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Initial antimicrobial effect of controlled-release doxycycline in subgingival sites

M. G. Jorgensen, A. Safarian, N. Daneshmand, R. J. Keim, J. Slots
University of Southern California School of Dentistry, Los Angeles, California, USA

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Objectives: To determine the ability of a 10% doxycycline hyclate controlled-release polymer (Atridox®) to suppress periodontopathic bacteria when placed subgingivally following scaling and root planing (Sc/Rp).

Methods: Eight males and seven females, mean age 48 years, with moderate to advanced periodontitis participated in the study. In each patient, bilateral periodontal pockets probing 6–7 mm were randomly assigned to treatment by Sc/Rp + doxycycline polymer or by Sc/Rp alone. Subgingival placement of doxycycline polymer was carried out according to the manufacturer's instructions. Sc/Rp was performed with hand instruments for at least 10 min in each study tooth. Subgingival samples were collected by paper-points at baseline, at 2 weeks and at 4 weeks post-treatment. Culture methodology was used to isolate and identify putative periodontal pathogens, including *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythia*, *Prevotella intermedia*/*Prevotella nigrescens*, *Campylobacter* species, *Eubacterium* species, *Fusobacterium* species, *Peptostreptococcus micros*, *Eikenella corrodens*, *Staphylococcus* species, enteric gram-negative rods, β-hemolytic streptococci and yeasts. The microbiologic examination was carried out blindly. Microbiological data were analyzed using a General Linear Model Analysis of Variance for within and between group effects.

Results: Sites receiving Sc/Rp + doxycycline polymer and sites receiving Sc/Rp alone exhibited similar levels of periodontal pathogens at baseline and did not differ significantly in total viable counts and proportional recovery of periodontopathic bacteria post-treatment.

Conclusions: Controlled-release doxycycline placed in moderate to deep periodontal pockets caused no significant additional reduction in the subgingival pathogenic microbiota compared to thorough Sc/Rp alone. Since controlled-release doxycycline may not significantly suppress several subgingival pathogenic microorganisms and seems to possess no distinct advantage over broad-spectra, safe and inexpensive antiseptics, the rationale for its employment in periodontal therapy remains unclear.

Michael G. Jorgensen, DDS, University of Southern California School of Dentistry, 925 West 34th Street, Room 4274, Los Angeles, CA 90089–0641, USA
Tel: +1 213 740 0717
Fax: +1 213 740 6778
e-mail: jorgensm@usc.edu

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The 'best practice' for controlling periodontal infections is still developing. The broad ranging types of periodontal infectious disease prohibit a rigid recommendation with respect to initial treatment and follow-up care. Apart from socio-economic factors, a recommended course of therapeutic action in periodontics would depend

on the biological behavior expected of the disease and the location and anatomy of the periodontal lesion. Current guidelines suggest that most patients with periodontitis should receive mechanical and chemical debridement, with surgery reserved for patients exhibiting poor response or advanced disease (1, 2). However, effective control of the pathogenic microbiota in periodontal sites has often proven more difficult by mechanical depuration than expected, providing a rationale for the adjunctive use of chemotherapeutic agents (3). Antimicrobial drug therapy may be beneficial for both periodontal treatment and prevention, and has been shown to reduce tooth loss and the need for surgical treatment in patients having advanced periodontitis (4).

Antiseptic and disinfective microbicides have more than a 100-year history of use as periodontal pocket disinfectants (1). Microbicides have a wide spectrum of activity that makes them effective against a broad range of bacteria, yeasts, protozoas and viruses; however, they are toxic to human tissue in systemic applications. Nevertheless, since topical antimicrobials give rise to minimal absorption into periodontal tissue (5), the risk of significant adverse events and drug interactions is virtually eliminated. The 1990s witnessed a resurgence of interest in microbicides due to the global emergence of antibiotic-resistant microorganisms (6). Microbicides show little propensity for inducing resistance because of their multiple intracellular targets, although acquired bacterial resistance is increasing to common antimicrobial agents in consumer products, such as quaternary ammonium compounds, bisguanides and triclosan (7–9). However, with the exception of a worldwide usage of chlorhexidine mouth rinses, antiseptics for periodontal treatment were for many years mostly employed in developing countries where other types of subgingival medication are often unavailable or too costly (1).

