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Locally delivered doxycycline improves the healing following non-surgical periodontal therapy in smokers

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Abstract

Objective: The outcome of non-surgical periodontal therapy is known to be inferior in smokers compared to non-smokers. In the present study, the question was asked whether such a difference in healing response may be less evident following adjunctive use of locally delivered controlled-release doxycycline.

Methods: One hundred and three patients (42 smokers, 61 non-smokers), each having at least eight periodontal sites with PPD (probing pocket depth) $\geqslant 5$ mm, were following stratification for smoking randomly assigned to two different treatment protocols; non-surgical scaling/root planing (Control) or ultrasonic instrumentation+application of a 8.5% w/w doxycycline gel (Atridox™) (Test). Instructions in oral hygiene were given to all patients. Clinical examinations of plaque, PPD, clinical attachment level (CAL) and bleeding following pocket probing were performed at baseline and after 3 months. Primary efficacy endpoints were changes in PPD and CAL. Patient mean values were calculated as basis for statistical analysis (multiple regression analyses).

Results: The baseline examination revealed no significant difference in mean PPD between treatment groups or between smokers and non-smokers (mean PPD 5.7–5.9 mm). The mean PPD reduction in the control group at 3-month was 1.1 mm (SD = 0.45) for smokers and 1.5 mm (0.67) for non-smokers. In the test group the PPD reduction was 1.4 mm (0.60) and 1.6 mm (0.45) for smokers and non-smokers, respectively. The mean CAL gain for smokers and non-smokers amounted to 0.5 mm (0.56) and 0.8 mm (0.71), respectively, in the control group, and to 0.8 mm (0.72) and 0.9 mm (0.82), respectively, in the test group. Multiple regression analysis revealed that smoking and initial PPD negatively influenced the treatment outcome in terms of PPD reduction and CAL gain, while the use of doxycycline had a significant positive effect.

Conclusion: Locally applied controlled-release doxycycline gel may partly counteract the negative effect of smoking on periodontal healing following non-surgical therapy.

Key words: debridement; doxycycline; local drug delivery; multicenter; periodontitis; randomised controlled trial; scaling and root planing

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The outcome of non-surgical periodontal therapy has been shown to be inferior in smokers compared to non-smokers (Grossi et al. 1997, Kinane & Radvar 1997, Preber & Bergström 1986, Palmer et al. 1999, Ryder et al. 1999). Hence, smokers show less probing pocket depth reduction, less gain in probing attachment and frequently also less recession of the gingival margin. Furthermore, Kinane & Radvar (1997) showed that the adverse effect of smoking on the clinical outcome of non-surgical periodontal therapy was especially related to sites with initially deep pockets.

Some recent studies have indicated that smokers may benefit from the use

of locally delivered antibiotics in conjunction with non-surgical periodontal therapy. Ryder et al. (1999) concluded, based on data from a clinical study comparing the effects of locally delivered doxycycline alone and scaling/root planing, that the utilization of the doxycycline treatment protocol resulted in similar degree of probing pocket depth reduction and attachment gain in smokers and non-smokers, while smoking negatively affected the treatment outcome following scaling/root planing. Williams et al. (2001) analyzed the clinical effects of minocycline microspheres in combination with scaling and root planing (SRP) in smokers and non-smokers and reported a positive effect of the local antibiotic therapy on pocket reduction in both categories of patients, and also that the added benefit of minocycline was found to be greater in smokers than in non-smokers. Palmer et al. (1999), on the other hand, did not observe any improved outcome in clinical parameters following the adjunctive use of a locally administrated 25% metronidazole gel. The different results of the studies referred to may be attributed to differences in the pharmacokinetic performance and the expected time of antibiotic activity. With the metronidazole gel used in the study by Palmer et al. (1999) an effective concentration of the drug can be expected to be maintained for a maximum of 1 day (Stoltze 1992), while the doxycycline gel and minocycline microspheres provides gingival crevicular fluid concentrations of $>300\,\mu$g/ml for at least 7–10 days (Stoller et al. 1998, Williams et al. 2001). Hence, the two latter local drug delivery systems should provide more efficient conditions for an antimicrobial influence on the subgingival ecology. In addition, doxycycline and other chemically modified tetracyclines possess a number of non-antimicrobial properties, e.g. potential to counteract tissue degradation enzymes such as collagenase, MMP8 and elastase (Golub et al. 1995, Llavaneras et al. 1999, Korostoff et al. 2000, Grenier et al. 2002). Such an activity of tetracyclines may offer an additional explanation for the positive effect of these drugs on periodontal wound healing in smokers, since smokers show suppressed levels of the serum protease inhibitors α-1-antitrypsin and α-2-macroglobulin in gingival crevicular fluid at sites with periodontal lesions (Gustafsson et al. 1994, Persson et al. 2001).

