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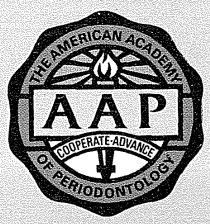

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Long-Term Treatment With Subantimicrobial Dose Doxycycline Exerts No Antibacterial Effect on the Subgingival Microflora Associated With Adult Periodontitis

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Background: The purpose of this study was to determine whether treatment with subantimicrobial dose doxycycline (SDD), 20 mg bid, exerted an antimicrobial effect on the microflora associated with adult periodontitis.

Methods: Following the approval of the protocol and informed consent forms by the respective IRBs at the University of Florida and West Virginia University, 76 subjects with adult periodontitis were entered and randomly assigned to receive SDD or placebo. A split-mouth design was utilized, with each subject receiving subgingival scaling and root planing (SRP) in two quadrants immediately following baseline data collection, while the remaining two quadrants were left unscaled (non-SRP). Microbial samples were collected prior to treatment, after 3, 6, and 9 months of treatment, and after 3 months of no treatment. The samples were examined by microscopy and by enumeration on selective and non-selective media.

Results: All treatments resulted in statistically significant decreases in the proportions of spirochetes and motile rods ($P < 0.05$) and in an increase in the proportion of coccoid forms ($P < 0.0001$) relative to baseline. No between-treatment differences were detected between the SDD and placebo treatments in either the SRP or non-SRP design, with the exception of the small and large spirochetal groups. The spirochetal proportions present in the SDD group were significantly lower ($P < 0.05$) than the paired placebo group during the 9-month treatment and was preceded by a significant decrease ($P < 0.01$) in the proportion of microbiologic sample sites that bled on probing. No between-treatment differences were detected in any of the other microbial parameters.

Conclusion: The microbial differences observed were attributed to the anticollagenase and anti-inflammatory properties of SDD and not to an antimicrobial effect. *J Periodontol 2000;71: 1465-1471.*

KEY WORDS

Periodontitis/microbiology; doxycycline/therapeutic use; clinical trials, controlled.

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Subantimicrobial dose doxycycline (SDD) consisting of 20 mg doxycycline hyclate§ bid has been approved as an adjunct to periodontal scaling and root planing (SRP) for the treatment of adult periodontitis. Doxycycline, like tetracycline and minocycline, in addition to being a broad-spectrum antimicrobial agent, also has inhibitory activity on host-derived collagenases and other matrix metalloproteinases by mechanisms independent of its antimicrobial properties. Specifically, tetracyclines inhibit the activity of mammalian neutrophil and osteoblast collagenases that appear crucial in the destruction of Type I and II collagen found in the periodontal ligament.[1,2] Apart from their anticollagenase activity, tetracyclines are also reported to have anti-inflammatory properties and to be potent inhibitors of osteoclast function.[3] Doxycycline is the most potent anticollagenase inhibitor of the commercially available tetracyclines with IC50 values of 16 to 20 μM for collagenases from PMNs, dental plaque, and gingival tissue.[4,5] Several short-term clinical studies have reported that SDD resulted in a decrease in collagenase activity which was accompanied by a beneficial and significant improvement in attachment

§ Periostat, CollaGenex Pharmaceuticals, Inc., Newtown, PA.

levels and probing depths.[6,7] More recently, a long-term, multi-centered clinical study compared the efficacy of a 9-month regimen of SDD following SRP to a placebo control and found that the use of SDD/SRP showed statistically significant improvements in attachment level and probing depth relative to SRP with a placebo.[8]

Substantial evidence indicates that the adjunctive use of SDD provides a significant benefit to SRP due to its anticollagenase and anti-inflammatory activities rather than to its antimicrobial activity. However, serious concern has been expressed that even subantimicrobial levels of doxycycline may exert a detrimental effect on the subgingival flora. Such an effect could result in the disruption or suppression of the normal flora and lead to its colonization or overgrowth by periodontal or opportunistic pathogens. The purpose of this study was to stringently evaluate the effects of a 9-month regimen of 20 mg doxycycline bid relative to a placebo control on the subgingival flora.