Antibiotics were introduced clinically in the 1940s, and systemic antibiotics became widely used in periodontal therapy in the 1980s to combat periodontopathic bacteria having the ability to invade periodontal tissue, inhabit subgingival sites that are difficult to reach by topical means, or colonize various oral domains from which they may translocate to periodontal sites (10). Inherent problems with systemic antibiotics include limited spectra of activity at clinical dosing, and thus inability to affect all pathogens present in polymicrobial periodontal infections. An incorrect decision in the choice of antibiotics not only causes a delay of effective antimicrobial treatment and potential loss of periodontal attachment, but may also subject patients to adverse effects from unnecessary medication, including possible induction of antimicrobial resistance and hypersensitivity reactions (6, 11). Since periodontal clinical findings are virtually never revealing enough to make a definite diagnosis of the causative pathogens, selection of the best and most specific therapeutic strategies utilizing systemic antibiotics may sometimes require a microbiological analysis (1, 12).

In the past couple of decades, antibiotic products with high drug content for direct subgingival placement have become commercially available (13, 14). However, although vigorously promoted, the efficacy of topical periodontal antibiotic therapy has not been convincingly demonstrated (15). A recent investigation of topical doxycycline therapy in periodontitis patients reported no significant reduction in the occurrence of periodontal pathogens (16). The present study investigated the antimicrobial action of a 10% doxycycline hyclate controlled-release polymer (Atridox®, CollaGenex Pharmaceuticals, Newtown, PA, USA) when placed subgingivally following scaling and root planing (Sc/Rp) in the treatment of human periodontitis. Information on the ability of locally applied doxycycline to suppress periodontopathic bacteria *in vivo* may help shed light on the usefulness of this mode of antimicrobial periodontal treatment.

Materials and methods

Fifteen periodontitis patients (seven females, eight males; 20–72 years of age; mean age, 48 years) treated at the Advanced Periodontics clinic at the University of Southern California School of Dentistry consented to participate in the study. Patient selection criteria included the following:

- presence of contralateral periodontal sites probing at least 6 mm;
- no history of allergic reaction to doxycycline;
- no antibiotic prophylaxis required for dental treatment;
- no pregnancy or nursing;
- not medically compromised;
- no periodontal or antibiotic treatment within the past 6 months.

The institutional review board of the University of Southern California approved the protocol of the study and written informed consent was obtained from all subjects.

Baseline data included probing depths, gingival recession, plaque index (17) and subgingival microbial analysis. In each patient, bilateral periodontal pockets probing 6–7 mm were randomly assigned to groups receiving either Sc/Rp followed by placement of a 10% doxycycline hyclate controlled-release polymer or Sc/Rp alone. Study patients received a series of oral hygiene instructions. Each study site received Sc/Rp under local anesthesia with hand instruments for at least 10 min; doxycycline polymer was applied according to the manufacturer's instructions. Periodontal dressings (non-eugenol or 2-octyl cyanoacrylate) were not used to enhance drug retention. Subgingival microbial analysis was repeated after 2 and 4 weeks. No further periodontal treatment was performed until after the 4-week follow-up, at which time each patient was offered appropriate additional periodontal therapy.

After removing supragingival plaque, two fine endodontic paper-points were inserted to the depth of each study periodontal pocket for 10 s and transferred to VMGA III transport medium (18). Samples were processed within 2 h of collection. Anaerobic microbiological isolation and identification of putative periodontal pathogens were carried out with no knowledge of the source of the specimens following previously described

procedures (19). Periodontal pathogens identified were *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythia*, *Prevotella intermedia/Prevotella nigrescens*, *Campylobacter* species, *Eubacterium* species, *Fusobacterium* species, *Peptostreptococcus micros*, *Eikenella corrodens*, *Staphylococcus* species, enteric gram-negative rods, β-hemolytic streptococci and yeasts. Bacteria designated as major periodontal pathogens included *A. actinomycetemcomitans, P. gingivalis, D. pneumosintes* and *T. forsythia*. The percentage recovery of periodontal pathogens was determined by the colony count of each microbial taxon in relation to total viable count. The data were analyzed using a General Linear Model Analysis of Variance for within and between group effects, employing the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

In the 15 study subjects, baseline probing depths were 6–8 mm for sites receiving Sc/Rp + doxycycline as well as for sites receiving Sc/Rp alone. Gingival recession at baseline was 0–2 mm for sites receiving Sc/Rp + doxycycline and 0–1 mm for sites receiving Sc/Rp alone. Tooth surfaces revealing supragingival plaque ranged from 10 to 30% among the study subjects. No adverse treatment effects were noted at any time during the study.