The aim of this study was to clinically evaluate if adjunctive, locally delivered controlled-release doxycycline might counteract the negative effect of smoking on periodontal wound healing following non-surgical pocket instrumentation.

Material and Methods

The data analyzed in the present report was generated from a three-center, single-blinded, randomized, clinical study published by Wennström et al. (2001). Briefly, 103 patients (42 smokers, 61 non-smokers; Table 1), each having at least eight periodontal sites with PPD (probing pocket depth) $\geqslant 5$ mm, were following stratification for smoking randomly assigned to two different treatment protocols (Test and Control treatment groups). The subjects of the test group received at baseline a single episode of full-mouth supra-/subgingival debridement by ultrasonic instrumentation. Immediately following the pocket instrumentation, a 8.5% w/w doxycycline gel (Atridox™; Block Drug Corporation, Inc., Jersey City, NJ, USA) was applied in all sites with probing depth $\geqslant 5$ mm in two experimental jaw quadrants. The patients randomized to the control group were subjected to a single episode of full-mouth supra- and subgingival scaling/root planing using an ultrasonic scaler and curettes but no application of antibiotic gel. Instructions in oral hygiene were given to all patients.

Clinical examinations were performed before treatment (baseline) and after 3 months and involved assessments of PPD, location of the gingival margin (GM), bleeding following pocket probing (BoP+) and visible plaque. The measurements of PPD and GM, which were performed by the use of a UNC no. 15 manual periodontal probe and recorded to the nearest whole millimetre, were repeated at the end of each examination and the mean value of the duplicate assessments for PPD and GM was calculated. Clinical attachment level (CAL) was determined based on the PPD and GM assessments using the formula CAL = PPD − GM. Prior to the study, the examiner at each of the three centers was calibrated to levels of accuracy and reproducibility for the various clinical variables to be included in the evaluations, consistent with current standards for clinical periodontal studies (Polson et al. 1997). All examiners were maintained blinded throughout the study period with respect to the design of the trial.

Information regarding smoking habits was obtained through a questionnaire. As smokers were considered all patients who reported that they currently were regular smokers (80% of the subjects was smoking more than 10 cig/day for an average of 25.9 years). Approval of the study was obtained by the Ethics Committee at each study center. Informed consent agreement was obtained from the participants before the start of the study.

Data analysis

The primary efficacy endpoints in the present trial were changes in probing pocket depth and clinical attachment level. Patient mean values were calculated as a basis for the statistical analysis. For description of the data, mean values, standard deviations and cumulative frequency distributions were computed for smokers and non-smokers, respectively, in the control (scaling/root planing) and test (ultrasonic instrumentation/doxycycline application) treatment groups. In addition, the individual mean PPD and CAL changes at 3-month were plotted against initial mean PPD and regression lines were calculated for illustration of a potential relation between the variables.

