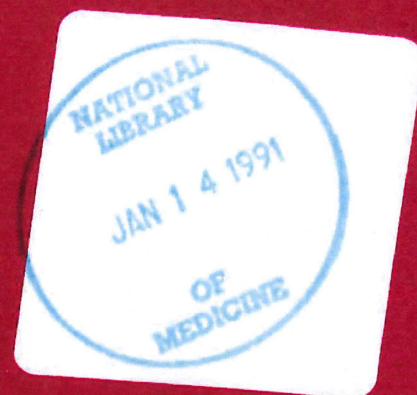
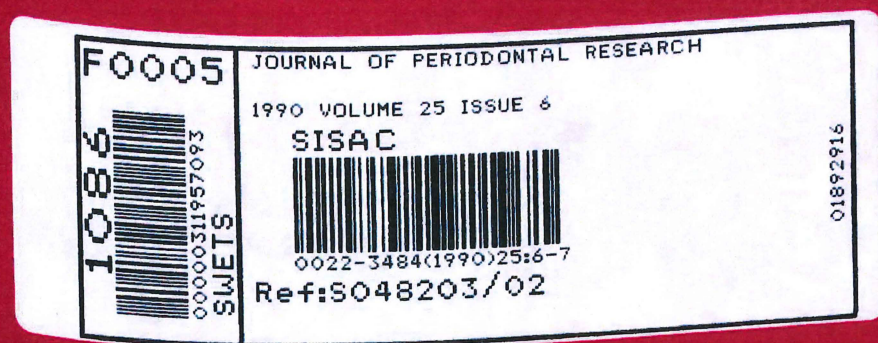


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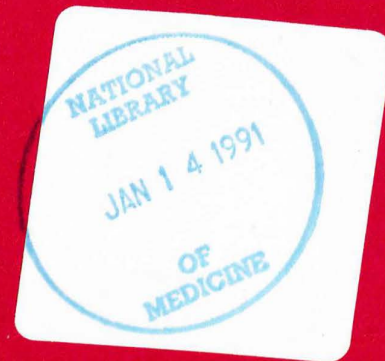
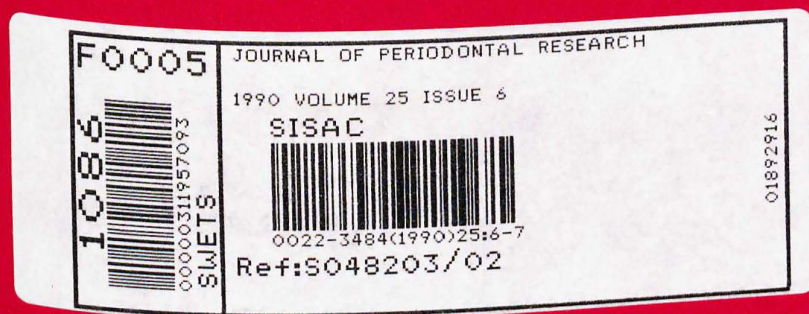
Vol. 25 No. 6: 321-377
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Journal of Periodontal Research (ISSN 0022-3484) is published bimonthly by Munksgaard International Publishers, 35, Nørre Søgade, P.O. Box 2148, DK-1016 Copenhagen, K, Denmark. USA Subscription price is USD 210.00 including airspeed delivery. Second class postage paid at Jamaica, NY 11431. USA POSTMASTER for N.A. subscribers: Send address changes to Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003. Air freight and mailing in the USA by Publications Expediting. Printed in Denmark by P.J. Schmidt, Vojens.

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

L. M. Golub, S. Ciancio*,
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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 [³H-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.

Accepted for publication March 13, 1990

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 phosphoramidates (8). [These are in addition to the endogenous physiologic inhibitors, α_2 -macroglobulin, small cationic proteins and tissue inhibitor of metalloproteinases, or TIMPs (12)]. Chloranil (quinone derivative), as well as phosphoramidate 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 $IC_{50}=15\ \mu M$) was a more potent collagenase inhibitor than two other tetracyclines, minocycline ($IC_{50}=190\ \mu M$) and tetracycline ($IC_{50}=350\ \mu M$).

