Specificity of the Anticollagenase Action of Tetracyclines: Relevance to Their Anti-Inflammatory Potential

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The concentrations of doxycycline and 4-de-dimethylaminotetracycline required to inhibit 50% of collagenase activity were found to be 15 to 30 μM for human neutrophil and gingival crevicular fluid collagenases. Fibroblast collagenase was relatively resistant to inhibition by tetracyclines; the 50% inhibitory concentrations of doxycycline and 4-de-dimethylaminotetracycline were 280 and 510 μM, respectively.

Interstitial collagenases (EC 3.4.24.7) are considered to be key initiators of collagen degradation during the progression of inflammatory diseases such as rheumatoid arthritis, corneal ulceration, and periodontal diseases. Elevated tissue levels of collagenase have been detected in these inflammatory diseases characterized by excessive collagen degradation. Both the amount of the enzyme and its conversion to an active form, possibly mediated by the action of proteinases and/or reactive oxygen metabolites, are increased during inflammation. Although the cellular origin(s) of collagenases in these diseases remains unclear, resident fibroblasts and epithelial cells as well as infiltrating leukocytes (neutrophils and macrophages) are considered potential sources of the enzymes (1, 4, 14, 25). Fibroblast-type interstitial collagenase (matrix metalloproteinase 1 or MMP-1), which is also produced by epithelial cells and monocyte/macrophages, and neutrophil interstitial collagenase (MMP-8) are distinct gene products and differ in their immunologic characteristics and substrate specificities (7, 12, 24). In addition, the physiological inhibitors α2-macroglobulin and tissue inhibitor of metalloproteinases have been found to inhibit the fibroblast collagenase more efficiently than the neutrophil collagenase (3, 27).

Recently, Golub et al. (9) discovered a new, nonantimicrobial property of tetracyclines—an ability to inhibit the activity of interstitial collagenases detected in these inflammatory cell and tissue sources. This effect has been confirmed by other investigators (4, 15). Moreover, a chemical modification of the tetracycline molecule that eliminates its antimicrobial efficiency does not result in a loss of its ability to inhibit collagenase (10). The specificity of the effect was partially addressed in a study showing that the tumor cell-derived type IV collagenase/gelatinase (MMP-2) can also be inhibited by tetracyclines (29). However, the ability of these drugs to inhibit different types of interstitial collagenases has not yet been investigated. We report here the differential susceptibility of human neutrophil and fibroblast interstitial collagenases to inhibition by a commercial antimicrobial tetracycline, doxycycline (DOXY) and by a chemically modified nonantimicrobial tetracycline (4-de-dimethylaminotetracycline or CMT-1) (10). Furthermore, we addressed the cellular source of collagenase in the inflammatory exudate of the human periodontal pocket (also called the gingival crevicular fluid) by using tetracycline inhibition as a probe.

4-Aminophenylmercuric acetate and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma (St. Louis, Mo.). DOXY and CMT-1 were obtained from L. M. Golub's laboratory. All other chemicals used were of reagent grade.

Purified human synovial fibroblast interstitial collagenase (matrix metalloproteinase 1) was kindly provided by Ko Suzuki and Hideaki Nagase, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas. (17). Human neutrophil collagenase was purified as described previously (18, 19).

Gingival crevicular fluid samples were collected from deep (>5 mm) periodontal pockets of patients with adult periodontitis. The buccal and interproximal tooth surfaces were cleaned of supragingival plaque, dried gently with air, and kept dry with cotton rolls. Filter paper strips were placed into the gingival margin approximately 1 mm into the sulcus for 5 min. The absorbed fluid was eluted with 25 μl of 0.2 M NaCl-10 mM CaCl2-0.5 mM phenylmethylsulfonyl fluoride in 50 mM Tris-HCl, pH 7.5, per strip. The samples were centrifuged at 1,000 × g for 15 min, and aliquots of the supernatant were used for collagenase assays (18, 19).