Stepwise regression analysis was used to identify factors predicting the primary outcome variables (PPD and CAL change). A simple correlation analysis was first carried out and the variables found significantly correlated with any of the outcome variables were

Table 1. Description of the patient sample

	Smokers		Non-smokers		Total sample
	control	doxycycline	control	doxycycline	
no. of patients	20	22	32	29	103
mean age	46.7	47.9	47.6	46.7	47.2
gender (male/female)	6/14	11/11	19/13	17/12	53/50

included as dependent variables in the stepwise regression models. The variable ''smoking'' was always included. The SPSS 11.0 (SPSS Inc., Chicago, IL, USA) software package was used for the data analysis and a *p*-value of <0.05 was considered as statistically significant.

Results

The description of the baseline characteristics of the sites qualifying for treatment, i.e. sites with probing pocket depth (PPD) of $\geqslant 5$ mm and BoP+, is given in Table 2. The subjects contributed with an average of 22 sites. The mean initial PPD varied between 5.7 and 5.9 mm in the four treatment groups ($p > 0.05$). The proportion of pockets $\geqslant 7$ mm amounted to about 25% in all groups except for smokers in the control treatment group (17%).

Figure 1 describes the mean % of sites with visible plaque and BoP+ at the baseline and the 3-month examinations. At 3-month the plaque score was $<25\%$ in all groups. The proportion of sites with BoP+ had decreased from 100% at baseline to 58–60% in the control groups and to 48–58% in the test groups. The lowest mean value was observed for the smokers in the test group.

The mean change in PPD at 3-month is presented in Figs 2 and 3. The mean PPD reduction in the control treatment group amounted to 1.1 mm (SD = 0.45) for smokers and 1.5 mm (0.67) for non-smokers, while in the doxycycline treatment group the PPD reduction was 1.4 mm (0.60) and 1.6 mm (0.45) for smokers and non-smokers, respectively. Of the smokers in the control group, 55% showed a mean PPD reduction of at least 1 mm (Fig. 3). The corresponding figure for smokers in the doxycycline group was 68%. A mean PPD reduction of $\geqslant 1.5$ mm was observed in 20% of the smokers following SRP and 32% of the smokers treated with adjunctive doxycycline.

The mean change in clinical attachment level (CAL) at 3-month is presented in Figs 2 and 4. The mean CAL gain for smokers and non-smokers in the control group amounted to 0.5 mm (0.56) and 0.8 mm (0.71), respectively, and to 0.8 mm (0.72) and 0.9 mm (0.82), respectively, in the doxycycline group (Fig. 2). Of the smokers in the control group, 75% presented an improvement in the mean CAL at 3 months (Fig. 4). Among the smokers treated with doxycycline the corresponding figure was 86%. A mean CAL gain of $\geqslant 1$ mm was found for 41% of the patients in the doxycycline group compared to 10% in the control group.

Two stepwise regression models were formulated to statistically analyze the relative influence of various factors on the treatment outcome, expressed as mean PPD and mean change in CAL at 3 months (Tables 3 and 4). The independent variables included in the models were those showing significant correlation based on an initial correlation analysis. The regression model with individual mean PPD at 3 months as the dependent variable (Table 3) could explain 55% of the variability in the mean PPD ($p < 0.000$). The explanatory variables that entered into the model and showed a negative influence on the 3-month PPD were mean baseline PPD, smoking and 3-month plaque score, while treatment modality positively influenced the outcome variable. A similar model with CAL change at 3 months (Table 4) revealed statistical significance only for the variables mean baseline PPD (negative influence) and treatment modality (positive influence). However, although the model was statistically significant ($p = 0.016$), it could only explain 8% of the observed variability in CAL change.

The mean PPD and CAL changes for smokers and non-smokers in the two treatment groups were also plotted against the mean baseline PPD to further evaluate the interaction between the variables (Figs 5 and 6). In non-smokers, the slope of the regression lines for the two treatments were similar, with about 0.2 mm greater mean PPD reduction and mean CAL gain for the test than the control treatment, independent of the mean initial PPD.

