group	5.72 ± 1.39	4.98 ± 1.10*	5.06 ± 1.33*	5.00 ± 1.40*	5.02 ± 1.44*
Papilla bleeding index					
SDD group	2.98 ± 0.71	0.44 ± 0.26*	0.50 ± 0.38*	0.48 ± 0.35*	0.47 ± 0.31*
Placebo group	2.78 ± 0.89	0.49 ± 0.30*	0.59 ± 0.38*	0.37 ± 0.19*	0.47 ± 0.24*
Plaque index					
SDD group	4.16 ± 0.72	1.92 ± 0.55*	1.46 ± 0.51*	1.23 ± 0.60*	1.24 ± 0.55*
Placebo group	4.05 ± 0.81	1.89 ± 0.42*	1.64 ± 0.60*	1.57 ± 0.36*	1.44 ± 0.43*

*Significant difference from baseline ($P < 0.0125$).

Table 3. **Per-patient mean probing depth (PD) reduction and clinical attachment level (CAL) gain from baseline at diseased sites (PD 4–6 mm and ≥7 mm at baseline) in sub-antimicrobial dose doxycycline (SDD) and placebo groups (mean ± SD)**

	Months			
	3	6	9	12
PD 4–6 mm				
SDD group	1.74 ± 0.52*	1.81 ± 0.57*	1.78 ± 0.66*	1.76 ± 0.64
Placebo group	1.52 ± 0.40*	1.46 ± 0.55*	1.62 ± 0.50*	1.63 ± 0.50*
CAL 4–6 mm				
SDD group	1.00 ± 0.43*	1.12 ± 0.44*	1.14 ± 0.55*	1.05 ± 0.62*
Placebo group	1.00 ± 0.33*	0.78 ± 0.64*	1.01 ± 0.39*	0.99 ± 0.47*
PD ≥ 7 mm				
SDD group	3.05 ± 0.87*	3.38 ± 0.77*†	3.44 ± 0.98*	3.50 ± 0.89*
Placebo group	2.78 ± 0.63*	2.57 ± 0.96*	2.63 ± 1.30*	2.90 ± 1.02*
CAL ≥ 7 mm				
SDD group	1.84 ± 0.68*	1.89 ± 0.71*	2.16 ± 0.64*	2.22 ± 0.73*
Placebo group	1.74 ± 0.89*	1.76 ± 0.96*	1.56 ± 1.21*	1.60 ± 1.15*

*Significant difference from baseline ($P < 0.0125$); †Significant difference from placebo group ($P < 0.05$) effect size = 0.85.

Table 4. **Clinical parameters of sampling sites in sub-antimicrobial dose doxycycline (SDD) and placebo groups (mean ± SE)**

	Months				
	0	3	6	9	12
PD (mm)					
SDD group	7.39 ± 0.65	3.77 ± 1.01*	3.46 ± 0.88*	3.50 ± 1.27*	3.50 ± 0.18*
Placebo group	7.31 ± 0.86	3.85 ± 0.80*	4.00 ± 0.82*	3.78 ± 0.83*	3.56 ± 0.73*
CAL (mm)					
SDD group	8.70 ± 1.97	6.77 ± 2.32*	6.31 ± 2.18*	6.20 ± 1.62*	6.40 ± 2.91*
Placebo group	8.39 ± 1.98	6.39 ± 1.90*	6.54 ± 1.61*	6.67 ± 2.06*	6.44 ± 1.74*
PBI					
SDD group	3.39 ± 0.96	0.46 ± 0.52*	0.39 ± 0.51*†	0.30 ± 0.48*	0.40 ± 0.52*
Placebo group	3.31 ± 0.86	0.31 ± 0.48*	0.85 ± 0.56*	0.56 ± 0.73*	0.56 ± 0.53*
PI					
SDD group	3.62 ± 0.87	1.76 ± 0.73*	1.39 ± 0.65*	1.00 ± 0.82*	0.90 ± 0.88*
Placebo group	3.54 ± 1.27	1.62 ± 0.51*	1.62 ± 0.65*	1.44 ± 0.53*	1.44 ± 0.53*
GCF (μl)					
SDD group	0.44 ± 0.22	0.27 ± 0.16*	0.27 ± 0.15*	0.24 ± 0.19*	0.20 ± 0.05*
Placebo group	0.43 ± 0.20	0.23 ± 0.18*	0.18 ± 0.12*	0.22 ± 0.20*	0.24 ± 0.13*

*Significant difference from baseline ($P < 0.0125$); †Significant difference from placebo group ($P < 0.05$).
PD, probing depth; CAL, clinical attachment level; PBI, papilla bleeding index; PI, plaque index; GCF, gingival crevicular fluid.