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 $-80^{\circ}C$ 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

measure collagenase activity in the gingival specimens was essentially the same as that described previously (16, 34). In brief, the gingival tissues from each sextant (one right side- and one left side-sample per subject) were thawed, weighed and minced with scissors (all procedures at 0–4°C). A known amount of minced tissue was then homogenized, extracted (100 mg wet weight gingival tissue/5 ml buffer) first with 10 mM Tris-HCl buffer (pH 7.5) containing 0.4 M sucrose and 5 mM CaCl_2 , and then with 50 mM Tris-HCl containing 0.2 M NaCl, 5 mM CaCl_2 and 5 M urea. After exhaustive dialysis (and after centrifugation) $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to produce 60% saturation and the precipitate was collected by centrifugation and dissolved in the Tris-NaCl- CaCl_2 buffer. The enzyme preparation was exhaustively dialyzed against the same buffer and analyzed immediately for collagenase activity. As described by us previously (14, 16), 70 μl of partially purified gingival extract was incubated with 10 μl of [^3H -methyl] collagen (113 000 DPM; 10 μg collagen) as substrate [the nativity of the collagen was ensured by its resistance to trypsin digestion and by circular dichroism measurements confirming its helical conformation (14, 16, 49)] and 10 μl of p-aminophenylmercuric acetate (APMA) was added in a final concentration of 1.2 mM. The incubation was carried out for 24 h at 22°C and the collagen components and degradation products were examined, after thermal denaturation, by a combination of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography (14). The conversion of the collagen α components to the α^A collagenase digestion products was calculated (31) after the fluorographs were analyzed densitometrically using a Beckman DU8 spectrophotometer equipped with a film tracing attachment.

Additional aliquots of the gingival extract from the subjects treated either with no medication, placebo, or low-dose doxycycline caps were incubated with the [^3H -methyl] collagen at 27°C for 18 h, the reaction was stopped and the undigested collagen was precipitated by the addition of phenanthroline, dioxane and non-radioactive methylated collagen as carrier. The radiolabeled degradation products were counted in a liquid scintillation spectrometer (see Golub *et al.* (14, 16) for details).

Following the analyses of the gingival tissues for each subject, the remaining gingival extracts, obtained only from the subjects during the first surgical procedure (prior to therapy with either the doxycycline or placebo capsules), were pooled and aliquots were incubated with the ^3H -collagen at 22°C, 18 h. The purpose was to determine whether different tetracyclines, including two which were chemically-modified to eliminate their anti-

crobial efficacy (35, 36), could directly inhibit human gingival tissue collagenase. Minocycline or either of two chemically-modified tetracyclines, 4-de-dimethylaminotetracycline (prepared and characterized in our laboratory; 23, 25), or 6-deoxy 6-demethyl 4-de-dimethylaminotetracycline (provided by Johnson & Johnson Dental Care, Inc., New Brunswick, N.J.) were added to the incubation mixture in a final concentration of 20 $\mu\text{g}/\text{ml}$ and collagenase activity in the presence or absence of these tetracyclines was analyzed using the SDS-PAGE/fluorography technique.

Collection and analysis of GCF (study no. 2)

In the second study, 4 adult humans with moderately severe periodontal disease at the sites monitored (see Fig. 4 for the pretreatment or time=0 mean values for pocket depth, gingival index and GCF flow) were administered a 2-wk regimen of low-dose doxycycline (20 mg capsules, b.i.d.; this formulation was selected to determine whether the "low-dose regimen" described in study no. 1 could be reduced even further and still be able to inhibit collagenase activity in the periodontal pocket). The subjects were monitored, as described below, immediately before receiving their prescription (Time=0) and at 1 wk (Time=1) and 2 wk (Time=2) after initiating medication. No dental treatment was administered during the experimental protocol and the subjects stated that they had not received dental treatment including antibiotics for at least 4 months prior to Time=0.

