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Aim and Scope

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Low-dose doxycycline therapy: Effect on gingival and crevicular fluid collagenase activity in humans

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Golub LM, Ciancio S, Ramamurthy NS, Leung M, McNamara TF: Low-dose doxycycline therapy: Effect on gingival and crevicular fluid collagenase activity in humans. J Periodont Res 1990; 25: 321–330.

Tetracyclines are now recognized to have non-antimicrobial properties with therapeutic potential – for example, these agents can inhibit pathologic collagenolysis by blocking mammalian collagenases and other matrix-degrading metalloproteinases. In the current study, adult human subjects with moderate chronic periodontitis were administered specially formulated capsules of doxycycline, containing lower-than-usual amounts of this semi-synthetic tetracycline, on a daily basis for 2 weeks prior to a full-thickness flap procedure; control subjects were administered placebo capsules. The gingiva excised during this surgical procedure were extracted, the extracts partially purified and analyzed for collagenase activity using [^{3}H-methyl] collagen as substrate and the techniques of SDS-PAGE/fluorography or liquid scintillation spectrometry. In the absence of any drug pre-treatment, or after a 2-wk regimen of placebo capsules, the gingival extracts exhibited pathologically-excessive mammalian collagenase activity. The 2-wk regimen of low-dose doxycycline capsules reduced this activity by approximately 60–80% ($p < 0.05$ and < 0.01, respectively); *in vitro* exposure of the gingival extract to doxycycline also inhibited its collagenase activity. Collagenase activity in the crevicular fluid of periodontal pockets of an additional group of subjects was also significantly reduced, as was the severity of inflammation at the same gingival sites. The results suggest that a regimen of low-dose doxycycline capsules may provide a safe (other studies indicate that this regimen may not induce tetracycline resistance in the subgingival plaque) and effective adjunct to instrumentation therapy in the management of pathologic collagenolysis in the periodontal patient. However, further studies are necessary to confirm this hypothesis.

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Introduction

Collagen breakdown is an essential pathway in the pathogenesis of periodontal and other diseases such as rheumatoid arthritis, corneal ulcers, the skin blistering disorder epidermolysis bullosa, and excessive bone resorption. The activity of collagenase, one of a series of matrix-degrading proteinases generated by the host tissues, is a rate-limiting step in pathologic collagenolysis (1–6). Recent therapeutic concepts are beginning to address the potential value of *inhibitors* of mammalian collagenases (see below) and collagenases from periodontopathic microorganisms such as *Bacteroides gingivalis*, *Actinobacillus actinomycetemcomitans*, and species of Spirochetes and Bacillus (7, 8). Examples of inhibitors of mammalian collagenases (activity or production) include retinoic acid (9), phenytoin (5), eriochrome black T (10) and synthetic compounds such as CI-1 (11) and phosphonamidates (8). [These are in addition to the endogenous physiologic inhibitors, α2-macroglobulin, small cationic proteins and tissue inhibitor of metallo proteinases, or TIMPs (12)]. Chloranil (quinone derivative), as well as phosphonamidate and phosphoramidate, is being investigated as an inhibitor of bacterial collagenases (8, 13).

Consistent with this potential therapeutic approach, Golub and colleagues reported, using different experimental systems and in humans, that tetracycline antibiotics (long advocated as useful adjuncts in periodontal therapy) can inhibit mam-