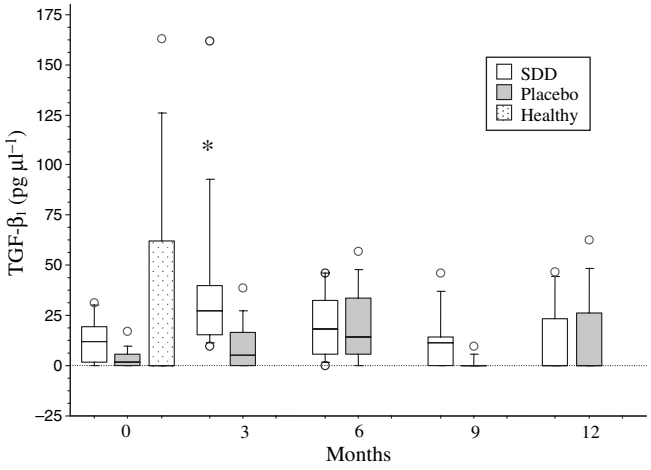

Fig. 3. Gingival crevicular fluid TGF-β_1 concentration (pg1 μl^{-1}) of sampling sites in SDD, placebo and healthy groups. Box plots show median values, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as open circles. *Significantly different from baseline, 6, 9 and 12 months ($P < 0.0125$) and placebo group ($P < 0.05$).

Laboratory findings of sampling sites

Subantimicrobial dose doxycycline, placebo and healthy groups had similar GCF TGF-β_1 total amount at baseline ($P > 0.017$). At 3 months GCF TGF-β_1 total amount of SDD group significantly increased when compared to baseline ($P < 0.0125$) and to healthy group ($P < 0.017$). At other time intervals GCF TGF-β_1 total amount of SDD group gradually decreased and found to be similar to baseline levels ($P > 0.0125$) and to that of healthy group ($P > 0.017$). GCF TGF-β_1 total amount of placebo group was found to be similar to baseline ($P > 0.0125$) and to that of healthy group ($P > 0.017$) at all time-points. Total amount of SDD group was significantly higher than that of placebo group at 3 months ($P < 0.017$). SDD and placebo groups exhibited similar GCF TGF-β_1 total amount at 6, 9 and 12 months ($P > 0.05$) (Fig. 2).

GCF TGF-β_1 concentration of SDD, placebo and healthy groups were found to be similar at baseline ($P > 0.017$). GCF TGF-β_1 concentration of SDD group showed significant improvement at 3 months in comparison to baseline ($P < 0.0125$), however, was similar to baseline levels at 6, 9 and 12 months ($P > 0.0125$). On the other hand, GCF TGF-β_1 concentration of placebo group was similar to baseline levels at all time intervals ($P > 0.0125$). At 3 months, SRP plus SDD therapy revealed a significant increase in GCF TGF-β_1 concentration over the placebo group ($P < 0.017$). GCF TGF-β_1 concentrations of both groups were found to be similar ($P > 0.017$) at 6, 9 and 12 months (Fig. 3).

Discussion

It is well documented that the efficacy of non-surgical therapy at an individual site is related to the baseline PD and deeper pockets have more potential of PD reduction and CAL gain (1). In the present study sites were stratified by baseline PD and the effectiveness of non-surgical therapy was evaluated separately for these sites as well as determining whole mouth clinical parameters. As one might expect, significant improvements in clinical parameters were observed following both SRP plus SDD and SRP plus placebo therapy and these improvements were maintained throughout the study. This consequence is attributable to resolution of inflammation following non-surgical therapy together with regular maintenance therapy that keeps patients highly motivated.