The techniques for collecting and analyzing the GCF samples have been described previously (16, 17). In brief, for each subject, GCF was collected on sterile filter paper strips (Periopaper[®]; IDE Interstate, Amityville, N.Y.) inserted into 7–8 interproximal pockets in the maxillary arch for a 30-second time period. The fluid volume was immediately determined on a calibrated Periotron 6000 (IDE Interstate) and the filter paper strips (each placed into a coded microfuge tube) were then frozen on dry ice. After 1 h, the collagenase activity on each strip was analyzed using [^3H -methyl] collagen as substrate (16); note, the GCF samples were not activated prior to or during the incubation (18 h, 27°C) with the radiolabeled collagen. The gingival index (33) and pocket depth were measured, at the sites donating the GCF, immediately after fluid collection.

In addition, 2 GCF samples were collected from one of these subjects at T=0 (before drug administration), the samples were activated by a brief exposure to trypsin (the trypsin was then inactivated by addition of a 5-fold molar excess of soybean trypsin inhibitor; see Golub *et al.* (16) for details),

Table 1. The effect of low-dose doxycycline therapy on collagenase activity in human gingiva*

Gingival tissue obtained after no drug or doxycycline therapy	Treatment with		Statistical Significance
	No Drug (right side)	Doxycycline (left side)	
Gingival Index	0.89 ± 0.05 (48)	0.85 ± 0.06 (40)	N.S.
Pocket Depth (mm)	4.7 ± 1.5 (48)	4.6 ± 1.6 (40)	N.S.
Gingival Collagenase Activity (% Lysis)**	69.0 ± 3.7 (4)	12.8 ± 12.8 (4)	p < 0.01

* Each value represents the mean ± S.E.M.; (number of determinations).

** Estimated by densitometric analysis of fluorograms:

$$\frac{4/3(a_1^A + a_2^A)}{4/3(a_1^A + a_2^A) + (a_1 + a_2)} \times 100 \text{ (see ref. 31)}$$

pooled and extracted in 450 μ l Tris-NaCl-CaCl₂ buffer (1 h, 22°C). Seventy-microliter aliquots of extract were incubated with [³H-methyl] collagen (see above for details) ± EDTA or doxycycline added in a final concentration of 25 mM and 20 μ g/ml, respectively, and the collagen reaction products assessed by SDS-PAGE/fluorography.

Results

The 8 human subjects in study no. 1 were selected because they exhibited moderate adult periodontitis and no differences in the severity of gingival inflammation and pocket depth in their right and left maxillary posterior arches. At the time of surgery, after a standardized protocol of pre-surgical therapy, the administration of a 2-wk regimen of low-dose doxycycline or placebo had no significant effect on the gingival index or pocket depth (compare left side vs. right side; Tables 1 and 2). All of the gingival specimens from the subjects who were not administered either type of capsule (right side) showed electrophoretic evidence of tissue collagenase activity (Figs. 1 and 2) as did the gingival tissues (left side) from the subjects who were administered the placebo capsules (Fig. 2). Incubating these gingival extracts with radio-labeled collagen resulted in the partial loss of α collagen components and the formation of 3/4 cleavage products of α , called α^A , which are charac-

teristically produced by mammalian, including human, collagenases (Figs. 1 and 2). In contrast, the gingival extracts (left side) from the subjects treated with low-dose doxycycline showed either no detectable (Fig. 1) or a reduction (separate fluorogram not shown) in collagenase activity. Densitometric analysis of the fluorograms shown in Figs. 1 and 2 indicated that placebo administration produced no effect on collagenase activity in the gingival tissues (Table 2), whereas low-dose doxycycline therapy reduced gingival tissue collagenase activity by about 81% (p < 0.01; Table 1). When aliquots of the same gingival extracts were incubated with the [³H-methyl] collagen (but at 27°C rather than 22°C) and the collagen breakdown products were measured in a liquid scintillation spectrometer, the *in vivo* administration of low-dose doxycycline also produced a reduction (about 63%; p < 0.05) in collagenolytic activity and again the placebo therapy had no effect (Table 3). Incubating the gingival extracts (from subjects prior to drug administration) *in vitro* with three different tetracyclines (minocycline, a semi-synthetic tetracycline, which, like doxycycline, was previously shown to inhibit mammalian collagenases (16), and two different chemically-modified analogs) produced a similar inhibition of gingival collagenase activity (Fig. 3) as *in vivo* low-dose doxycycline therapy.