MATERIALS AND METHODS

Study Design

Clinical and microbial data were collected at the University of Florida and West Virginia University from subjects with adult periodontitis during a 9-month treatment period followed by a 3-month no-treatment period. Microbiological samples of subgingival plaque were collected prior to the initiation of treatment (baseline), after 3, 6, and 9 months of treatment, and at 3 months post-treatment. A total of 76 subjects (38 at each study site) with adult periodontitis who met the inclusion and exclusion criteria set forth in the experimental protocol were entered into the placebo-controlled, double-blind treatment phase.

The design of the study was as follows: A split-mouth design was used where two quadrants in each subject received scaling and root planing (SRP) while the opposite two quadrants did not (non-SRP). The quadrants selected to receive SRP were required to have a minimum of two sites with probing depth (PD) and loss of attachment level (AL) of ≥5 but ≤9 mm and that bled on probing. The non-SRP quadrants may or may not have met this criteria. Each subject was then randomly assigned to receive either SDD or placebo treatment. Thus, in effect, there were four treatment groups: SRP-SDD, non-SRP-SDD, SRP-placebo, and non-SRP-placebo. SRP-placebo was considered as a positive control, while non-SRP placebo was a true negative control. Thus, the study was considered to consist of two parallel experiments. SRP-SDD and non-SRP-SDD were paired as were non-SRP-SDD and non-SRP-placebo so that the SDD was the variable tested.

All subjects who completed the 9-month treatment phase were invited to continue in a 3-month no-treatment phase. Of the 67 subjects who completed the 9-month treatment phase, 27 of 36 and 26 of 29 subjects at the University of Florida and West Virginia University, respectively, returned for sampling at the end of the 3-month no-treatment period.

A total of 4 sites, distributed in a minimum of 3 quadrants (4 quadrants were selected where possible), with PD ≥5 mm but ≤8 mm were selected in each subject for microbial sampling; two sites were from the SRP quadrants and two from the non-SRP quadrants. These sample sites were retained throughout the study. Plaque samples were collected using sterile endodontic paper points as previously described.[9] The two microbial samples collected from the SRP sites were pooled by subject and then processed, as were the two samples from the non-SRP sites.

Microbial Enumeration

Immediately following collection, samples were transported to the microbiology laboratories. The samples were gently sonicated to dispense adherent plaque and then processed. Each sample was examined by direct microscopy and by culture on selective and non-selective media.

Microscopy. A 10 µl aliquot of the sample was removed under anaerobic conditions and placed on a clean slide for examination at 1,000× by dark-field microscopy. Eight distinct cellular morphotypes were distinguished and enumerated as previously described.[10]

Selective and non-selective media. Following a series of 10-fold dilutions in pre-reduced, anaerobic-sterilized Ringers solution, performed under strict anaerobic conditions, 0.1 ml aliquots were dispensed onto agar plates and spread with sterile glass rods. The following taxa were enumerated on selective and non-selective media: total anaerobic counts, total facultative counts, total *Streptococcus,* total *Actinomyces, Actinobacillus actinomycetemcomitans, Eikenella corrodens, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus,* enteric bacteria, *Staphylococcus aureus,* and *Candida.* Estimates of obligate anaerobic bacteria were determined by subtracting the total facultative count from the total anaerobic count. If the facultative count was greater than the anaerobic count, a zero value was entered for the obligate anaerobes. Bacteria tentatively identified as *P. intermedia* are, in reality, *P. intermedia sensu lato* since *P. intermedia* was not differentiated from *P. nigrescens.*

Statistical Analyses

The study was considered to consist of two parallel experiments, each of which was designed to test for differences between doxycycline treatment and a placebo control. One design sought for differences following conventional periodontal treatment consisting of mechanical scaling and root planing (SRP), and the second sought for differences without the initial peri-

odontal therapy of scaling and root planing (non-SRP). With this in mind, the resulting data sets were analyzed with the subject as the statistical unit to detect if differences existed at any sample period between doxycycline-treated and placebo-treated subjects.