Previous double-blind, placebo-controlled studies evaluating the efficacy of adjunctive SDD therapy in chronic periodontitis patients have revealed additive effect of this treatment modality on mean PD reduction and CAL gain when compared to SRP plus placebo (12–18). The findings of the present study are in accordance with the findings previously reported. In the present study mean probing depth reduction of initially deep pockets was found significantly higher in SDD group than that of placebo at 6 months. It is generally considered that an effect size of 0.8 or higher and NNT values <4 indicate large statistical and clinical significance (29). Moreover, Caton *et al.* (13) have proposed that determining per-patient mean values may be of less clinical significance than determining sites that exhibit improvements exceeding threshold levels. As have been stated by the authors probing depth reductions 3 mm or more might represent marked improvements of individual sites and might be of clinical importance. In the present study, when the percentage of initially deep sites exhibiting probing depth reduction at least 3 mm from baseline was taken into consideration a significantly greater percentage of sites (23% and 29% more sites than that of placebo at 6 and 9 months respectively) in adjunctive SDD group was observed to attain this threshold level than adjunctive placebo. In addition, NNT values for PD reduction ≥3 mm from baseline of severe sites were approximately 4 or less at 6 and 9 months which denotes clinical significance (29). Similar results were found at 6 months regarding 4 mm or more probing depth reduction of deep pockets (18% more sites than that of placebo). These results could be of particular significance as sites with a baseline probing depth ≥7 mm are potential candidates for surgical procedures. Therefore, it could be hypothesized that 3-month adjunctive SDD therapy might reduce the probability and

resultant discomfort and cost of further periodontal therapies thus might provide a clinical significance (30, 31). These findings overall suggest that clinical improvements observed in SDD group might not be solely attributed to the efficacy of SRP, but could also be due to long-term host-modulatory benefits of sub-antimicrobial dose doxycycline therapy (7).

TGF-β_1 plays an important role in wound healing by stimulating fibroblast proliferation, increasing the synthesis of extracellular matrix molecules and inhibitors of MMPs as well as inhibiting MMP synthesis (20). In the present study both total amount and concentration of GCF TGF-β_1 increased following both adjunctive SDD and placebo therapy at 3 months and then exhibited a gradually decreasing trend. Similarly, Stein *et al.* (32) have demonstrated an elevation of TGF-β_1 following mechanical instrumentation at early stages of healing. Kuru *et al.* (33) also have reported elevated GCF TGF-β_1 following both regenerative and conventional surgical periodontal therapy. Conversely, Wright *et al.* (34) demonstrated a substantial decline of GCF TGF-β_1 during resolution of gingivitis. In the present study the increase in cytokine levels following elimination of microbial factors at 3 months might reflect the effort to restore the periodontium in a rapid and effective manner (35) and might suggest that anti-inflammatory and reparatory properties of TGF-β_1 dominate in resolution of chronic periodontitis. At later stages of healing decreasing but still detectable TGF-β_1 activity might indicate to some extent constant healing besides normalizing health status of the periodontium.

In the present study it has been shown that GCF TGF-β_1 total amount and concentration in adjunctive SDD group was significantly higher than that of placebo group at 3 months. TCs are known to increase collagen production in connective tissue (36, 37). It is well documented that TGF-β_1 could increase the production of various connective tissue matrix constituents including collagen and mediates the equilibrium between proteases and their inhibitors (20). In this regard, it could be proposed that the collagen increase following TC application might be partly regulated by TGF-β_1. It was demonstrated that doxycycline increases mRNA levels of TGF-β receptors (38). Several *in vitro* studies have demonstrated that TGF-β_1 application reduced synthesis of MMP forms which are key to periodontitis pathogenesis including MMP-1, -2, -8, -9 and -13 (39–42). Therefore, it could be speculated that elevation of TGF-β_1 that regulate proteinase mechanism might mediate connective tissue healing and collagenous matrix production and might be one of the inhibitory pathways for matrix degradation that SDD possess. Nevertheless, this possible mode of action remains to be verified.

Within the limitations of the present data and sample size it might be speculated that adjunctive SDD therapy improves clinical parameters and increases GCF TGF-β_1 levels together with resolution of deep pockets significantly better than adjunctive placebo in patients with severe, generalized chronic periodontitis. Therefore severe, generalized chronic periodontitis patients might benefit from 3-month use of SDD in combination with SRP therapy.

