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The effects of the initial treatment phase and of adjunctive low-dose doxycycline therapy on clinical parameters and MMP-8, MMP-9, and TIMP-1 levels in the saliva and peripheral blood of patients with chronic periodontitis

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Abstract

Introduction: The treatment of periodontal disease can consist of bacterial plaque reduction, risk factor elimination, and metalloproteinase inhibitor medication. The level of matrix metalloproteinases (MMPs) are regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs) as well as therapeutic low-dose doxycycline. The aim of the study was to evaluate the effect of the initial phase of periodontal treatment and the effect of doxycycline on clinical parameters and the MMP-8, MMP-9, and TIMP-1 concentrations in the saliva and peripheral blood of patients with chronic periodontitis.
Materials and Methods: The study group consisted of 33 patients with chronic periodontitis. Conventional periodontal treatment (scaling and root planing) was conducted on all the patients and doxycycline (20 mg orally) was administered twice daily for three months. Thirty-three controls received the conventional treatment only. Clinical scores (PI, BI, PD, CAL) were recorded before and three months after the treatment. MMP-8, MMP-9, and TIMP-1 concentrations in saliva and peripheral blood were measured by ELISA before and after the treatment of 20 patients from the study group and 13 of the controls.
Results: The application of doxycycline 20 mg resulted in significant improvement in clinical parameters compared with the conventional periodontal treatment. Doxycycline did not produce significant reductions in MMP-8 and MMP-9 levels in saliva observed after the conventional treatment. The study revealed increases in the TIMP-1 concentration and the MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios in saliva and blood after treatment with doxycycline.
Conclusions: The study confirmed the modulating effect of doxycycline on the host response in chronic periodontitis.

Key words: chronic periodontitis, therapy, metalloproteinases.

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INTRODUCTION

Periodontitis develops as a result of an imbalance between dental plaque microorganisms that act on periodontal tissues and host defense mechanisms, which are also affected by risk factors. Bacteria and their metabolites stimulate periodontium cells to produce proinflammatory cytokines that enhance the further production of other cytokines and enzymes. Among the effects of bacterial enzymes and those derived from host cells is degradation of the collagen fibers in connective tissue attachment, clinically manifested as disruption of the connection between the gingiva and the tooth surface. Damage to epithelial attachment and progressive loss of connective tissue attachment lead to the formation of periodontal pockets [4].

The degradation of proteins in the extracellular matrix of periodontal connective tissue, including collagen, is caused by matrix metalloproteinases (MMPs), which are released by fibroblasts, other periodontal cells, and cells of the inflammatory infiltrate [7]. MMPs are a family of extracellular zinc-dependent proteolytic enzymes (endopeptidases). Under physiological conditions, MMPs participate in connective tissue remodeling in the developmental phase and are involved in the healing of injured tissue [5, 6]. In the course of inflamma-

tion, bacterial products, cytokines, and growth factors act on periodontal and inflammatory cells, which may then produce and release increasing amounts of MMPs [29, 30]. Increased expression of MMPs in the cells of the periodontium and in inflammatory cells leads to the elevation of MMP levels in body fluids such as gingival crevicular fluid (GCF) and saliva. According to Uitto et al. [39], polymorphonuclear leukocytes are the main source of so-called salivary MMPs. MMPs derived from inflammatory infiltrate cells, but not stationary cells, are mainly responsible for the destruction of tooth-supporting tissues in the course of chronic periodontitis, previously known as adult periodontitis [10, 36, 40]. These MMPs include the neutrophil collagenase MMP-8 and gelatinase MMP-9.