Figures 1–3 describe the microbiological findings. Average total colony counts were at baseline, at 2 weeks post-treatment and at 4 weeks post-treatment, respectively, 5,278,000, 757,000 and 653,000 for sites receiving Sc/Rp + doxycycline, and 4,890,000, 748,000 and 687,000 for sites receiving Sc/Rp alone (Fig. 1). The average percentage of total cultivable periodontal pathogens was at baseline, at 2 weeks post-treatment and at 4 weeks post-treatment, respectively, 35%, 12% and 22% for sites receiving Sc/Rp + doxycycline, and 32%, 19% and 17% for sites receiving Sc/Rp alone (Fig. 2). The average percentage of total isolates that were designated as major periodontal pathogens was at baseline, at 2 weeks post-treatment and at 4 weeks post-treatment, respectively, 10%, 1% and 4% for sites receiving Sc/Rp + doxycycline, and 13%, 3% and 3% for sites receiving Sc/Rp alone (Fig. 3).

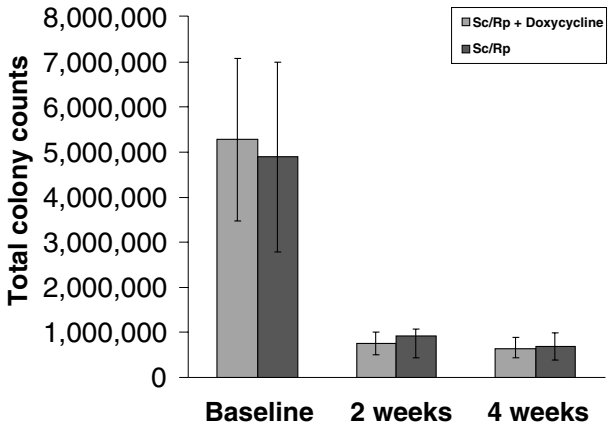

Fig. 1. Total viable microbial counts (± standard error) after scaling and root planing (Sc/Rp) with and without subgingival placement of controlled-release doxycycline.

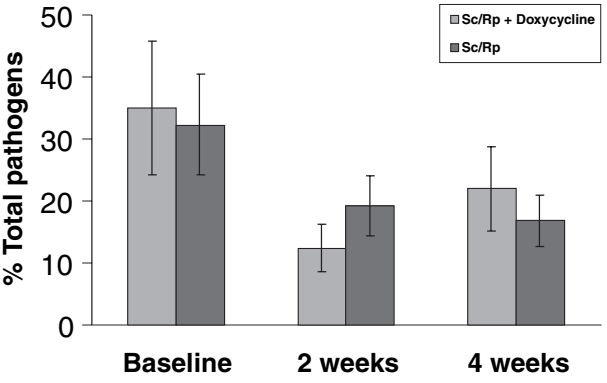

Fig. 2. Percentage of total periodontal pathogens (± standard error) after scaling and root planing (Sc/Rp) with and without subgingival placement of controlled-release doxycycline.

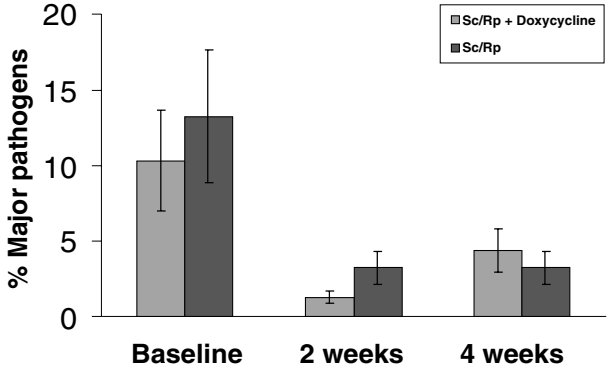

Fig. 3. Percentage of major periodontal pathogens (± standard error) after scaling and root planing (Sc/Rp) with and without subgingival placement of controlled-release doxycycline.