References

1 Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. *J Clin Periodontol* 2002; **29 (Suppl. 2):** 6–16.

2 Suvan JE. Effectiveness of mechanical nonsurgical pocket therapy. *Periodontol 2000* 2005; **37:** 48–71.

3 Offenbacher S. Periodontal diseases: pathogenesis. *Ann Periodontol* 1996; **1:** 821–878.

4 Salvi GE, Lang NP. Host response modulation in the management of periodontal diseases. *J Clin Periodontol* 2005; **32 (Suppl. 6):** 108–129.

5 Kantarcı A, Hastürk H, van Dyke TE. Host-mediated resolution of inflammation in periodontal diseases. *Periodontol 2000* 2006; **4:** 144–163.

6 Ingman T, Sorsa T, Suomalainen K *et al.* Tetracycline inhibition and the cellular source of collagenase in gingival crevicular fluid in different periodontal disease. A review article. *J Periodontol* 1993; **64:** 82–88.

7 Golub LM, Lee HM, Ryan ME *et al.* Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv Dent Res* 1998; **12:** 12–26.

8 Golub LM, Lee HM, Greenwald RA *et al.* A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 1997; **46:** 310–319.

9 Golub LM, McNamara TF, Ryan ME *et al.* Adjunctive treatment with subantimicrobial doses of doxycycline: effects on gingival fluid collagenase activity and attachment loss in adult periodontitis. *J Clin Periodontol* 2001; **28:** 146–156.

10 Choi DH, Moon IS, Choi BK *et al.* Effects of sub-antimicrobial dose doxycycline therapy on crevicular fluid MMP-8, and gingival tissue MMP-9, TIMP-1 and IL-6 levels in chronic periodontitis. *J Periodontal Res* 2004; **39:** 20–26.

11 Golub LM, Ciancio S, Ramamurthy NS, Leung M, McNamara TF. Low-dose doxycycline therapy: effect on gingival and crevicular fluid collagenase activity in humans. *J Periodontal Res* 1990; **25:** 321–330.

12 Ciancio S, Ashley R. Safety and efficacy of sub-antimicrobial-dose doxycycline therapy in patients with adult periodontitis. *Adv Dent Res* 1998; **12:** 27–31.

13 Caton JG, Ciancio SG, Blieden TM *et al.* Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planning in patients with adult periodontitis. *J Periodontol* 2000; **71:** 521–532.

14 Novak MJ, Johns LP, Miller RC, Bradshaw MH. Adjunctive benefits of subantimicrobial dose doxycycline in the management of severe, generalized chronic periodontitis. *J Periodontol* 2002; **73:** 762–769.