Table 2. Baseline characteristics of qualified experimental sites

	Smokers		Non-smokers	
	control	doxycycline	control	doxycycline
Probing pocket depth				
mean (SD) (mm)	5.7 (0.35)	5.9 (0.45)	5.7 (0.38)	5.9 (0.56)
$\geqslant 7$ mm (%)	17	26	23	25
5–6 mm (%)	83	74	77	75
Bleeding on probing (%)	100	100	100	100
Plaque score (%)	23	25	38	28

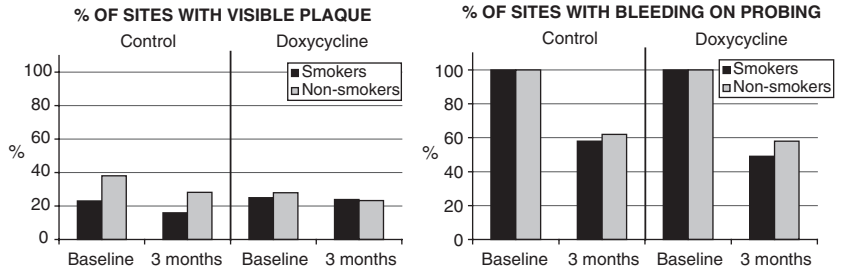

Fig. 1. Plaque and gingivitis score (BoP) at the various examination intervals according to treatment group and smoking habits.

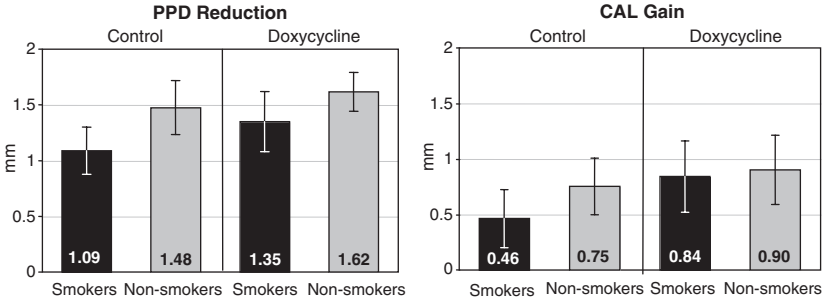

Fig. 2. Mean PPD reduction and CAL gain at 3 months according to treatment group and smoking habits. Bars indicate the 95% confidence interval.

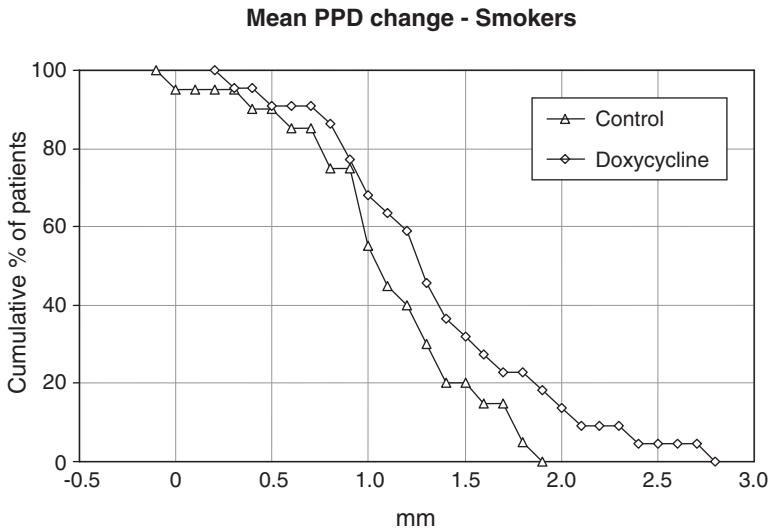

Fig. 3. Cumulative % distribution of smokers with respect to mean PPD change.

