

Reduction of Matrix Metalloproteinase 8-Neutrophil Collagenase Levels during Long-Term Doxycycline Treatment of Reactive Arthritis

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The aim of this work was to determine whether human polymorphonuclear neutrophilic interstitial collagenase (matrix metalloproteinase 8 [MMP-8]) levels are reduced during long-term doxycycline treatment in humans with reactive arthritis. Serum MMP-8 levels were reduced (mean \pm standard error of the mean, 678.9 ± 185.6 versus 491.2 ± 144.8 ng of MMP-8 per ml), but not statistically significantly. However, the reduction of salivary MMP-8 levels was statistically significant ($3,729 \pm 1,905.3$ versus $1,866 \pm 780.0$ ng of MMP-8 per ml, $P < 0.05$). This study demonstrated that a 2-month regimen of doxycycline can reduce MMP-8 levels in serum and especially in body fluids (i.e., saliva) containing inflammatory exudates and thus may contribute to reduced tissue destruction.

Since the discovery of human interstitial collagenase and other members of the matrix metalloproteinase (MMP) family, their production, the proteolytic and oxidative activation mechanisms of latent proforms, and their excessive activity especially in diseased states (in which this activity overcomes the protective endogenous inhibitory potential of α 2-macroglobulin and tissue inhibitors of MMPs) are considered to be key rate-limiting steps in the pathologic collagen destruction seen especially in inflammatory diseases such as arthritides, periodontal and skin diseases, and the development of malignancies (4, 10, 15, 16). Therefore the chemotherapeutic inhibition of excessive collagenase MMP activities has now emerged as an exciting concept even though, until recently, this was considered a difficult and perhaps impractical task. Tetracyclines can, independent of the drugs' antimicrobial efficacy, directly inhibit in vitro the activities of human interstitial collagenases and other MMPs (4, 6), especially preferring neutrophil collagenase (matrix metalloproteinase 8 [MMP-8]) (14), and, further, prevent oxidative activation of the latent procollagenases (8, 12). Recent data suggest that the members of the tetracycline family of antibiotics can reduce tissue destruction events in arthritides (2, 4, 6, 7, 17). In a recent randomized, double-blind, placebo-controlled study it was found that a 3-month lymecycline (tetracycline-L-methylenelysine, a form of tetracycline) treatment significantly reduced the duration of *Chlamydia trachomatis*-induced reactive arthritis (ReA) (7). In addition, among all the ReA patients studied, the number of patients with tissue destruction, as determined by radiographic examination, was significantly lower among those treated with lymecycline than among those treated with placebo (7). Yu et al. have also recently shown that prophylactic 2-month oral doxycycline administration reduced the severity of canine osteoarthritis (17). However, the systemic chemotherapeutic modification of MMP-8 levels has not been

described before although doxycycline therapy was previously found to reduce the activity of collagenase in humans (3). The aim of the present work was to determine whether MMP-8 levels reduce during long-term doxycycline treatment in ReA.

The protocol of the present study was approved by the appropriate institutional ethics committee. Adult patients of the outpatient or inpatient departments of the Aurora Hospital, Helsinki, Finland, who had clinically evident ReA as described in a previous study by Lauhio et al. (7) were included in the study. The exclusion criteria were (i) previous tetracycline or doxycycline treatment of the current episode of ReA, (ii) pregnancy, (iii) breastfeeding, and (iv) oral or salivary gland diseases. The triggering infections of the 10 ReA patients included in the present study were verified by culture and serology as described in detail previously by Lauhio et al. (7). ReA patients were treated with 150 mg of doxycycline once a day plus 100 mg of ketoprofen three times a day for 2 months. No concomitant medication was permitted. Before and after a 2-month period of doxycycline and nonsteroidal anti-inflammatory drug (NSAID) therapy, serum samples (according to Bergman et al. [1]) and saliva samples (according to Ingman et al. [5a]) were collected and assayed by specific enzyme-linked immunosorbent assay technique for MMP-8 (1).

The antibody for MMP-8 was prepared and characterized as described previously by Bergman et al. (1). Microtiter plates were coated with 100 μ l of antibody at 4°C overnight at a concentration of 2 mg/liter in coating buffer (50 mmol of sodium carbonate per liter, pH 9.6). After being washed twice with PBST (0.2 mol of sodium phosphate per liter [pH 7.4], 0.15 mol of NaCl per liter, 0.5 g of Tween 20 per liter), standards and samples were diluted in 10 g of bovine serum albumin (BSA)-PBST per liter and incubated overnight at 4°C. After four washes with PBST, 100 μ l of the immunoglobulin G-peroxidase conjugate solution in 10 g of BSA-PBST per liter was placed in wells and incubated for another 2 h at room temperature. After six washes with PBST, the substrate reac-

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TABLE 1. Clinical characteristics of the ReA patients before and after 2 months of doxycycline treatment