No significant differences in microbiological findings were identified for sites receiving Sc/Rp + doxycycline

and sites receiving Sc/Rp alone ($p = 0.91$ for total colony counts, $p = 0.93$ for percentage of total pathogens, $p = 0.46$ for percentage of major pathogens).

Discussion

Limitations of the present investigation should be recognized. First, as discussed elsewhere (20), the split-mouth design employed in the present study requires fewer study patients than a parallel study design but carries the risk of underestimating the efficacy of the antimicrobial agent tested. Second, the present study was limited to a 4-week follow-up period, which, however, may be sufficient to evaluate the microbiological impact of the treatments rendered, because controlled-release doxycycline can be expected to exert the most profound antimicrobial effects in the first weeks post-treatment when the drug is detectable in subgingival sites (21). Third, this study examined the antimicrobial efficacy of controlled-release doxycycline employed together with thorough Sc/Rp, and not as a monotherapy or in conjunction with less intensive root debridement as described by Garrett *et al*. (22). Fourth, the present study did not apply a periodontal dressing in an attempt to enhance subgingival retention of the doxycycline polymer (21).

Since tetracyclines are essentially not absorbed into adjacent gingival tissue by subgingival application (5), they can be expected to exert clinical benefits only through antimicrobial effect in the periodontal pocket and not through modulation of matrix metalloproteinase activities within periodontal tissues (23). Doxycycline is a broad-spectrum antibiotic, although yeasts and some enteric gram-negative rods exhibit intrinsic resistance (24). However, despite the wide-ranging antimicrobial activity of doxycycline, the present topical therapy failed to eradicate several bacteria that *in vitro* were susceptible to the antibiotic. Similarly, Salvi *et al*. (16) reported a failure of subgingival doxycycline placement to suppress various periodontopathic species to below detection level. The presence of doxycycline-susceptible bacteria post-treatment may be due to the biofilm phenomenon that helps protect periodontal bacteria against antimicrobial assault (25), or to rapid pocket recolonization from supragingival bacterial reservoirs. Furthermore, due to technical difficulties in applying the doxycycline polymer throughout the entire periodontal pocket and to a rapid outflow of gingival crevice fluid from inflamed periodontal sites (26), some subgingival domains may have been exposed to doxycycline at levels too low to inhibit resident microorganisms. In the same way, a chlorhexidine controlled-release device plus Sc/Rp yielded no more suppression of subgingival pathogens than Sc/Rp alone (27). In contrast, using a similar study design, subgingival irrigation with povidone-iodine for 5 min plus Sc/Rp was recently shown to cause a greater reduction of periodontopathogens than that obtained by thorough Sc/Rp alone (20).

In conclusion, although chemotherapeutics are recognized conceptually to constitute valuable supplements to mechanical periodontal therapy (1, 28), subgingival treatment with a 10% doxycycline controlled-release polymer showed no significant decrease in putative periodontal pathogens beyond that achieved by thorough Sc/Rp alone. The chief drawbacks to topical antibiotic therapy in periodontics are an insufficient spectrum of antimicrobial activity for even broad-spectra antibiotics and the risk that, with repeated usage, bacteria become resistant to the antibiotic and may even develop multiple-drug resistance. In the absence of microbiological testing data, periodontal therapy should preferentially employ antimicrobial agents exhibiting the broadest spectrum of chemotherapeutically significant antimicrobial activity with low potential for adverse reactions. If topical tetracyclines are nevertheless preferred, dental practitioners may choose less expensive modes of delivery than controlled-release devices from commercial sources (29). The approval by the US Food and Drug Administration of antibiotic devices for subgingival placement is based on the ability of the products to reduce signs of periodontal disease compared with a placebo, and not on testing against other antimicrobial agents in comparative trials. In order not to overlook less expensive generic chemotherapeutic alternatives, dentists need data from head-to-head studies on the efficacy of various periodontal antimicrobial agents to answer questions pertaining to both science and cost-quality trade-offs. Otherwise, the dental profession may miss out on superior, low-cost generic drugs, such as potent antiseptics, for which there is no major backing from industry.

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