Measurement of peptidase activity and evaluation of effectiveness of administration of minocycline for treatment of dogs with periodontitis

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Objective—To determine clinical, enzymatic, and microbiologic effects of controlled-release localized administration of minocycline on dogs with periodontitis.

Animals—Five adult Beagles with periodontitis.

Procedure—After tooth scaling and root planing, 2 treatment, 1 placebo, and 1 control site were selected for each dog. Treatment sites (n = 10) received a periodontal formulation of minocycline hydrochloride, placebo sites (5) received ointment without minocycline, and control sites (5) did not receive ointment. Treatments were administered 4 times at weekly intervals. Peptidase activity and clinical and microbiologic effects were evaluated and compared among sites for 17 weeks.

Results—Bleeding of the gums on probing (BOP) and pocket depth (PD) improved at the treatment site and were maintained for 13 weeks after treatment. However, BOP and PD in placebo and control sites increased from weeks 9 to 17. Peptidase activity in the periodontal pocket decreased noticeably from week 1 to 17 compared with baseline values for the treatment site. However, peptidase activity for placebo and control sites increased and were above baseline values on week 9 and week 13, respectively. Total bacterial counts decreased by 90% for treatment sites and remained at that value for 13 weeks. However, for placebo and control sites, bacterial counts increased and reached the baseline value on week 17.

Conclusions and Clinical Relevance—Increased peptidase activity is correlated with the progression of periodontitis in dogs. Treatment with minocycline, using a localized delivery system, was effective in dogs for at least 13 weeks after cessation of drug administration. (Am J Vet Res 2000;61:1349–1352)

A number of studies have been conducted on controlled-release localized delivery of antimicrobial agents for treatment of humans with periodontitis. Although there is a great deal of interest in use of this method for treatment of dogs with periodontitis, few controlled studies have been reported. In a previous study conducted by our laboratory group, localized delivery of a slow-release periodontal formulation of minocycline directly into the gingival crevice after tooth scaling and root planing resulted in greater reduction in the number of putative periodontal pathogens deep in periodontal pockets, improving the clinical condition. However, those results were analyzed on the basis of comparing baseline values with values obtained at week 4 of treatment. Thus, we considered it necessary to conduct long-term observations on effects of this drug in dogs. In the study reported here, we determined the long-term clinical efficacy of subgingival delivery of minocycline into periodontal pockets after tooth scaling and root planing. We also examined whether the enzymatic diagnostic methods commonly used for analysis of periodontitis in humans are useful as diagnostic indicators of periodontitis in dogs.

Materials and Methods

Animals—Five Beagles, 4 to 7 years old, with periodontitis that ranged from moderate to severe were included in the study. Dogs were housed separately in cages, and a diet formulated for dogs was fed. Water was available ad libitum. Dogs generally were healthy and had not received antibiotics prior to the study. Studies were conducted in accordance with ethical guidelines for animal experiments established for the Animal Research Center of Nihon University School of Dentistry at Matsudo.

Clinical examination—Clinical variables were recorded prior to treatment (week 0; baseline) and 4, 9, 13, and 17 weeks after treatment. Clinical variables measured were pocket depth (PD), measured to the nearest millimeter by use of a standard periodontal probe; probing depth, measured at the deepest site of the periodontal pocket on the buccal surface of the tooth; and bleeding of the gums on probing (BOP), classified as negative or positive after probing with a 0.5-mm diameter probe. For all examinations, dogs initially were sedated with droperidol, and they then were anesthetized with halothane.

Procedures—Tooth scaling and root planing were performed after examination for baseline measurements (ie, prior to treatment). Immediately after obtaining clinical data and collecting specimens for microbial culture, minocycline (treatment sites) or ointment (placebo sites) was injected to fill the periodontal pockets of sites. Sites for sample collection in the dogs were selected on the basis of existence of a PD ≥ 5 mm or detection of BOP. Twenty periodontal pockets (4 sites/dog [2 treatment sites, 1 placebo site, and 1 control site]) were used to evaluate the effect of minocycline. Main ingredient of the dental ointment was 2% minocycline hydrochloride microcapsulated in an ointment base. The ointment was composed of hydroxyethylcellulose, magnesium chloride, aminomethyl methacrylate copolymer, triacetin, and glycerin, which served as a slow-release system of this formulation for localized drug delivery. An ointment base without minocycline was administered to placebo sites. Untreated sites (scaling and root planing only) were used as control sites.

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For each test site, minocycline or ointment was inserted 4 times at 1-week intervals. All investigations were performed as placebo-controlled randomized double-blind clinical trials. Throughout the course of the 17-week investigation, these dogs did not receive any prophylactic treatment that could have affected subgingival plaque microorganisms.

