Regulation of Cartilage Collagenase by Doxycycline

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ABSTRACT. ***Objective.*** To investigate the ability of doxycycline to modulate collagenases, cytokines, and cytokine receptors in chondrocytes from osteoarthritic (OA) cartilage.

Methods. Chondrocytes were isolated from human OA cartilage and treated with doxycycline. Synthesis of collagenases, cytokines, and cytokine receptors was quantified by Northern and Western blot analysis and RNase protection assay.

Results. We observed significant inhibition of matrix metalloproteinases (MMP-1) and MMP-13 mRNA and protein production by chondrocytes, isolated from OA cartilage, after treatment with doxycycline. The decrease in collagenase protein level paralleled a decrease in mRNA for these enzymes, suggesting a transcriptional/posttranscriptional level of control. In addition, treatment with 10 μg/ml doxycycline resulted in 2.2-fold upregulation of transforming growth factor (TGF-ß3) and a significant decrease of interleukin 1α (IL-1α), IL-1ß, and IL-6 mRNA. Upregulation of TGF-ß RI and TGF-ß RII was also detected. These cytokines are known to affect collagenase expression and could contribute to inhibition of MMP-1 and MMP-13 production by OA chondrocytes. A decrease in IL-1α, IL-1ß, and IL-6 would reduce stimulation of MMP production, while an increase in TGF-ß3 would lead to downregulation of local proinflammatory cytokine production as well as of the collagenases themselves.

Conclusion. Our findings show that a decrease in MMP-1 and MMP-13 collagenase production by articular chondrocytes in response to treatment with doxycycline can be explained by a regulatory effect of doxycycline on the production of cytokine and cytokine receptors. (J Rheumatol 2001;28:835–42)

Key Indexing Terms:
DOXYCYCLINE COLLAGENASE INHIBITION OSTEOARTHRITIS

Tetracycline and its analogs have been shown to be of therapeutic benefit in several animal models of disease and in human arthritis[1,2]. The precise mechanism of action is not fully understood but appears to be related to nonantibiotic properties of these compounds and in particular to their ability to inhibit matrix metalloproteinases (MMP). There have been numerous reports that tetracyclines directly inhibit some but not all MMP.

The collagenase subfamily of MMP consists of 3 different enzymes: fibroblast collagenase (MMP-1)[3], neutrophil collagenase (MMP-8)[4], and collagenase-3 (MMP-13)[5]. These enzymes mediate the degradation of native type II collagen, the major structural protein of cartilage. The tetracycline analog most studied in regard to MMP inhibition is doxycycline. Doxycycline has been shown to decrease both collagenase and gelatinase activity[1,6-12]. Although it has been shown to directly inhibit each of these enzymes, the concentrations required have generally been higher than is clinically achievable[12,13]. Recently, Greenwald and co-workers[14] presented new evidence showing that doxycycline and other tetracyclines in the presence of physiologic concentration of Ca^{++} (1 mM) can significantly inhibit collagenase activity in serum at concentrations achievable after oral administration of these drugs.

Because of the discrepancy between the ability of doxycycline to prevent the development of experimental osteoarthritis (OA) and the level required to directly inhibit collagenase, we have investigated other modes by which doxycycline could inhibit collagenase activity. We have reported[10] that doxycycline at concentrations as low as 1 μg/ml (2.08 μM) are capable of downregulating the production of MMP-1 and MMP-13. It has also been shown by Jonat and co-workers that 1 μM doxycycline (0.48 μg/ml) will inhibit stromelysin transcription[15]. Thus it is likely that doxycycline not only acts directly on collagenase to inhibit its activity but also acts indirectly to reduce collagenase levels. It could do this either by a direct effect on collagenase expression or indirectly by modulating cytokines or cytokine receptors that regulate collagenase.

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In this study we examined the ability of doxycycline to modify the synthesis of collagenase in chondrocytes from patients with OA. We found that doxycycline inhibits constitutive expression of collagenases from chondrocytes taken from both involved and uninvolved areas of articular cartilage. In addition, we determined the effect of doxycycline on cytokine and cytokine receptor mRNA levels to identify potential mechanisms by which it could act.

MATERIALS AND METHODS

Chondrocyte preparation. Joint tissue was obtained from 16 patients with OA (ages 60–75 yrs) undergoing implant surgery for total knee replacement. Articular cartilage within 2–3 mm of the macroscopic OA lesion was surgically removed and separated from adjacent nonlesion cartilage that appeared to be morphologically normal. Cells isolated from these 2 different areas were considered to be lesional or nonlesional chondrocytes, respectively. These studies excluded the most extreme examples of advanced OA where very little cartilage tissue could be recovered and differentiation of lesion versus nonlesion cartilage could not be made. Chondrocytes from both locations were isolated as described[10,16]. Briefly, cartilage pieces were incubated with pronase (0.5 mg/ml; Worthington Biochemicals, Freehold, NJ, USA) and type II collagenase (2 mg/ml; Worthington) in F-12K medium. After isolation chondrocytes were resuspended in F-12K medium containing 10% fetal bovine serum (FBS). Viability of the chondrocytes was assessed by trypan blue exclusion and ranged from 90 to 95%.

Cell culture treatment. After isolation, chondrocytes were plated to a density of 2.2×10^7 cells in 75 cm^2 flasks and allowed to recover overnight in 10 ml F-12K supplemented with 10% FBS. Prior to stimulation, each monolayer was washed and cultured for 24 h in serum-free F-12K medium. To examine the effect of doxycycline on collagenase synthesis, endogenous cytokine, and cytokine receptor mRNA levels, OA chondrocytes were treated with 10 (20.8 μM) or 25 μg/ml (52 μM) of doxycycline in F-12K serum-free medium for 24 h. Supernatants were removed for Western blot analyses, viability was determined, and the cells were extracted for RNA isolation.