The levels and activities of MMPs in body tissues and fluids are regulated by inhibitors such as tissue inhibitors of metalloproteinases (TIMPs). The most common inhibitor, TIMP-1, is released by most stationary cells of the periodontium (fibroblasts, keratinocytes, endothelial cells) and cells of the inflammatory infiltrate (monocytes/macrophages). Under physiological conditions, inhibitors of MMPs are required for the normal remodeling of connective tissue. An imbalance between the levels of active MMPs and their tissue inhibitors may lead to excessive degradation of extracellular matrix proteins by MMPs [33, 34]. The levels and activities of MMPs may also be regulated by exogenous inhibitors of these enzymes. Tetracyclines, originally used as antibiotics in the treatment of periodontitis, are the most thoroughly investigated MMP inhibitors. Studies by Golub et al. [18, 20, 21] showed that tetracyclines additionally reduce host-cell collagenase activity and thus inhibit the breakdown of extracellular matrix proteins in the connective tissue. Moreover, the activity of tetracyclines in inhibiting collagen degradation is independent of their antibacterial action, while the adverse effects may be decreased by appropriate dose reduction. Soumalainen et al. [37] first demonstrated the sensitivity of MMP-8 to low-dose doxycycline. As a result of further studies, the so-called "sub-antimicrobial dose of doxycycline" (SDD), i.e. the minimum dose of doxycycline that inhibits collagenase activity but does not lead to the emergence of antibiotic-resistant bacterial strains, was established. At the SDD, adverse effects are significantly reduced and long-term use of the antibiotic is possible [16]. In 1998 the Food and Drug Administration approved Periostat® (from CollaGenex) for use in the USA. Periostat® contains 20 mg of doxycycline in tablets to be taken orally twice a day for at least three months as adjunctive therapy in patients with chronic periodontitis undergoing the initial phase of periodontal treatment (scaling and root planing).

Determining the levels of selected MMPs (e.g. MMP-8 and MMP-9) and their inhibitors (e.g. TIMP-1) as markers of periodontal disease activity or progression may be a good and simple method for monitoring and assessing the effectiveness of treatment with medications that are MMP inhibitors and modulators of the inflammatory response.

The objective of the study was to assess the effect of the initial phase of periodontal treatment and of a low dose of doxycycline on the clinical parameters of the periodontal tissues and on MMP-8, MMP-9, and TIMP-1 concentrations in patients with chronic periodontitis. As earlier articles [14, 24, 26, 27, 39] showed that salivary proteases (particularly MMP-8 and MMP-9) reflect changes in the periodontal condition after conventional treatment, the authors chose saliva as a safely and easily obtained material. Plasma levels of MMPs were also examined to check for systemic effects of doxycycline.

MATERIALS AND METHODS

Patient selection and clinical examination

The study group (periostat patients – PP) consisted of 33 patients with chronic periodontitis aged 20–56 years (18 females and 15 males, mean age: 43 years). All patients underwent the first phase of periodontal treatment and at the same time took doxycycline (Periostat) 20 mg orally twice a day 1 h before or 2 h after a meal for 3 months. The first phase of conventional periodontal treatment consisted of scaling and root planing and all procedures were performed by the same operator.

The control group (control patients – CP) consisted of 33 patients with chronic periodontitis aged 23–63 years (18 females and 15 males, mean age: 44 years). The control patients underwent only the initial phase of conventional periodontal treatment, which also lasted three months. The patients were randomly divided into the study group and the control group.

In all patients, the diagnosis of chronic periodontitis was made on the basis of history, clinical assessment, and X-rays. The diagnosis was in accord with the current classification of periodontal diseases [1]. None of the patients gave a history of any concurrent systemic disease. None were regularly taking any kind of medication. Pregnant and lactating women as well as patients with a history of acute or chronic disease of the joints (including rheumatoid arthritis), kidneys, liver, heart, and hemopoietic system or allergic reactions to antibiotics were excluded from the study. Additionally, patients who in the previous 6 months had taken antibiotics or anti-inflammatory medication, especially NSAIDs, including aspirin, were also excluded from the study. All patients signed an informed consent form based on the study protocol approved by the Ethics Committee of the Medical University of Warsaw.