In the second study, 2 wk after low-dose doxycycline therapy (T = 2), the GCF collagenolytic activ-

Table 2. The effect of placebo therapy on collagenase activity in human gingiva*

Gingival tissue obtained after no drug or Placebo therapy	Treatment with		Statistical Significance
	No drug (right side)	Placebo (left side)	
Gingival Index	1.04 ± 0.19 (28)	1.15 ± 0.37 (26)	N.S.
Pocket Depth (mm)	4.9 ± 1.0 (28)	4.7 ± 1.0 (26)	N.S.
Gingival Collagenase Activity (% Lysis)**	67.3 ± 5.2 (4)	70.2 ± 7.4 (4)	N.S.

* Each value represents the mean ± S.E.M.; (no. of determinations).

** Estimated by densitometric analysis:

$$\frac{4/3(a_1^A + a_2^A)}{4/3(a_1^A + a_2^A) + (a_1 + a_2)} \times 100$$

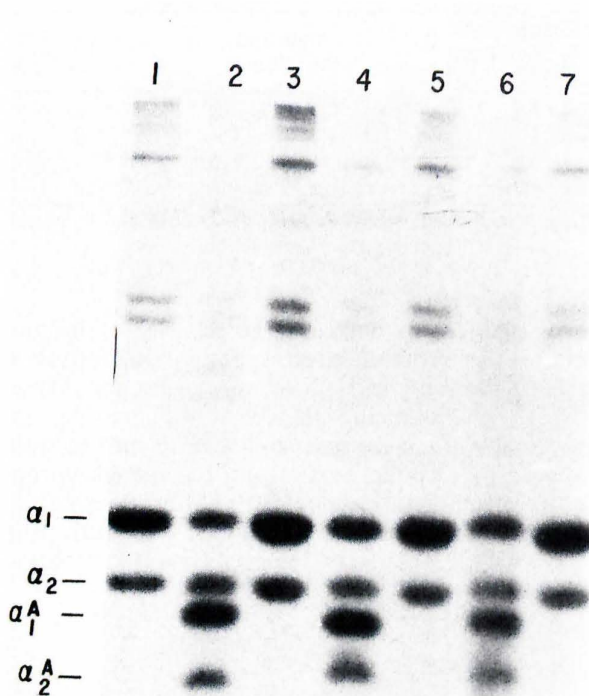


Fig. 1. Fluorograph of [^3H -methyl] collagen and its degradation products after SDS-polyacrylamide gel electrophoresis (PAGE). The ^3H -collagen was incubated at 22°C for 24 h with partially-purified extracts of human gingiva. The gingival tissues were removed during periodontal surgery first from the right side and, 2 wk later, from the left side of the maxillary arch of 3 adult humans. Lane 1 = ^3H -collagen with no gingival extract; lanes 2, 4 & 6 = ^3H -collagen after incubation with gingiva from the right side of 3 human subjects *without* drug pre-treatment; lanes 3, 5 & 7 = ^3H -collagen after incubation with gingiva from the left side of the maxillary arch of the same 3 subjects after a 2-wk regimen of low-dose doxycycline capsules.

ity, GCF flow and gingival index were significantly reduced by 54%, 39% and 31%, respectively, compared to baseline ($T=0$) values ($p < 0.01$ for all 3 measurements); pocket depth showed no reduction at the 1-wk or 2-wk time periods (Fig. 4). As shown in Fig. 5, an extract of GCF obtained at $T=0$ (before low-dose doxycycline therapy), incubated with radiolabeled collagen *in vitro*, showed evidence of tissue collagenase activity (track 2) and adding 25 mM EDTA (Track 3) or 20 $\mu\text{g}/\text{ml}$ doxycycline (track 4) to the reaction mixture inhibited this activity.