	Characteristic at indicated time (months) ^a	
	0	2
Erythrocyte sedimentation rate (mm/h)	52.83 ± 31.9	20.9 ± 7.7 ^b
C-reactive protein (mg/liter)	15.1 ± 14.7	4.9 ± 8.0
Leukocytes (10 ⁹ /liter)	8.3 ± 2.6	7.6 ± 3.1
Hemoglobin (g/liter)	126.5 ± 9.0	131.2 ± 17.7
No. of swollen joints	2.6 ± 3.0	1.2 ± 1.3
No. of painful joints	2 ± 2.2	2.8 ± 3.9
Lower back pain (no. and % of patients)	9 (82)	2 (18.1) ^b
Enthesopathy (no. and % of patients)	6 (60)	4 (40)
Extraarticular manifestations (no. and % of patients)	3 (30)	1 (10)
Urethritis	2 (20)	0
Eye inflammation	1 (10)	0
Mucocutaneous lesion	3 (30)	1 (10)

^a Data are means ± standard deviation or numbers (percentages in parentheses) of patients ($n = 10$).

^b $P = 0.007$ by the Wilcoxon signed rank test.

tion was performed by incubation in 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid solution (1 g/liter in 0.1 mol of citric acid per liter, adjusted to pH 4.2 with Na₂HPO₄-0.5 g/liter-Tween 20-1.3 mmol H₂O₂). Absorbance was measured with a Dynatech Microreader MR 4000 (Denkendorf, Federal Republic of Germany) at 405 nm. Human serum and salivary samples were 5- or 10-fold diluted in 10 g of BSA-PBST per liter. The Wilcoxon signed rank test was used for statistical analysis.

Of the 10 ReA patients, the numbers with each of the indicated triggering infections were five with salmonella, two with *Yersinia enterocolitica* serotype 3, one with *C. trachomatis*, one with preceding urethritis with an unknown etiology, and one with preceding enteritis with an unknown etiology. Of these 10 ReA patients, 8 were HLA-B 27 positive. The clinical characteristics of the ReA patients before and after 2 months of doxycycline treatment are shown in Table 1. The adverse effects of the doxycycline were mild and did not lead to withdrawals from the study. Three of the 10 ReA patients had adverse drug effects: one had nausea, and two had mild diarrhea with negative stool cultures (sampled twice) for salmonella, shigella, *Yersinia enterocolitica*, *Campylobacter*, and *Clostridium difficile*; as well, there was no blood in fecal samples.

Serum MMP-8 levels were reduced, but not statistically significantly (mean ± standard error of the mean, 678.9 ± 185.6 versus 491.2 ± 144.8 ng of MMP-8 per ml). However, the reduction of salivary MMP-8 levels was significant (3,729.0 ± 1,905.3 versus 1,866.0 ± 780.0 ng of MMP-8 per ml; $P < 0.05$).

This study demonstrated that 2 months of doxycycline medication can reduce the levels of MMP-8 in serum and especially in body fluids (i.e., saliva) containing inflammatory exudates. This can be concluded since NSAIDs do not appear to directly affect MMP activities (5); however, these anti-inflammatory drugs may facilitate the anticollagenolytic potential of members of the tetracycline family of antibiotics by reducing edema and thereby facilitating the entry of tetracyclines to the sites of inflammation in vivo (5, 9, 11). In support of this conclusion, Breedveldt et al. demonstrated that a 4-month treatment of rheumatoid arthritis patients with minocycline

and NSAIDs showed significant improvements in all clinical parameters (2). Also, Greenwald et al. recently administered chemically modified, nonantimicrobial but anticollagenolytic tetracycline (CMT) plus flurbiprofen to rats with adjuvant arthritis; combined therapy with CMT and NSAID resulted in significant reduction in bone erosion (5). It is of interest that doxycycline is a more efficient direct inhibitor of interstitial collagenases than minocycline or tetracycline (4, 6, 14). Thus, in regard to anticollagenolytic or antiproteolytic therapeutic action of tetracyclines, it appears to be most favorable to combine doxycycline with NSAIDs.

Since α 1-proteinase inhibitor, an endogenous inhibitor of serine proteinases and a substrate for interstitial collagenases and other MMPs, can be inactivated either oxidatively or proteolytically through MMP action, it may be possible that therapeutic levels of doxycycline can prevent general proteolytic events—in addition to specific collagenolysis—by maintaining the α 1-proteinase shield (13).

Overall, we have provided evidence that doxycycline treatment results in reduced levels of MMP-8 in vivo, which may contribute to the reduced tissue destruction in ReA (7) during long-term tetracycline or doxycycline medication. These in vivo observations suggest that it may be useful in the future to consider long-term tetracycline or doxycycline targeted to systemically reduce MMP-8 levels in order to control tissue destruction events in the treatment of inflammatory diseases such as ReA and other arthritides (9) as well as periodontal and skin diseases (4, 6).

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