In all 66 patients, clinical measurements of the periodontal tissues were performed prior to treatment and at three months. The clinical examinations of the patients were conducted by one person, the dentist, who initially did not know to which group, study or control, each patient would be assigned. Clinical measurements were performed by the dentist in charge of the patients and under the same conditions (the same examining room, standard lighting). The set of diagnostic instru-

ments used consisted of a dental mirror, a probe, dental dressing forceps, and a WHO 621 periodontal probe. Additionally, the diagnostic Florida Probe (FP32) set was used. The system consists of a computer-linked fixed-pressure periodontal probe which allows standardized, automatically recorded measurements. Oral hygiene was assessed by the simplified plaque index (PI, %) according to O'Leary, which describes the percentage of tooth surfaces with plaque in relation to all assessed surfaces. Four surfaces (medial, distal, buccal, and lingual) of all teeth were assessed. Active periodontal disease was assessed by the simplified sulcus bleeding index (BI, %) according to Ainamo and Bay, which describes the percentage of sites bleeding on examination with a periodontal probe. Six sites were assessed for each tooth (mesially, centrally, and distally to the buccal/labial surface and to the lingual/palatal surface). The severity of pathological changes in the periodontium was assessed by measuring the periodontal pocket depth (PD, mm) and clinical attachment loss (CAL, mm). The measurements were performed at the same 6 sites for each tooth as in the evaluation of pocket bleeding, using the periodontal Florida Probe.

Saliva and blood sampling

Additionally, in 20 patients from the study group (PP1) aged 20–55 years (12 females and 8 males, mean age: 42 years) and 13 patients from the control group (CP1) aged 23–58 years (7 females and 6 males, mean age: 40 years), MMP-8, MMP-9, and TIMP-1 concentrations were measured in the saliva prior to and at three months of treatment. During the first visit and at three months, 5 ml of mixed saliva was collected following stimulation by chewing paraffin granules for 3 min. The saliva samples were collected between 9:00 and 1:00 p.m. at least 2 h after a meal. The saliva was frozen at –20°C (253 K) and stored at that temperature for biochemical assays. Additionally, ca. 10 ml of peripheral blood was drawn from the basilic vein, left for 30 min to coagulate, and then centrifuged at ca. 1000xg. The plasma was frozen at –70°C and stored at that temperature for biochemical assays. Salivary and plasma MMP-8, MMP-9, and TIMP-1 concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available Quantikine kits (R&D Systems, MN, USA) for quantitative determination of total human MMPs (the active enzyme and prometalloproteinases). Laboratory measurements of MMP and TIMP concentrations were done blindly. Each sample was numbered, and the enzyme concentrations were marked by means of ELISA tests, using all blind trials recommended by the test manufacturer. The manufacturer's instructions were closely followed and the tests performed on particular samples were reproducible. The test sensitivities were 0.02, 0.156, and 0.08 ng/ml for MMP-8, MMP-9, and TIMP-1 respectively. The ratio of MMP-8 and of MMP-9 to TIMP-1 was calculated in both the saliva and peripheral blood.

Statistical analysis

Statistical evaluation to compare the levels of particular parameters between two independent groups, i.e. the Periostat group and the control group, was performed with the Mann-Whitney test as the nonparametric alternative to Student's *t*-test. Student's *t*-test for dependent samples was used in the evaluation of the statistical significance of changes in clinical parameters within groups (comparison of baseline values and values at three months of treatment). In comparing clinical and biochemical outcomes with conventional and doxycycline treatment, changes from pre-treatment values were used for the statistical tests. In the entire study, the critical level of significance was assumed at $p=0.05$. To avoid type II-beta error, the power analysis was conducted. All calculations were performed using Statistica Stap Soft software.

RESULTS

Table 1 presents the mean values of the selected parameters describing the clinical condition of periodontal tissues prior to (T_0) and at three months after (T_1) starting the initial phase of treatment using conventional treatment alone (CP) or in combination with a low dose of doxycycline (PP). In both groups the PI was reduced and the difference between the groups was not statistically significant. The BI was decreased by treatment in both groups. The reduction in doxycycline-treated patients was higher and the difference between the groups was found to be statistically significant. In patients treated with the low dose of doxycycline, the mean PD significantly decreased. After conventional treatment alone, the decrease in this parameter was not as marked, but the difference between the groups was significant. A similar tendency, i.e. a more marked reduction in the doxycycline group, was observed in the proportion of pockets >4 mm (%PD>4 mm) and maximum PD (PD_{max}). On the other hand, the mean loss of attachment (CAL) was significantly reduced only in patients treated with the low dose of doxycycline, and the difference between the two groups was statistically significant.