The factoral ANOVA and Fisher's PLSD test were utilized to determine if statistically significant differences were present between the paired treatment groups at each sample period. The repeated measures ANOVA was used for longitudinal analyses to test for differences within a treatment. If differences were detected longitudinally, the paired *t* test was used to detect the location of the differences. In cases where outliers were suspected, e.g., microbial culture counts that could influence parametric analyses, the Wilcoxon signed rank, a non-parametric version of the paired *t* test, was used to verify statistical significance. Since the paired *t* test and Wilcoxon signed rank require matched samples from the same subject and the 3-month post-treatment data were derived from fewer subjects than the 9-month data set, it was necessary to construct a new data set limited to those subjects who consented to participate in the 3-month no-treatment phase for analyses seeking differences in the latter.

A total of 78 subjects were entered at the two study sites with the expectation that a minimum of 65 subjects would complete the 9-month treatment phase of the study. This sample size, if equally split, had a 90% power of detecting a difference of 1 $\log_{10}$ in microbial counts between SDD and the paired treatment. All statistical comparisons were based on $P \leq 0.05$.

RESULTS

Microscopic Enumeration

Differences between and within treatment groups were analyzed for each of the following morphological groups: small, intermediate, and large spirochetes; motile rods; coccoid forms; non-motile rods; fusiforms; and filamentous rods.

Between-treatment differences. No between-treatment differences were detected for any morphological group other than the spirochetes. In the SRP design, the proportion of small spirochetes (Table 1) present at the 3- and 6-month sample periods and the proportion of large spirochetes (Table 2) present at the 6-month sample were significantly lower in the SDD group than in the placebo group ($P < 0.05$). In the non-SRP design, the proportions of both the small and large spirochetal groups present at the 9-month sample were significantly lower in the SDD group than in the placebo group ($P < 0.05$).

Within-treatment differences. Differences within a treatment were analyzed using the paired *t* test to detect if the treatment had any significant effect on a particular morphologic group. Both the SDD and placebo treatments, regardless of SRP or non-SRP design, produced statistically significant reductions in both the intermediate and large spirochetal groups (Tables 2 and 3). In the SRP design, the SDD treatment yielded significant reductions in small spirochetes, relative to baseline, for all 9 months of treatment, while the placebo treatment demonstrated only significant reductions at the 9-month sample period (Table 1). Significant reductions in the proportion of motile rods were detected for all treatments at all sample periods relative to baseline (Table 4). Significant increases ($P < 0.0001$) were found in the proportion of coccoid forms, relative to baseline, for all sample periods (Table 5). No significant changes were noted during any treat-

Table 1.

Mean Percentage of Small Spirochetes Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	Treatment Phase 3 Months	6 Months	9 Months	3 Months Post
SRP	SDD	10.35	4.32*†	6.28*†	4.95†	6.98
	Placebo	10.36	9.59*	10.40*	5.89†	8.57
Non-SRP	SDD	9.98	6.26	7.36	6.58*	8.53
	Placebo	11.42	8.91	9.54	9.79*	10.25

* Statistically significant differences ($P \leq 0.05$) between SDD and placebo treatment groups.
† Statistically significant within-treatment differences ($P \leq 0.05$) relative to baseline.

Table 2.

Mean Percentage of Large Spirochetes Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	Treatment Phase 3 Months	6 Months	9 Months	3 Months Post
SRP	SDD	3.34	0.72†	0.13*†	0.58†	0.81†
	Placebo	4.29	1.99†	1.13*†	1.74†	1.06†
Non-SRP	SDD	3.22	0.81†	0.56†	0.56*†	0.47†
	Placebo	3.13	2.28	1.25†	1.79*†	1.76

* Statistically significant differences ($P \leq 0.05$) between SDD and placebo treatment groups.
† Statistically significant within-treatment differences ($P \leq 0.05$) relative to baseline.

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