Human neutrophil (18, 19) and synovial fibroblast interstitial collagenases (17) as well as samples of gingival crevicular fluid (8) were incubated with radioactive type I collagen (8) in the presence of 0 to 1,000 μM DOXY or CMT-1 at 22°C for 8 h. Under these circumstances the reaction proceeds within the linear range. To ensure measurement of total collagenase activity, 1 mM 4-aminophenylmercuric acetate, an organomercurial activator of latent collagenase (8, 18), was included in the incubations. After addition of Laemmli's sample buffer containing 40 mM EDTA, the samples were immediately heated to 100°C for 5 min. Subsequently, the degradation products, which were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8, 19) on 10% cross-linked gels and processed for fluorography, were quantitated by densitometric scanning with a LKB Ultrascan Laser Densitometer model 2202. The value representing αA-chains was multiplied by 4/3, and its proportion of total collagen in the sample was used as a measure of collagenase activity.

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The drug concentration (DOXY or CMT-1) required to inhibit 50% of the enzyme activity (IC$_{50}$) was found to be 15 to 30 μM for the collagensases from human neutrophils and gingival crevicular fluid (Fig. 1). In contrast, the human fibroblast collagenase was relatively resistant to tetracycline inhibition; the IC$_{50}$ for DOXY and CMT-1 were 280 and 510 μM, respectively (Fig. 1).

We suggest that systemic (e.g., serum and gingival crevicular fluid) levels of tetracyclines (8, 11, 22, 28) can inhibit collagenases derived from inflammatory (neutrophil) sources but not from fibroblasts, the latter MMP perhaps being more resistant to normal tissue remodeling and repair (8, 19). Therefore, the inhibition of pathologic collagenolysis in inflammatory diseases (e.g., rheumatoid arthritis, noninfected corneal ulcerations, periodontal diseases, and dystrophic epidermolysis bullosa) by tetracyclines may explain the recently described therapeutic anti-inflammatory efficiency of these drugs independent of their antimicrobial activity (2, 8, 13, 26). Tetracycline inhibition of neutrophil collagenase may also prevent other proteolytic events because this matrix metalloproteinase (MMP-8) (12) can degrade α1-proteinase inhibitor (5, 16); as an example of a potential therapeutic benefit, it is tempting to speculate that excess elastase activity, believed to mediate pulmonary emphysema (20), could be counteracted by tetracycline protection of α1-proteinase inhibitor (14A). It is also noteworthy that tetracyclines as well as their nonantimicrobial derivatives may prove to be therapeutically potent neutrophil collagenase inhibitors in vivo, since this enzyme, unlike fibroblast collagenase, is relatively resistant to the endogenous inhibitors, α2-macroglobulin, and tissue inhibitors of metalloproteinases (3, 27).

Human fibroblast-type collagenase is known to be constitutively de novo synthesized and secreted by fibroblasts, epithelial cells, and monocyte/macrophages (1, 14, 24, 25), whereas in human neutrophils collagenase is stored in subcellular specific granules, having been synthesized during neutrophil maturation in bone marrow (12, 23). Thus, the levels of this MMP released by mature circulating neutrophils are regulated by factors that affect degranulation rather than de novo biosynthesis of collagenase (1, 23, 25), and studied with cDNA probes to measure collagenase mRNA levels in cells and tissues may not completely address the specific roles of both fibroblast-type and neutrophil interstitial collagensases in connective tissue destruction (1, 12). In fact, to date, no practical technique has been described to differentiate the in vivo functional forms of these two MMPs in biologic and/or pathologic samples. Therefore, we propose that tetracycline IC$_{50}$ measurements appear to serve as a simple and practical probe to identify the cellular origin of collagenases present in tissue and biological fluid samples (e.g., rheumatoid synovial and tear fluid). In support of this view, the present data using this probe are consistent with those from recent studies (6, 19, 21) which identified the
neutrophil, not the fibroblast (epithelial cell and monocyte/macrophage), as the major source of collagenase in human adult periodontal lesions. Thus, these observations suggest a role for this approach in tissue and fluid samples from patients with other collagenolytic diseases such as rheumatoid arthritis and corneal ulcerations (4, 25).

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