Table 2 shows the mean concentrations of MMP-8, MMP-9, and TIMP-1 in the saliva of the patients with periodontitis prior to (T_0) and following (T_1) treatment with the low dose of doxycycline (study group, PP1) and conventional treatment (controls, CP1). In the doxycycline group, a significant increase in the mean concentration of TIMP-1 was observed in saliva (72.80 ng/ml vs. 138.76 ng/ml). In the control group, the change in the mean TIMP-1 concentration (80.86 vs. 84.12) was not significant. The difference in changes between the two groups was, however, statistically significant ($p<0.05$).

The mean salivary MMP-9/TIMP-1 ratio decreased accordingly in the doxycycline group (6.66–2.23). The difference was statistically significant. A decrease in the

Table 1. Mean values of selected clinical parameters prior to (T_0) and following treatment (T_1) with Periostat (PP) and conventional treatment (CP)

	Periostat group (PP) n=33			Control group (CP) n=33			p
	T_0 (SD)	T_1 (SD)	p	T_0 (SD)	T_1 (SD)	p	ΔT_1–T_0 (PP) vs. ΔT_1–T_0 (CP)
PI (%)	60.42 (22.98)	30.23 (18.04)	0.00000*	65.02 (20.49)	41.51 (23.63)	0.00000*	0.3998
BI (%)	39.71 (27.31)	11.92 (9.36)	0.00000*	45.87 (29.56)	33.82 (23.79)	0.00026*	0.0034*
PD (mm)	2.21 (0.65)	1.92 (0.45)	0.00002*	2.26 (0.64)	2.18 (0.5)	0.18305	0.0030*
% PD >4 mm	10.87 (14.88)	4.81 (6.32)	0.00348*	11.05 (15.87)	8.51 (10.78)	0.07361	0.0383*
PD_{max}	6.21 (2.14)	5.18 (1.94)	0.00000*	5.96 (1.72)	5.66 (1.59)	0.07664	0.0131*
CAL (mm)	3.27 (1.61)	2.94 (1.51)	0.00001*	2.88 (1.53)	2.84 (1.52)	0.68719	0.0396*

* $p<0.05$

Table 2. Mean saliva MMP-8, MMP-9, and TIMP-1 concentrations and ratios prior to and following Periostat treatment and conventional treatment

	Periostat group (PP1) n=20			Control group (CP) n=20			p
	T_0 (SD)	T_1 (SD)	p	T_0 (SD)	T_1 (SD)	p	ΔT_1–T_0 (PP) vs. ΔT_1–T_0 (CP)
SMMP-8 (ng/ml)	179.63 (170.5)	146.93 (132.23)	0.5018	241.76 (249.27)	63.07 (43.4)	0.0176*	0.1253
SMMP-9 (ng/ml)	372.13 (390.67)	268.06 (172.86)	0.2696	384.75 (389.22)	201.70 (176.63)	0.0980	0.5787
STIMP-1 (ng/ml)	72.80 (43.92)	138.76 (93.92)	0.0116*	80.86 (24.53)	84.12 (24.57)	0.6952	0.0164*
SMMP-8/ TIMP-1	2.97 (3.12)	1.43 (1.97)	0.0600	3.50 (3.95)	0.859 (0.8)	0.0235*	0.4483
SMMP-9/ TIMP-1	6.66 (6.68)	2.74 (3.4)	0.0215*	5.47 (6.42)	2.80 (2.76)	0.1510	0.5446

S – saliva, T_0 – prior to treatment, T_1 – following treatment, * $p<0.05$

mean MMP-9/TIMP-1 ratio was also observed in the patients receiving conventional treatment alone (5.47–2.80), but the difference was not statistically significant. No statistically significant difference was demonstrated between the two groups. On the other hand, conventional treatment alone resulted in a significant reduction in the mean salivary MMP-8 concentration 241.76–63.07 ng/ml. In the doxycycline group, the change in the mean MMP-8 concentration was not significant, but the difference between the two groups was statistically significant. A corresponding statistically significant decrease in the mean MMP-8/TIMP-1 ratio was found in the saliva of conventionally treated patients (3.50–0.85). The mean MMP-8/TIMP-1 ratio was also decreased in the doxycycline group (2.97–1.43), but the difference did not achieve statistical significance. Similarly, differences between the changes found in the two groups were not statistically significant.

