NOTES

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

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Received 17 June 1992/Accepted 15 December 1992

The concentration of doxycycline required to inhibit 50% (50% inhibitory concentration for serpinase activity) of alpha-1-antitrypsin (AAT; also called alpha-1-proteinase inhibitor or serpin) (3, 7, 9). Serpins are major endogenous inhibitors of serine proteases, particularly neutrophil elastase (9, 16). Of relevance to neutrophil-mediated collagen degradation (16), the tetracycline family of antibiotics is now known to inhibit mammalian collagenses (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 manganese mercapt 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 109/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 125I-labeled AAT (New England Nuclear Corp., Boston, Mass.) for 0, 2, 4, and 6 h at 37°C. Degradation of 125l-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 collagen 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 (IC50 of serpinase activity) was found to be approximately 20 µM (Fig. 1), a value similar to the IC50 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 proteases (4). However, in addition to directly inhibiting neutrophil collagenase (thus preventing colla-
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 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 proteinases, than previously recognized, not just from the MMP collagenase and gelatinase.

This study was financially supported by the Päivikki and Sakari Sohlberg Foundation, the Finnish Dental Society, the Academy of Finland, NIDR grant R37 DE-03987, U.S. Public Health Service grant HL-14262 (National Heart, Lung and Blood Institute), the New York State Office of Science and Technology (Stony Brook Biotechnology Center), and Cortech Inc.

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