malian collagenases and collagen breakdown by a mechanism *independent* of the antimicrobial efficacy of these drugs (14–25); this effect was recently confirmed in other laboratories (26, 27). Tetracyclines may also inhibit other metalloproteinases (gelatinase, type IV/V collagenase, macrophage elastase) involved in connective tissue and basement membrane degradation (24, 25, 28, 29) including a collagenase produced by the periodontopathogen, *Bacteroides gingivalis* (27). In several studies on humans, routinely prescribed, antimicrobially-effective doses of tetracyclines (tetracycline, minocycline, doxycycline) were found to reduce the collagenase activity in the fluid of the periodontal pocket (14–17) which originates from the adjacent host tissues (16, 30–32). The current study was carried out to determine whether a newer, semi-synthetic tetracycline, doxycycline, could be administered to humans in a low-dose regimen (20 or 30 mg capsules, rather than the 50 or 100 mg capsules which are commercially available) which would effectively inhibit collagenase activity in the *gingival tissue* as well as in crevicular fluid. Doxycycline was chosen, in part, because a recent study by Burns *et al.* (26), which compared the relative potencies of different collagenase inhibitors including tetracyclines, reported that doxycycline (with an $IC_{50} = 15$ μM) was a more potent collagenase inhibitor than two other tetracyclines, minocycline ($IC_{50} = 190$ μM) and tetracycline ($IC_{50} = 350$ μM).

Material and Methods

Selection and management of human subjects

Two studies (one on gingival tissue, the other on gingival crevicular fluid or GCF; see below) were conducted in which adult human subjects, between the ages of 35 and 56 years, with moderate adult periodontitis were prescribed a 2-wk regimen of a lower-than-usual dose of doxycycline, a newer semi-synthetic tetracycline. The subjects were determined by history to have had no dental treatment, including antibiotic therapy, for at least 4 months prior to starting the experimental trial and to have no contraindications (e.g. allergy to tetracyclines) to the doxycycline regimen. All subjects gave written informed consent using a document approved by the University's Human Use Committee. Special capsules of doxycycline were formulated, and dispensed for the studies by a licensed pharmacist, each containing 20 or 30 mg of Vibramycin® (Pfizer Pharmaceuticals, Groton, CT) with microcrystalline cellulose (Avicel®) used as the inactive filler in each capsule. The subjects were instructed to take one capsule in the morning and one at night, but not at mealtimes. Note that the recommended dose of doxycycline, when the drug is prescribed to combat infection, is 100 mg or 50 mg two times per day; the former regimen is prescribed for the first 24 h as a loading dose and the latter is administered daily thereafter as the maintenance dose.

Collection and analysis of gingival tissue (study no. 1)

The first study included 8 human subjects, 4 of whom received the test regimen (30 mg doxycycline b.i.d. for 2 wk), the other 4 received the Placebo (Avicel® caps b.i.d. for 2 wk), according to the following experimental protocol: Subjects were selected who exhibited similar moderate severity of adult periodontitis bilaterally in their maxillary right and left posterior sextants, and who were judged to require full-thickness flap surgery as part of their therapy in both areas. Each subject received a standardized regimen of presurgical therapy in the test quadrants consisting of oral hygiene instruction and scaling and root planing. Immediately prior to the surgical procedure in the right maxillary arch (and, 2 wk later, in the left maxillary arch), the gingival index (33) and pocket depth were measured on the mesial and distal surfaces of each tooth to be included in the surgical procedure. No prescription was given to the subjects prior to the first surgery (right side). The gingival tissues were removed during a full-thickness flap procedure by an inverse bevel incision beginning approximately 1–1/2 mm apical to the free margin of the gingiva and ending slightly buccal to the alveolar crest (care was taken to include the "col" area in the gingival tissue removed for analysis). Lidocaine 2% with 1/100 000 epinephrine was used as the local anesthetic for all surgical procedures in the study, with care being taken to inject the solution only in the periapical tissues away from the gingiva to be analyzed. The tissues, once removed, were immediately and briefly washed to remove blood and debris, blotted, placed in coded glass scintillation bottles and frozen at -80°C until analyzed. After completing the surgical procedure on the right side, each subject was given a vial containing a 2-wk supply (28 capsules) of low-dose doxycycline (30 mg caps; n = 4 subjects) or the placebo (Avicel® caps; n = 4 subjects) with instructions to take the capsules twice per day until the next surgical procedure. After the 2-wk regimen, the subjects were treated with the same type of surgical procedure in the left maxillary arch and the gingival tissue from this area was collected, washed and stored frozen in the coded bottles. The right and left side tissues were analyzed for collagenase activity, as described below, under blinded conditions.

The procedure to extract, partially-purify, and

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