Table 3 compares mean peripheral blood concentrations of MMP-8, MMP-9, and TIMP-1 in the patients with periodontitis prior to and after the initial phase of conventional treatment either alone or in combination with low doses of doxycycline. In the study group, the mean baseline MMP-8 concentration was 4.00 ng/ml. After treatment with doxycycline it rose to 5.70 ng/ml, but the difference was not statistically significant. On the other hand, the fall in the MMP-8 concentration in patients receiving conventional treatment was significant (3.84 ng/ml vs. 2.75 ng/ml). There was a tendency for peripheral blood MMP-9 concentrations to decrease and TIMP-1 concentrations to increase in both treatment groups. However, these changes were not significant, nor were most of the changes in plasma ratios.

DISCUSSION

Studies undertaken in recent years have shown that periodontal disease develops as a result of interaction between the bacteria present in dental plaque and the

Table 3. Mean plasma MMP-8, MMP-9 and TIMP-1 concentrations and ratios prior to and following Periostat treatment and conventional treatment

	Periostat group (PP1) n=20			Control group (CP) n=13			p
	T_0 (SD)	T_1 (SD)	p	T_0 (SD)	T_1 (SD)	p	$\Delta T_1–T_0$ (PP) vs. $\Delta T_1–T_0$ (CP)
MMP-8 (ng/ml)	4.00 (2.26)	5.70 (5.3)	0.3106	3.84 (2.41)	2.75 (1.01)	0.0465*	0.1533
MMP-9 (ng/ml)	43.34 (43.66)	29.56 (7.15)	0.2700	35.15 (15.05)	29.78 (13.35)	0.2769	0.9598
TIMP-1 (ng/ml)	86.40 (47.53)	126.33 (71.73)	0.0955	60.24 (20.57)	71.48 (47.38)	0.4074	0.1689
MMP-8/ TIMP-1	0.06 (0.07)	0.04 (0.02)	0.2734	0.07 (0.05)	0.04 (0.02)	0.1079	0.6138
MMP-9/ TIMP-1	0.51 (0.25)	0.27 (0.12)	0.0109*	0.64 (0.33)	0.55 (0.35)	0.3364	0.2642

T_0 – prior to treatment, T_1 – following treatment, * $p<0.05$.

host immune and inflammatory responses. These two factors, directly responsible for the destruction of periodontal tissues, are linked by proinflammatory cytokines and proteolytic enzymes, including MMPs. Modern periodontitis treatment modalities target the etiopathological factors with the goal of disrupting the chain of reactions evoked by bacteria in the human defense system. These reactions, which are additionally modulated by risk factors, result in the release of the cytokines and MMPs responsible for the breakdown of connective tissue and bone. This is why treatment may encompass reduction of dental plaque, elimination of risk factors, and the use of PGE_2 and MMP inhibitors.

