NOTES

Doxycycline in the Protection of Serum Alpha-1-Antitrypsin from Human Neutrophil Collagenase and Gelatinase

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The concentration of doxycycline required to inhibit 50% (50% inhibitory concentration for serpinase activity) of alpha-1-antitrypsin degradation by purified neutrophil collagenase was found to be approximately 20 μM, a value similar to the 50% inhibitory concentration of doxycycline required to inhibit collagen degradation by neutrophil collagenase. Doxycycline also efficiently inhibited phorbol myristate acetate-triggered neutrophil-mediated degradation of alpha-1-antitrypsin. This suggests that doxycycline can protect alpha-1-antitrypsin from collagenase and gelatinase in the presence of other proteases and biologically active molecules that are released by triggered neutrophils. The protection of a body's alpha-1-antitrypsin shield from serpinolytic activity of collagenase and matrix metallproteinases can result in inhibition of serine proteases such as neutrophil elastase. Tetracyclines may thus protect matrix constituents from a wider spectrum of neutral proteases than previously recognized, not just from the matrix metalloproteinases collagenase and gelatinase.

Interstitial collagenases, members of the matrix metalloproteinase (MMP) family, are considered to be key initiators of collagen destruction during various disorders such as rheumatoid arthritis, inflammatory skin and periodontal diseases, corneal ulcerations, and pathological bone resorption (4, 16). Recently, these neutral proteinases were found to efficiently degrade an additional (noncollagenous) substrate, the serum protein alpha-1-antitrypsin (AAT; also called alpha-1-proteinase inhibitor or serpin) (3, 7, 9). Serpins are major endogenous inhibitors of serine proteinases, particularly neutrophil elastase (9, 16). Of relevance to neutrophil-mediated collagen degradation (16), the tetracycline family of antibiotics is now known to inhibit mammalian collagenases (and some other MMPs) by a mechanism unrelated to the antimicrobial activity of this family of drugs (4, 13). In the study described here, we identified an additional nonantimicrobial mechanism by which tetracyclines may retard tissue breakdown during inflammatory diseases. In fact, this mechanism may explain in part the recent clinical successes described by Humbert et al. (6).

Human AAT, which was purified from human serum as described previously (3, 7, 9), was incubated for 120 min at 22°C with purified and optimally activated (by 1 mM aminophenyl mercuric acetate) human neutrophil interstitial collagenase (MMP-8) (8, 12, 17) in the presence of 0 to 1,000 μM doxycycline (13). The breakdown products of AAT that result from the serpinase activity of MMP-8 (3, 7, 9) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were quantitated densitometrically (3, 8, 10, 12, 13). Human neutrophils (polymorphonuclear leukocytes [PMNs]; 1 to 10⁶/ml) isolated by the

Ficoll-Hypaque method (9, 17) were stimulated with phorbol myristate acetate (PMA; final in vitro concentration, 250 nM [17]). Subsequently, PMN supernatants were incubated with ^{125}I-labeled AAT (New England Nuclear Corp., Boston, Mass.) for 0, 2, 4, and 6 h at 37°C. Degradation of ^{125}I-labeled AAT was assessed by laser densitometric quantitation of the SDS-PAGE and fluorographic analysis of the conversion of native 53-kDa AAT to the lower-molecular-mass forms (49 and 4 kDa) resulting from collagenase and gelatinase action (7, 9).

Purified MMP-8 degraded AAT, consistent with earlier reports (3, 7, 9). Doxycycline, which was added to the reaction mixture at concentrations of as low as 10 μM, which corresponds to levels that are readily achievable in vivo during therapy with this drug, produced detectable inhibition of the serpinase activity of MMP-8, although levels of 50 to 100 μM or greater were required to reduce AAT degradation by more than 75% (Fig. 1). The concentration of doxycycline required to inhibit 50% of AAT degradation by MMP-8 (IC$_{50}$ of serpinase activity) was found to be approximately 20 μM (Fig. 1), a value similar to the IC$_{50}$ of doxycycline required to inhibit collagen breakdown by MMP-8 (13). Figure 2 shows the effect of doxycycline on the ability of degranulating viable PMNs to degrade alpha-1-antitrypsin. It was found that with increasing time of incubation, the PMA-stimulated PMN supernatants degraded AAT to lower-molecular-mass forms (data not shown). However, when doxycycline was added to the incubation mixtures at final concentrations of both 10 μg/ml (20 μM) and 50 μg/ml (100 μM), the PMN-mediated degradation of AAT appeared to slow down (Fig. 2).