15 Emingil G, Atilla G, Sorsa T, Luoto H, Kırılmaz L, Baylas H. The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J Periodontol* 2004; **75:** 106–115.
16 Emingil G, Atilla G, Sorsa T, Savolainen P, Baylas H. Effectiveness of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid laminin-5 gamma2 chain levels in chronic periodontitis. *J Periodontol* 2004; **75:** 1387–1396.
17 Preshaw PM, Hefti AF, Novak MJ *et al.* Subantimicrobial dose doxycycline enhances the efficacy of scaling and root planing in chronic periodontitis: a multicenter trial. *J Periodontol* 2004; **75:** 1068–1076.
18 Emingil G, Gürkan A, Atilla G, Berdeli A, Çınarcık S. Adjunctive low-dose doxycycline therapy effect on clinical parameters and gingival crevicular fluid tissue plasminogen activator levels in chronic periodontitis. *Inflamm Res* 2006; **55:** 550–558.
19 Gürkan A, Çınarcık S, Hüseyinov A. Adjunctive subantimicrobial dose doxycycline: effect on clinical parameters and gingival crevicular fluid transforming growth factor- beta levels in severe, generalized chronic periodontitis. *J Clin Periodontol* 2005; **32:** 244–253.
20 Prime SS, Pring M, Davies M, Paterson IC. TGF-β signal transduction in oro-facial health and non-malignant disease (Part I). *Crit Rev Oral Biol Med* 2004; **15:** 324–336.
21 Wahl SM, Swisher J, McCartney-Francis N, Chen W. TGF-beta: the perpetrator of immune suppression by regulatory T cells and suicidal T cells. *J Leukoc Biol* 2004; **76:** 15–24.
22 Wahl SM, Chen W. Transforming growth factor-beta-induced regulatory T cells referee inflammatory and autoimmune diseases. *Arthritis Res Ther* 2005; **7:** 62–68.
23 Sodek J, Overall CM. Matrix metalloproteinases in periodontal tissue remodeling. *Matrix (Suppl.)* 1992; **1:** 352–362.
24 Laiho M, Keski-Oja J. Growth factors in the regulation of pericellular proteolysis: a review. *Cancer Res* 1989; **49:** 2533–2553.
25 Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase 72-kDa progelatinase and metalloproteinase inhibitor expression in human fibroblasts by transforming growth factor-β. *J Biol Chem* 1989; **264:** 1860–1869.
26 AAP Consensus report: chronic periodontitis. *Ann Periodontol* 1999; **4:** 38.
27 Saxer UP, Muhlemann HR. Motivation and education. *SSO Schweiz Monatsschr Zahnheilkd* 1975; **85:** 905–919.
28 Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc* 1962; **65:** 26–29.
29 Van Dyke TE. The clinical significance of new therapies for the management of periodontal disease. *J Int Acad Periodontol* 2005; **7 (4 Suppl.):** 191–196.
30 Addy M, Newcombe RG. Statistical versus clinical significance in periodontal research and practice. *Periodontol 2000* 2005; **39:** 132–144.
31 Braegger U. Cost-benefit, cost-effectiveness and cost-utility analyses of periodontitis prevention. *J Clin Periodontol* 2005; **32 (Suppl. 6):** 301–313.
32 Stein SH, Green BE, Scarbecz M. Augmented transforming growth factor- β_1 in gingival crevicular fluid of smokers with chronic periodontitis. *J Periodontol* 2004; **75:** 1619–1626.
33 Kuru L, Griffiths GS, Petrie A, Olsen I. Changes in transforming growth factor-beta1 in gingival crevicular fluid following periodontal surgery. *J Clin Periodontol* 2004; **31:** 527–533.
34 Wright HJ, Chapple ILC, Matthews , J.B . Levels of TGF-β_1 in gingival crevicular fluid during a 21-day experimental model of gingivitis. *Oral Dis* 2003; **9:** 88–94.
35 Ejeil A, Gaultier F, Igondjo-Tchen S *et al.* Are cytokines linked to collagen breakdown during periodontal disease progression? *J Periodontol* 2000; **74:** 196–201.
36 Sasaki T, Ramamurthy NS, Golub LM. Tetracycline administration increases collagen synthesis in osteoblasts of diabetic rats: a quantitative autoradiograph study. *Calcif Tissue Int* 1992; **50:** 411–419.
37 Schneir M, Ramamurthy NS, Golub LM. Minocycline treatment of diabetic rats increases skin collagen production and mass. *Matrix* 1990; **10:** 112–123.
38 Shlopov BV, Stuart JM, Gumanovskaya ML, Hasty KA. Regulation of cartilage collagenase by doxycycline. *J Rheumatol* 2001; **28:** 835–842.
39 Shlopov BV, Smith GN Jr, Cole AA, Hasty KA. Differential patterns of response to doxycycline and transforming growth factor β_1 in the down-regulation of collagenases in osteoarthritic and normal human chondrocytes. *Arthritis Rheum* 1999; **42:** 719–727.
40 Vaday GG, Schor H, Rahat MA, Lahat N, Lider O. Transforming growth factor-beta suppresses tumor necrosis factor alpha-induced matrix metalloproteinase-9 expression in monocytes. *J Leukoc Biol* 2001; **69:** 613–621.
41 Martelli-Junior H, Cotrim P, Graner E, Sauk JJ, Coletta RD. Effect of transforming growth factor-beta1, interleukin-6, and interferon-gamma on the expression of type I collagen, heat shock protein 47, matrix metalloproteinase (MMP)-1 and MMP-2 by fibroblasts from normal gingiva and hereditary gingival fibromatosis. *J Periodontol* 2003; **74:** 296–306.
42 Palosaari H, Wahlgren J, Larmas M *et al.* The expression of MMP-8 in human odontoblasts and dental pulp cells is down-regulated by TGF-beta1. *J Dent Res* 2000; **79:** 77–84.