Discussion

Tetracyclines are often advocated as useful adjuncts in periodontal therapy based on their effectiveness against periodontopathogens (37–41); an

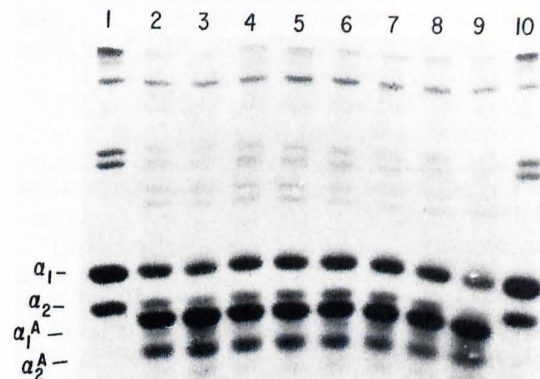


Fig. 2. Fluorograph of the SDS-PAGE pattern of ^3H -collagen and its degradation products after incubation with extract of gingival tissue, removed from the right and left sides of the maxillary arch of 4 additional human subjects. The experimental protocol is the same as in Fig. 1 except that these subjects were administered a 2-wk regimen of placebo capsules prior to the surgery on the left side. Lane 1 & 10 = ^3H -collagen alone; lanes 2, 4, 6 & 8 = ^3H -collagen after incubation with gingiva from the right maxillary arch of the 4 subjects *without* drug pre-treatment; lanes 3, 5, 7 & 9 = ^3H -collagen after incubation with gingiva from the left side of the same 4 subjects after a 2-wk regimen of placebo capsules.

additional advantage is their unique ability, among antibiotics, to be highly concentrated within the fluid of the periodontal pocket (40, 42). Recently, novel properties of these drugs have been discovered which help explain their clinical effectiveness and may also expand their future applications *beyond* their current use as antimicrobials. As one example, tetracyclines now appear to possess anti-inflammatory properties when administered to patients with certain skin diseases, diseases such as rosacea, dermatitis herpetiformis and pyoderma gangrenosum which are *not* believed to have a microbial etiology (43, 44, 58, 59). Several non-antimicrobial mechanisms may be involved, including tetracycline's ability to inhibit prostaglandin production (at least in high concentrations of the drug), to suppress leukocyte activity, and to function as scavengers of superoxide radical (45–47). In addition, the object of the current study – an ability of tetracyclines to inhibit mammalian collagenase and other metalloproteinase activities (see

Table 3. The effect of low-dose doxycycline or placebo administration on collagenolytic activity in human gingiva*

Gingival tissue obtained after no drug, doxycycline or Placebo therapy (Rx)	Gingival Collagenolytic Activity: % ^3H -collagen lysed at 27°C	Statistical Significance
No drug (right side)	16.0 \pm 3.4	p < 0.05
Doxycycline Rx (left side)	5.9 \pm 1.7	
No drug (right side)	13.2 \pm 0.4	N.S.
Placebo Rx (left side)	14.4 \pm 0.7	

* Each value represents the mean \pm S.E.M. for gingival specimens from 4 human subjects (4 subjects per control and 4 subjects per experimental group).

Introduction) – may also contribute to the anti-inflammatory efficacy of these drugs. In this regard, Chang *et al.* (48) reported that collagen breakdown products are chemotactic for polymorphonuclear leukocytes (PMNs). Thus, a reduction in collagen degradation due to the anticollagenase properties of tetracyclines could contribute to the

drug's ability to decrease the severity of inflammation. Of supreme interest, patients with dystrophic epidermolysis bullosa, a serious and sometimes life-threatening skin-blistering disorder associated with excessive collagenase production, appear to respond to the anti-collagenase properties of minocycline (58, 59) and tetracycline administration was recently reported to significantly