Doxycycline and other tetracyclines do not directly inhibit serine proteinases (4). However, in addition to directly inhibiting neutrophil collagenase (thus preventing colla-
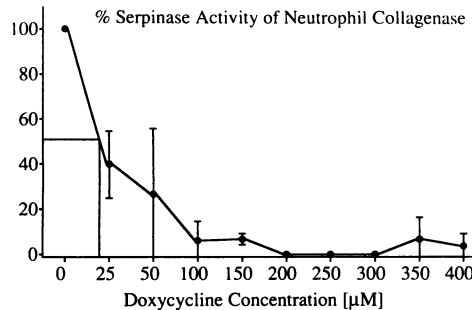
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FIG. 1. Inhibition of serpinolytic activity of purified human neutrophil collagenase (MMP-8) by doxycycline. The concentration of doxycycline required to inhibit 50% (IC_{50}) of serpinase activity of MMP-8 is indicated. The serpinase activity of MMP-8 was measured as described by Desroches et al. (3) and Michaelis et al. (9). The values represent means ± standard deviations of four determinations.

genolysis) (4, 13), pharmacologic levels of doxycycline may indirectly retard the breakdown of other matrix constituents that are susceptible to serine proteinases, such as elastin, fibronectin, proteoglycan and basement membrane. The protection of the body's AAT shield from the serpinolytic activity of MMP-8 would result in inhibition of the activity of serine proteinases such as neutrophil elastase and cathepsin G, and accordingly, this links the drug (doxycycline)-dependent inhibition of collagenolysis to the inhibition of elastinolysis. Importantly, the inhibition of neutrophil MMP-dependent degradation of AAT was found to occur at the neutrophil cell level in the presence of other proteases and biologically active molecules released by triggered PMNs. Additional protective mechanisms of these drugs may include their ability to scavenge neutrophil-generated reactive oxygen species, such as hypochlorous acid (8, 10, 12, 15–17). This may protect AAT from direct oxidative inactivation or
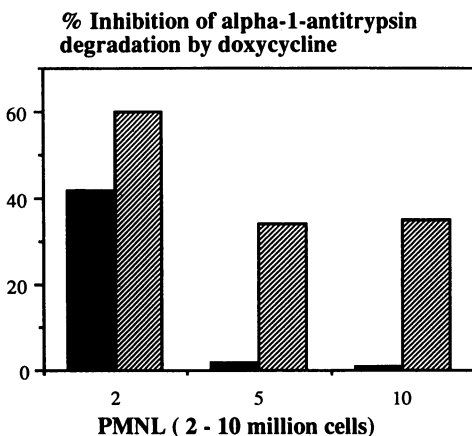

FIG. 2. Effect of doxycycline on the ability of degranulating viable PMNs to degrade AAT. ^{125}I-labeled AAT was incubated with different concentrations of viable PMNs at 37°C for 6 h. The PMNs were triggered to be degranulated by 250 nM PMA. The ability of degranulating PMNs to degrade radiolabeled AAT was assessed by detecting the conversion of 52-kDa AAT to lower-molecular-mass fragments by a quantitative SDS-PAGE fluorographic assay. Doxycycline added to reaction mixtures at both 10 µg/ml (20 µM) and 50 µg/ml (100 µM) inhibited PMN-mediated degradation of AAT. ■, 10 µg of doxycycline per ml; ▨, 50 µg of doxycycline per ml.

degradation as well as prevent the oxidative activation of latent procollagenase to the actively destructive form of the enzyme (2, 8, 10, 12, 15–17).

The tetracycline-mediated protective pathways described above may contribute to the anticollagenolytic efficacies of these drugs in patients with a variety of noninfectious diseases including rheumatoid arthritis (1, 5), osteoarthritis (18), sterile corneal ulcers (11), and tumor-induced angiogenesis (14). In addition, a series of inflammatory skin diseases not believed to be of infectious origin, including rosacea, pyoderma gangrenosum, dermatitis herpetiformis, recessive dystrophic epidermolysis bullosa, and bullous pemphigoid, also respond favorably to tetracyclines (4). The most recent expression of this nonantimicrobial protective effect of doxycycline in patients with skin disease was described by Humbert et al. (6). Approximately 10% of most European populations are genetically deficient in serum AAT, which predisposes these people to inflammatory-mediated diseases. Doxycycline administration to patients suffering from one of these diseases, AAT deficiency panniculitis, which involves the breakdown of erythematous nodules to form necrotic ulcers, resulted in complete remission of the condition (3). The rationale for using doxycycline was its anticollagenase activity (4, 13). Indeed, tetracyclines may protect matrix constituents from a wider spectrum of neutral proteinases, i.e., neutrophil elastase, cathepsin G, and other serine proteases, than previously recognized, not just from the MMP collagenase and gelatinase.

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