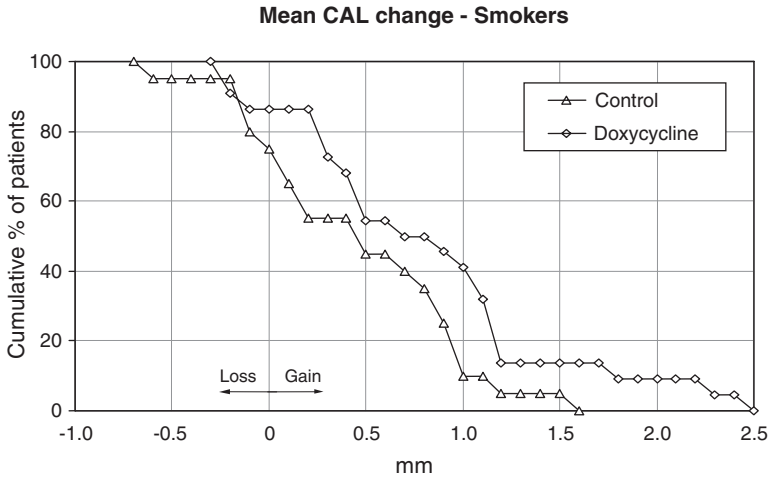

Fig. 4. Cumulative % distribution of smokers in the two treatment groups with respect to mean CAL change.

Table 3. Multiple regression analysis with mean probing pocket depth (PPD) at 3 months as dependent variable (mean 4.4 mm, SD 0.80); adjusted $R^2 = 0.55$

	Coefficient	SE	*p*-Value
constant	3.332	0.697	0.000
mean baseline PPD	– 1.291	0.120	0.000
smoking (0 = no, 1 = yes)	– 0.368	0.108	0.001
3-month plaque score	– 0.243	0.093	0.011
treatment (0 = control, 1 = doxycycline)	0.258	0.109	0.019

Table 4. Multiple regression analysis with mean clinical attachment level change at 3 months as dependent variable (mean 0.8 mm, SD 0.73); adjusted $R^2 = 0.08$

	Coefficient	SE	*p*-Value
constant	3.094	0.894	0.001
mean baseline probing pocket depth	– 0.430	0.156	0.007
treatment (0 = control, 1 = doxycycline)	0.323	0.141	0.024

In smokers, on the other hand, the slope for the regression lines markedly differed for the two treatments. Hence, SRP resulted in inferior healing results with regard to both PPD reduction and CAL gain with increasing mean initial PPD (coefficient of determination 0.47 and 0.42, respectively), whereas the outcome following the test treatment including doxycycline application was unrelated to the mean initial PPD ($R^2 = 0.00$–0.01). In other words, the difference between the two treatment modalities in smokers became more pronounced with increased severity of the disease.

Discussion

The results of the present study demonstrated that locally applied controlled-release doxycycline gel might partly counteract the negative effect of smoking on periodontal healing following non-surgical therapy.

The two non-surgical treatment protocols utilized in the present study both resulted in a significant improvement of the periodontal conditions at 3 months. The SRP performed in the control group resulted in a mean PPD reduction of 1.3 mm and a mean improvement in CAL of 0.6 mm. This magnitude of improvement is consistent with recently published data from a systematic review describing changes following SRP of periodontal pockets initially $\geqslant 5$ mm in depth (Van der Weiden & Timmerman 2002). The authors presented a weighted mean PPD reduction of 1.18 mm 3–4 months after SRP, while the weighted mean value for the gain in CAL amounted to 0.64 mm. Other recent literature reviews (Cobb 1996, Hung & Douglass 2002) also described comparable figures with respect to the reduction in clinical signs of chronic periodontitis 3–6 months after SRP. In these reviews, the PPD reduction of initial medium deep pockets at non-molar sites was calculated to be 1.02–1.29 mm, with a CAL gain amounting to 0.53–0.55 mm.