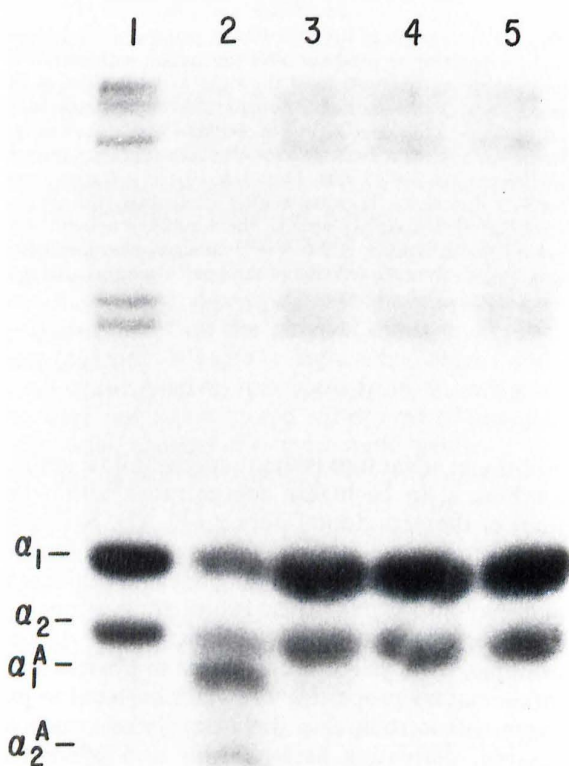


Fig. 3. Fluorograph of the SDS-PAGE pattern of ^3H -collagen incubated (22°C, 24 h) with pooled extract of human gingival tissue. Only tissues removed from the subjects without any drug pre-treatment (i.e. from the right maxillary arch) were used in this experiment. Lane 1 = ^3H -collagen alone; lanes 2, 3, 4 and 5 = ^3H -collagen after incubation with extract of gingiva in the presence of no added drug (lane 2), or with minocycline (lane 3), 4-de-dimethylaminotetracycline (lane 4), or 6-deoxy 6-dimethyl 4-de-dimethylaminotetracycline (lane 5) added to the incubation mixture in a final concentration of 20 $\mu\text{g}/\text{ml}$.

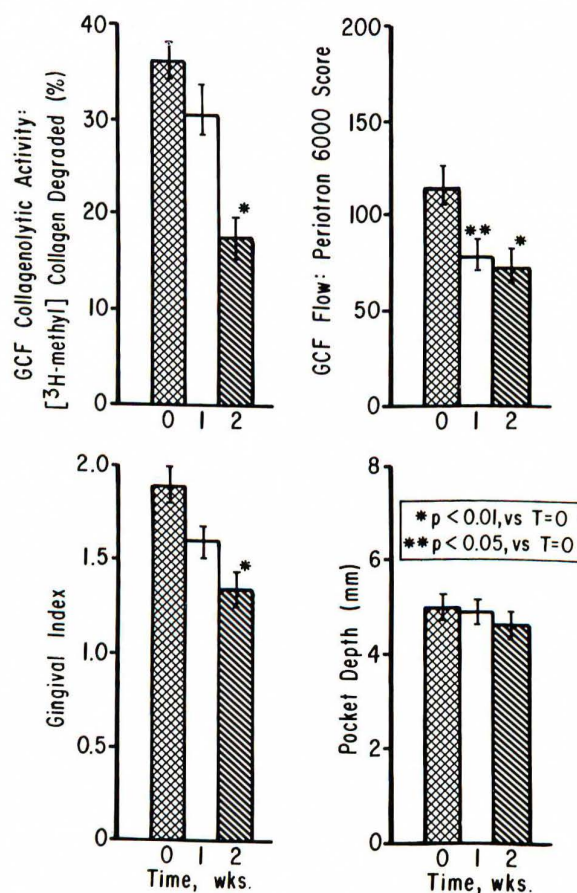


Fig. 4. The effect of a 2-wk regimen of low-dose doxycycline on GCF flow and collagenolytic activity and on gingival inflammation (G.I.) and pocket depth in 4 adult human subjects. Each value represents the mean \pm S.E.M. at time = 0 (before drug treatment) and 1 and 2 wk after initiating drug treatment.