Microbiologic examination—A sample of gingival crevicular fluid was collected prior to tooth scaling and root planing and administration of treatment (baseline) as well as 4, 9, 13, and 17 weeks after treatment. After supragingival irrigation with sterile saline (0.9% NaCl) solution, gingival crevicular fluid was obtained from each site by use of paper points inserted in position for 10 seconds, which were quickly removed and placed in 500 μl of reduced transport fluid and then immediately placed in an anaerobic box. Each sample was sonicated, diluted, and plated for culture on anaerobic agar with 5% rabbit blood containing 0.0005% hemin and 0.0001% menadione, and each plate was incubated at 37°C for 5 days. Growing colonies were counted, and number of bacteria was determined on the basis of number of colony forming units per milliliter.

Measurement of peptidase activity—Peptidase activity was measured by use of the SK-013 method, using a commercially developed periodontal diagnostic kit. In brief, 3 paper points were positioned in a pocket for 30 seconds, and they then were removed. These paper points were inserted into a small vial containing substrate, chromogen, and ascorbic oxidase solution; the vial then was agitated for 10 seconds. The mixture was incubated at 37°C for 15 minutes. Optical density was measured spectrophotometrically at a setting of 666 nm. Enzymatic activity was calculated from a standard curve and recorded as the number of trypsin units per milliliter.

Statistical analysis—Data were analyzed, and means were calculated. Differences among means for the treatment, placebo, and control sites were evaluated, using the Student t-test. Values were considered significantly different at \( P < 0.01 \).

Results
Changes in clinical variables over time were examined for each site. We did not detect differences in clinical variables for baseline measurements among sites. The BOP for the treatment site was significantly reduced, compared with that for the control site at weeks 4, 9, 13, and 17 and compared with that for the placebo site at weeks 13 and 17 (Table 1). The BOP for the placebo site did not differ significantly from that of the control site at week 17.

The treatment site had received substantial benefits, as determined on the basis of change in mean PD. The decrease in PD for the treatment site was significantly greater than that for control sites during the study (Fig 1). The PD of the control site at week 4 was less than the baseline value. However, the PD of the control site was greater than the baseline value at week 13 and increased throughout the remainder of the study. The placebo site had a decrease in mean PD at weeks 4 and 9, which were significantly less than the baseline value. However, this site had a rebound in PD by week 13.

Shifts in the number of trypsin units for the 3 sites were examined (Fig 2). Peptidase activity in periodontal pockets of the 3 sites was decreased, compared with the baseline value determined after tooth scaling and root planing. Peptidase activity of the control site continued to increase from week 2 until the end of the study. Peptidase activity for the placebo site began to increase noticeably and reached a value greater than the baseline value at week 13. The treatment site still had low trypsin activity (mean, 0.35 trypsin units/ml) at week 17 of the study.

Table 1—Results of clinical examination for bleeding of gums after probing at minocycline-treated, placebo, and control sites in 5 dogs with periodontitis

<table>
<thead>
<tr>
<th>Site</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
</tr>
<tr>
<td>Placebo</td>
<td>5/5</td>
</tr>
<tr>
<td>Minocycline-treated</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Values reported are No. of sites with bleeding/No. of sites examined. Week 0 = Before tooth scaling and root planing and prior to administration of treatment.
Values differ significantly ($P < 0.01$) from values for control site.

**Values differ significantly ($P < 0.01$) from baseline value for that site. CFU = Colony-forming units.

Figure 3—Change in total number of cultivable microorganisms for minocycline-treated ($\square$), placebo ($\triangle$), and control ($\bigcirc$) sites. $\star$Values differ significantly ($P < 0.01$) from values for control site. $\star\star$Values differ significantly ($P < 0.01$) from baseline value for that site.

**Discussion**

In the study reported here, we investigated the effects of a slow-release periodontal formulation of minocycline on clinical, enzymatic, and bacteriologic variables to assess clinical effectiveness after administration directly into periodontal pockets following tooth scaling and root planing. Bleeding was not evident at treatment sites during the study period, although bleeding was evident at all placebo and control sites by week 17. By week 17, the treatment sites had a significant decrease in pocket depth, whereas improvements were not observed for the placebo or control sites. Thus, inflammation in periodontal pockets had resolved in the minocycline-treated sites. In contrast, deterioration of clinical condition associated with low values of peptidase activity was observed throughout the study in minocycline-treated sites. In contrast, deterioration of clinical condition was observed throughout the study in placebo and control sites after documentation of high peptidase activity. Multivariate analysis of the data reported here suggested that the number of trypsin units at baseline can be used to predict clinical condition and progression of periodontitis in dogs. We believe that the placebo and control sites were not affected by the minocycline that was locally delivered at treatment sites, using the procedure of a split-mouth design for nonadjacent sites.

Analysis of results of the study reported here confirmed that treatment with minocycline administered by a controlled-release localized delivery system was effective for at least 17 weeks after treatment. However, multiple administrations of minocycline may cause pain to dogs. The development of a new ointment base or drug that can maintain long-term effectiveness in periodontal tissue by 1-time drug administration is expected. Furthermore, analysis of our findings suggested that the peptidase test we used was useful for identifying sites at risk for periodontal disease in dogs.

References


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