The treatment response to SRP was found to be less favorable in smokers compared to non-smokers. Hence, the smokers showed about 30% less PPD reduction (1.1 mm versus 1.5 mm in non-smokers) and CAL gain (0.5 versus 0.8 mm), which corroborate the results of previous studies comparing the outcome of various periodontal treatment modalities in smokers and non-smokers (Preber & Bergström 1986, Ah et al. 1994, Grossi et al. 1997, Kinane & Radvar 1997, Palmer et al. 1999, Scabbia et al. 2001). In a recent study by Biddle et al. (2001), it was suggested that the poorer response to non-surgical

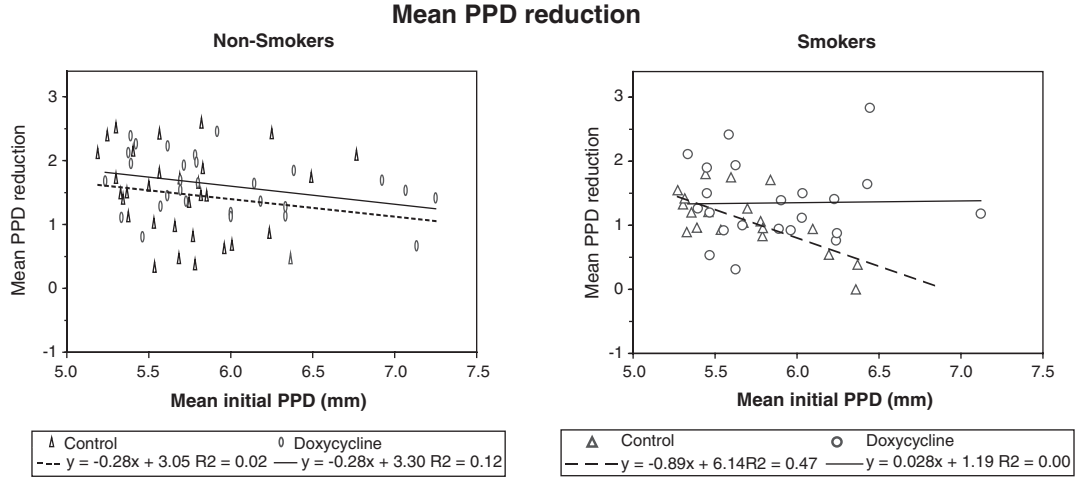

Fig. 5. Scatter plots, regression lines and equations of the PPD reduction against individual mean initial probing pocket depth according to smoking habits and treatment group.