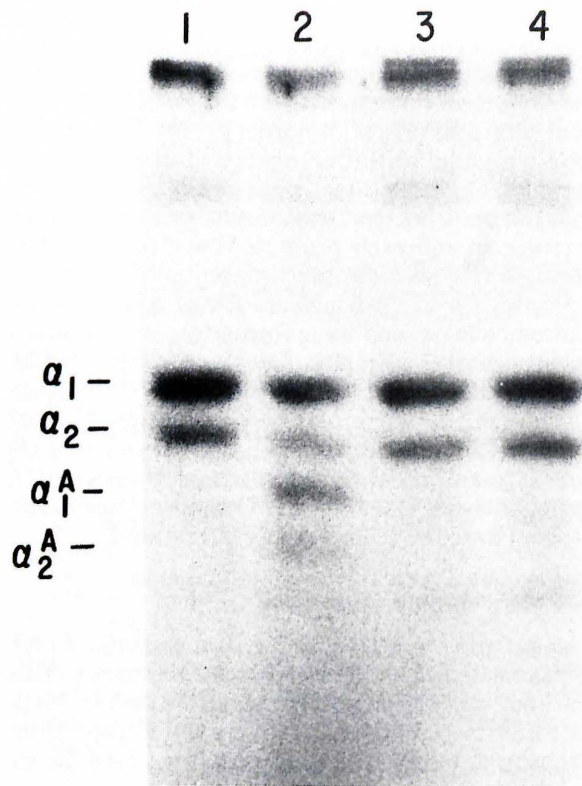


Fig. 5. Fluorograph of the SDS-PAGE pattern of ^3H -collagen after incubation (22°C , 18 h) with a trypsin-activated extract of GCF; the GCF was collected on filter paper strips from a human subject *before* doxycycline administration. Lane 1 = ^3H -collagen alone; lanes 2, 3 & 4 = ^3H -collagen after incubation with GCF alone (lane 2) or with GCF plus EDTA (lane 3) or doxycycline (lane 4) added in a final concentration of 25 mM and 20 $\mu\text{g}/\text{ml}$, respectively.

reduce clinical signs of rheumatoid arthritis in a 4-month study on humans (63).

In the current study, a low-dose regimen of doxycycline appeared to substantially reduce the collagenolytic activity in the fluid of the periodontal pocket (GCF) and in the gingiva of humans with adult periodontitis. This is the first study to demonstrate an anti-proteinase effect of a tetracycline on human gingival tissues and the reduced collagenolytic activity in this tissue (and in GCF) reflects an inhibiting of a specific matrix-degrading metalloproteinase derived from the host, namely mammalian collagenase. The evidence includes the ability of the drug, both *in vivo* and *in vitro*, to inhibit the breakdown of undenatured collagen by an EDTA-inhibitable neutral proteinase, thus preventing the production of α^A cleavage fragments characteristically produced by host collagenases. Previous studies on humans demonstrated that regular doses of different tetracyclines, as well as low

doses of minocycline, produced similar reductions in GCF collagenase activity (14–17, 51). A direct inhibition of this metal-dependent proteinase by a portion of the tetracycline molecule (one of its metal-binding sites) not involved in the drug's antimicrobial activity was the mechanism proposed (23). In fact, experiments are now being carried out to localize the anti-collagenase site on the tetracycline molecule and these will be reported elsewhere.

The following additional evidence argues *against* some aspect of the clinical protocol – rather than the drug regimen itself – being responsible for the *in vivo* reduction in collagenase activity: (a) In the gingival tissue study (study No. 1), the low-dose doxycycline regimen resulted in a 60–80% reduction in gingival tissue collagenase activity. However, when a separate group of subjects was administered placebo capsules and the same clinical protocol was followed, no change in collagenase activity could be detected in their gingival tissues; and (b) In the GCF study (study no. 2), a 2-wk regimen of low-dose doxycycline resulted in a 54% reduction in collagenase activity within the periodontal pocket. An additional group of subjects administered placebo capsules was not included in this study because Golub *et al.* (51) reported previously that a control group of human subjects, administered a 2-wk regimen of vitamin C capsules, exhibited no change in GCF collagenase activity (and no change in severity of gingival inflammation assessed clinically or by GCF flow), whereas the collagenase activity was significantly reduced in this experiment when a separate group of subjects was prescribed a low-dose regimen of minocycline.