Our study demonstrated that the initial phase of treatment for periodontitis led to a significant decrease in the PI and BI. However, improvements in the mean PD, the proportion of pockets deeper than 4 mm, and maximum PD were less pronounced, while loss of attachment (CAL) remained virtually the same. Our findings confirmed the reports of other authors who found that in cases of severe periodontitis and pockets deeper than 6 mm, the reduction of bacterial infection alone failed to produce an effective cure and led only to remission of the disease [3, 31]. Surgery was then required with concurrent drug treatment to modify the host response. In our study, additional administration of doxycycline 20 mg twice a day for three months in patients with severe periodontitis produced considerable improvement of clinical parameters compared with conventional causal treatment. We achieved a statistically significant reduction in PI and BI, mean and maximum PD, the proportion of pockets deeper than 4 mm, and mean CAL. Additionally, except for PI, doxycycline treatment efficacy was significantly better than conventional initial phase treatment. These findings are reflected in the available literature. Crout et al. [12] demonstrated that the use of low doses of doxycycline produced a significantly higher reduction of CAL and PD compared with placebo, although the PI and BI did not differ between the two groups. A study by Ashley [2] showed a greater improvement in CAL and PD values and BI in patients treated with low doses of doxycycline. Novak et al. [28] and Caton et al. [8, 9] (a study of 190 patients) found that treatment of periodontitis with subantibacterial doses of doxycycline produced a significantly greater reduction in PD and the CAL compared with scaling and root planing alone, with the best results observed for the deepest pockets (>6 mm). A similar improvement in CAL was seen by Golub et al. [19]. Recent studies by Emingil et al. [13], Choi et al. [11], and Lee et al. [25] demonstrated that both the conventional initial phase of treatment and the use of low doses of doxycycline resulted in a reductions in PI, BI, PD, and CAL, with the reduction in PD always being significantly higher in the doxycycline group.

In our study, MMP-8 values in the saliva and peripheral blood were significantly decreased following the initial phase of treatment, which lasted three months. These findings confirm the report by Chen et al. [10], who observed decreased MMP-8 levels in the GCF of patients with periodontitis as early as two weeks after scaling and root planing. Earlier studies by Gangbar et al. [14], Uitto et al. [39], and Makela et al. [26] also confirmed a decrease in the salivary concentrations of active collagenases and gelatinases following the initial phase of treatment.

Our study did not demonstrate any changes in the TIMP-1 concentrations in saliva and peripheral blood following scaling and root planning, in contrast to the findings by other authors. Studies by Hayakawa et al. [23] and Tüter et al. [38] showed that TIMP-1 concentrations in saliva and GCF significantly increased after the initial phase of treatment, reaching values found in normal subjects. On the other hand, Haerian et al. [22] found that the follow-up phase of treatment decreased the TIMP-1 levels in GCF.

Although our findings only partly support those of other authors, they clearly indicate that scaling and root planing are adequate measures to reduce salivary MMP-8 and MMP-9 concentrations. The most likely cause is the reduction in the number of bacteria responsible for the migration of inflammatory cells and stimulation of their

MMP release. Also, the freeze-thawing of our saliva samples would have released cellular MMPs and so it is possible that our MMP-8 and MMP-9 measurements were indirect reflections of gingival inflammation rather then direct indications of enzyme activity. A reduced load of virulence factors, however, may be insufficient to bring about an increase in the salivary concentration of TIMP-1, which also inhibits the activity of other MMPs and can be released by cells that do not come from the inflammatory infiltrate. Still, we observed some improvement produced by the initial phase of treatment seen as a decrease in the salivary MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios. Tüter et al. [38] observed a similar tendency for the MMP-1/TIMP-1 ratio in GCF.

The improvement in clinical markers produced by treatment with the low dose of doxycycline was not reflected in the concentrations of the two MMPs and TIMP-1 measured in the saliva. It had been assumed that the use of doxycycline in at a low dose would cause a significant reduction in the concentrations of MMP-8 and MMP-9 in the saliva of patients with periodontitis. Reduced salivary MMP-8 and MMP-9 concentrations were indeed found following the treatment with doxycycline, but the difference was not statistically significant. Also, the decrease in the enzyme concentrations in the group of patients receiving conventional periodontal treatment alone was significantly higher. Our findings do not fully agree with the results reported by other authors. Sorsa et al. [35] demonstrated that therapeutic doses of doxycycline decreased the activity and concentrations of MMP-8 in the gingiva, GCF, and saliva of patients with periodontitis. Ashley et al. [2], who investigated three groups of patients with periodontitis, found decreased collagenase activity in GCF following both conventional periodontal treatment (scaling and root planing) and doxycycline 20 mg twice a day and once a day. The highest reduction was seen in the group treated with doxycycline twice a day, with statistical significance being achieved after 12 weeks of treatment. Caton et al. [9], Crout et al. [12] and Golub et al. [15, 17] demonstrated that treatment with doxycycline 20 mg twice a day for one, two, and three months produced a considerable decrease in the GCF concentrations of collagenases. No changes of this kind were observed in controls. In comparisons it must be remembered that the studies described above measured the activity of collagenases, while our study focused on the concentrations of specific MMPs using ELISA methods which would also detect inactive enzymes.