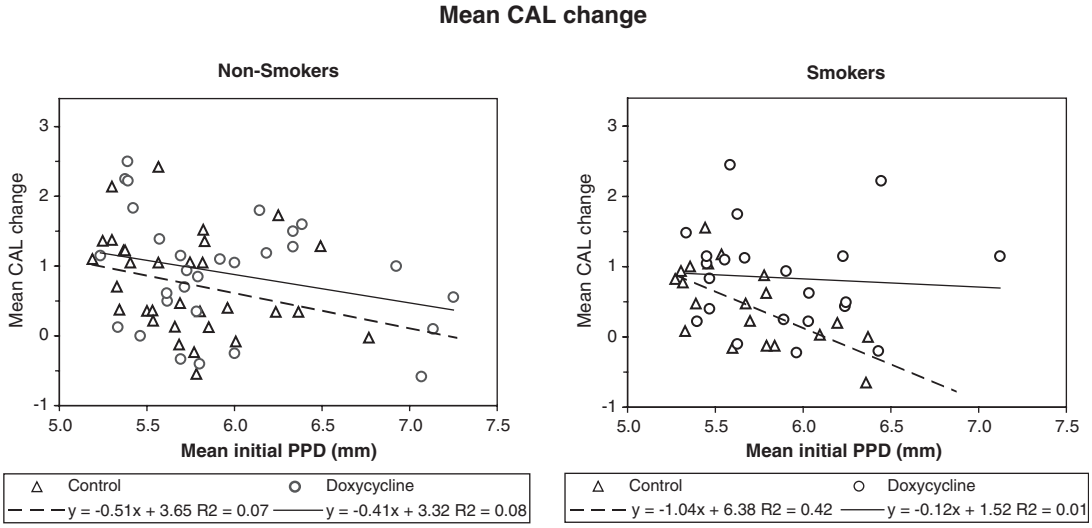

Fig. 6. Scatter plots, regression lines and equations of the CAL change against individual mean initial probing pocket depth according to smoking habits and treatment group.

treatment observed in smokers may in part be explained by less probe tip penetration of the tissue in smokers, particularly in sites measuring 5 mm or more. The authors based their conclusion on a comparison of clinical probing measurements at human molar tooth sites and microscopic assessments of the connective tissue level at the same sites following extraction of the tooth. The reduced probe penetration found in smokers compared to non-smokers was suggested by the authors to be due to a lower degree of tissue inflammation, i.e. lower frequency of bleeding on probing, and a lower height of the suprabony connective tissue portion, which would entail less potential for reduction in probing assessments as a result of successful resolution of the inflammation.

In the present study, the response to SRP in smokers was found to be poorer with increasing severity of the disease, i.e. increased mean baseline PPD (Table 3), a finding that is in accordance with observations reported by Kinane & Radvar (1997) in a 6-week follow-up study of non-surgical periodontal therapy. One explanation for this could be that the ecological environment of deep periodontal pockets in the smoker is more difficult to alter by SRP, an interpretation that is supported by the observation that periodontally untreated as well as treated smokers harbor a subgingival microflora that shows a higher prevalence of e.g. *Bacteroides forsythus* than non-smokers (Zambon et al. 1996, Darby et al. 2000, Boström et al. 2001, Haffajee & Socransky 2001, van Winkelhoff et al. 2001). The smokers in the test group, who, in addition to the pocket instrumentation, received local application of the doxycycline gel, showed improved treatment outcome compared with the smokers in the control group. Furthermore, the improvement became more evident with increasing severity of the disease (Figs 5 and 6). Beneficial effects of locally delivered doxycycline and minocycline in the treatment of periodontal disease in smokers, with a more evident effect in deep pockets, were also reported by Ryder et al. (1999) and Williams et al. (2001).

Since the doxycycline gel used in the present study provides gingival crevicular fluid concentrations ranging from over 1900 μg/ml at placement to about 300 μg/ml at 7 days (Stoller et al. 1998), it is likely that the enhanced treatment outcome is attributed to a change in the subgingival ecology as a result of antimicrobial effects. However, doxycycline as well as other chemically modified tetracyclines (CMT) also possesses non-antimicrobial properties that may positively contribute to improved healing. In vitro studies have demonstrated that doxycycline inhibits proteases by blocking the conversion of latent proteases into active mature forms and the activation of MMP's by chelating metal ions (Korostoff et al. 2000, Grenier et al. 2002). In a rat model, CMT reduced the activity of tissue degradation enzymes such as collagenase, gelatinase, MMP8 and elastase (Llavaneras et al. 1999), and down-regulated bone resorption (Bezerra et al. 2002, Ramamurthy et al. 2002). Also, systemic administration of low doses of doxycycline in humans, which have negligible antimicrobial effects, resulted in reduction of collagenase in gingival crevicular fluid (Golub et al. 1995, 1997, 2001, Ashley 1999, Novak et al. 2002). Hence, since an increased protease activity is associated with smoking, these non-antimicrobial properties of doxycycline and other tetracyclines may offer an additional explanation to the observed improved treatment outcome in smokers who received locally delivered doxycycline compared to smokers subjected to mechanical instrumentation only.

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