Sorsa *et al.* (50) recently addressed the origin of collagenase in the GCF and in extracts of inflamed human gingiva. Based on the molecular weight, substrate specificity, and activation profile, they concluded that PMNs were the major source of this enzyme in inflamed periodontal tissues and that fibroblasts provided the collagenase for connective tissue remodeling in normal gingiva. Of supreme interest, tetracyclines were recently found to be more effective in blocking the activity of collagenase from PMNs than that from fibroblasts (52). This observation suggests that tetracycline administration, particularly in low doses, could provide a safe and effective therapeutic approach for reducing pathologically-excessive collagenase activity in inflammatory lesions without interfering with normal connective tissue remodeling. It should be noted, however, that fibroblasts (or other cells) may also contribute to the pool of collagenase found in the fluid of the periodontal pocket (60).

In addition to the effect of low-dose doxycycline

on collagenase activity in inflamed human gingiva and pocket fluid, this regimen also appeared to reduce the severity of inflammation assessed by the gingival index (33) and by measuring GCF flow (16, 53). In study no. 2, this effect was significant and was consistent with earlier studies, using a similar experimental protocol, in which patients were administered regular or low doses of minocycline (14–17, 51). However, in study no. 1, in which a more complex clinical protocol was followed to obtain excised gingival specimens, the severity of inflammation in the gingival tissues did not appear to be reduced by the low-dose doxycycline therapy, even though collagenase activity in these tissues was suppressed. This apparent inconsistency can be explained as follows: The first set of gingival specimens was surgically removed from the control and experimental groups of human subjects *without* pre-treatment with a placebo or doxycycline regimen. This provided inflamed gingival tissues from both groups exhibiting detectable collagenase activity using both SDS-PAGE/fluorography and liquid scintillation spectrometry techniques. At the same appointment, immediately following surgery on one side of the maxillary arch, the control and experimental groups were placed on a 2-wk regimen of placebo or low-dose doxycycline capsules and the gingival specimens were surgically removed from the opposite side of the maxillary arch 2 wk later. Because the doxycycline (as well as placebo) was administered during wound healing following the first surgical procedure, oral hygiene and plaque control undoubtedly were poor, which could have prevented a significant reduction in clinically assessed gingival inflammation.

The safety aspect of a low-dose regimen of this tetracycline is also beginning to be addressed. In preliminary reports, Golub *et al.* (17, 51) described the effectiveness of a low-dose regimen of minocycline in reducing GCF collagenase activity in the periodontal pocket although no significant changes in the make-up of the crevicular microflora were detected. In addition, a recent preliminary study suggested that subgingival plaque microorganisms (*Fusobacterium nucleatum*, *Actinomyces spp.*, *Bacteroides spp.*) do not develop tetracycline resistance when patients are administered a 2-wk regimen of low-dose doxycycline capsules (61). In contrast, when patients were administered regular-dose doxycycline capsules (50 mg, b.i.d.), the subgingival plaque appeared to become resistant (minimum inhibitory concentration of doxycycline = 25–100 µg/ml in these subjects) to expected tissue fluid levels (1–5 µg/ml) of this antibiotic (62).

Additional studies are being conducted to identify the optimal formulation (× mg/capsule) and duration (2 wk, 3 wk, etc...) of this low-dose

doxycycline regimen over more extended periods of time. The purpose is to develop a safe and effective chemotherapeutic adjunct to instrumentation therapy (scaling, root, planing, surgery) in the long-term management of the periodontal patient. Previous clinical reports (39, 54) suggest that long-term administration of regular-dose (250 mg) capsules of tetracycline, prescribed less than the usual four times per day, could prevent further destruction and even promote tissue regeneration during severe periodontitis; inhibition of tissue collagenase is a likely beneficial factor although enhanced collagen and bone formation, and antimicrobial activity, may also play a role (39; 55–57). We hypothesize that specially formulated low-dose doxycycline capsules may yield the same clinical efficacy without inducing the well-known side-effects (e.g. antibiotic-resistant microorganisms (39), gastro-intestinal upset) of long-term antibiotic usage. However, further study is required.

Acknowledgment

The authors thank Ms. H. M. Lee and Ms. A. M. Vidal for their excellent technical assistance. This research was supported by grants from the Natl. Institute for Dental Research (DE-03987) and from Johnson & Johnson Dental Care, Inc., New Brunswick, NJ.

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