The measurement of collagenase activity would seem more appropriate than ELISAs for evaluation of the MMP inhibitor. Nevertheless, several other studies have used ELISAs (Choi et al. [11], Emingil et al. 13], Lee et al. [25]), reporting slightly different results for MMP-8 concentrations and totals in GCF samples. Salivary MMP-8 levels are probably dependent on the total amounts in GCF entering the oral cavity, and Emingil et al. [13] reported a significant decrease in total MMP-8 immunoreactivity in 30-sec GCF samples after three months of treatment in both doxycycline and placebo groups. However, all the studies found that the difference between such groups did not reach statistical significance until 4–9 months of treatment (Choi et al. [11], Emingil et al. [13], Lee et al. [25]). In our study, salivary MMP concentrations were measured after three months of treatment. The significant reduction in the MMP-8 and MMP-9 concentrations may have resulted from the conventional treatment alone. Further studies, e.g. at 6 and 12 months may possibly reveal the expected differences between the two groups, favoring treatment with low doses of doxycycline. A reduction in the number of bacteria may decrease the inflammatory response within the periodontal pocket when proper oral hygiene is maintained, but the immune response does not return to normal. Periodic increases in the number of bacteria can cause immediate aggravation of the clinical condition. On the other hand, doxycycline 20 mg a day gradually blocks increasing numbers of MMP molecules. In the initial phase of treatment, MMP activity is reduced, but their release later decreases and their concentration at inflamed sites declines.

Our study showed a significant increase in TIMP-1 concentrations in the saliva of doxycycline-treated patients. In the group receiving conventional treatment alone, the salivary inhibitor concentrations remained virtually the same and the difference between the two groups was statistically significant. The increase in the TIMP-1 concentrations observed in the study group could not have resulted from the conventional treatment as it did not occur in the control group. Also, it cannot have been produced by a mere decrease in the concentrations of MMPs, which can block active TIMP-1 molecules, because the changes in salivary MMP-8 and MMP-9 concentrations was not significant in the group receiving the low dose of doxycycline. Doxycycline inhibition of MMPs may rather leave more free TIMP-1 for detection by the ELISA method which we used. Pourtaghi et al. [32] confirmed that topical applications of tetracyclines (tetracycline strips and minocycline gel) in patients with chronic periodontitis caused a significant increase in the TIMP-1 concentrations in the GCF while scaling and root planing did not produce any changes in that level.

In our study, the salivary MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios fell in both groups. The two treatment modalities regulated the ratios of the MMPs to TIMP-1, but the underlying mechanism was different in each group. The conventional periodontal treatment brought about a reduction in the number of bacteria and in inflammation with the resulting decrease in the MMP-8 and MMP-9 concentrations, while Periostat, which is an exogenous inhibitor, contributed indirectly to the increase in TIMP-1 levels.

The administration of an exogenous MMP inhibitor such as doxycycline may restore the balance between the concentrations of MMPs and those of their tissue inhibitors and inhibit the disintegration of the periodontium. Treatment with a low dose of doxycycline pro-

duced a significant improvement in several clinical parameters (BI, PD, %PD >4 mm, max PD, and CAL) compared with the initial phase of conventional periodontal treatment. Treatment of chronic periodontitis consisting of a reduction of dental plaque with concurrent modulation of the host response is clinically more effective than antibacterial management alone and should be used in cases of severe chronic periodontitis.

As no significant decreases in salivary MMP-8 and MMP-9 levels were found in patients treated with the low dose of doxycycline compared with conventional periodontal treatment, further studies should be conducted to measure the long-term levels of these enzymes. The observed increases in the TIMP-1 concentrations and the MMP8/TIMP-1 and MMP-9/TIMP-1 ratios in saliva and plasma produced by doxycycline confirm the modulating effect of the exogenous MMP inhibitor doxycycline on the host response in the course of chronic periodontitis.

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