RNA isolation and RNase protection assay. Total RNA was isolated from OA chondrocytes by guanidinium thiocyanate phenol-chloroform in a single-step extraction method (RNA Isolation Kit, Stratagene, La Jolla, CA, USA) as described[16]. RNase protection assays for cytokines and cytokine receptors were performed using the RiboQuant Multi-Probe RNase Protection Assay System (PharMingen, San Diego, CA, USA). For human cytokine detection hCK-2 and hCK-3 multiprobe template sets containing interleukin (IL)-1α, IL-1ß, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-8, IL-10, IL-12, tumor necrosis factor alpha (TNF-α), TNF-ß, lymphotoxin beta (LTß), interferon gamma (IFN-γ), IFN-ß, transforming growth factor beta (TGF-ß1), TGF-ß2, and TGF-ß3 were used (PharMingen). For human cytokine receptor detection a hCR-4 multiprobe template set containing DNA templates for IL-1 receptor I (RI), IL-1 RII, IL-6 R, TNF RI, TNF RII, TGF-ß RI, and TGF-ß RII was used (PharMingen). The housekeeping genes L-32 and GAPDH were included on all template sets.

Probe syntheses and hybridizations were performed according to the PharMingen RiboQuant instruction manual. Briefly, the DNA templates were collectively transcribed by DNA-dependent RNA T7 polymerase into an α^{32}P labeled anti-sense RNA probe set. Labeled probes were hybridized for 16 h at 56°C to 15 μg of total RNA. The samples were treated with RNase mixture, phenol-chloroform extracted, and precipitated in the presence of ammonium acetate. The remaining "RNase protected" probes were purified and then resolved on 5% acrylamide-urea sequencing gel (the labeled and undigested probes serve as a molecular weight marker). The radiolabeled blots were exposed to a phosphor imaging screen that was scanned using a Storm phosphor imaging detection system (Molecular Dynamics Inc., Sunnyvale, CA, USA) and quantitated by computer based image analysis software. The quantity of each mRNA species was determined based on the intensity of the appropriate size protected probe fragments. After normalization of the mRNA based on GAPDH levels, cytokine and cytokine receptor mRNA levels were compared.

Northern blot analysis. For Northern blot analysis the isolated RNA was transferred to Gene Screen Plus hybridization membranes and immobilized by UV crosslinking at 120,000 microjoules/cm^2 as described[16]. After prehybridization, the blots were hybridized overnight at 42°C with specific cDNA MMP-1 and MMP-13 probes labeled by random primer extension with α^{32}P dCTP and autoradiographed. For MMP-13, bands migrating at 3.0 and 2.5 kb were both quantitated. The radiolabeled blots were scanned and analyzed using a Storm phosphor imaging detection system and computer based image analysis software. A radiolabeled G3PDH probe served to standardize the amount of RNA in each lane.

Western blot analysis. A 30 μl sample of culture supernatant from human chondrocytes was separated on 10% sodium dodecyl sulfate-polyacrylamide gels and immediately transblotted into a nitrocellulose membrane as described[17]. For detection of collagenase proteins, polyclonal rabbit antisera specific for either MMP-1 (the gift of Dr. Howard Welgus, Parke-Davis, Morris Plains, NJ, USA) or MMP-13 (the gift of Dr. Peter Mitchell, Pfizer Central Research, Groton, CT, USA) were used at a dilution 1:1000. Transblots were incubated with each antiserum overnight at 4°C and washed extensively. Horseradish peroxidase labeled second antibody conjugate, donkey anti-rabbit IgG (Amersham Life Science, Buckinghamshire, England), was added at a dilution of 1:10,000 and incubation continued for 30 min at room temperature. The blot was developed using the enhanced chemiluminescence method (Amersham Life Science) and analyzed using the Storm imaging system.

Statistical analysis. After normalization, cytokine mRNA and protein levels were compared using the Microsoft Excel version 7.0a statistical program. Results are presented as the mean ± standard error of the mean. Comparisons between the different groups were performed using a standard Student t test.

RESULTS

Doxycycline inhibits collagenase production by OA chondrocytes. We used different concentrations of doxycycline to determine its cytotoxic effect on chondrocytes. We found that 100 μg/ml was toxic, 50 μg/ml had some cytotoxicity. Concentrations of doxycycline that we normally use — 1, 10, and 25 μg/ml — are not toxic for human chondrocytes (data not shown). The direct effect of doxycycline on MMP-1 and MMP-13 mRNA and protein was evaluated in cultures of OA chondrocytes. In chondrocytes cultured with 25 mg/ml doxycycline, MMP-1 and MMP-13 were downregulated to 19.2% and 13% ($p \leq 0.05$), respectively, compared with untreated control chondrocytes (Figure 1). Protein levels of MMP-1 and MMP-13 in chondrocyte supernatants were assayed by Western blot. Protein levels correlated with changes in collagenase mRNA and were decreased to 23.3% and 18.2% ($p \leq 0.05$), respectively (Figure 2).

The response of both lesional and nonlesional chondrocytes was compared. Basal level of MMP-13 was lower in nonlesional cells, as described[10,16]. Treatment of lesional OA chondrocytes with 10 μg/ml doxycycline resulted in inhibition of MMP-13 protein production to 35.3% ($p \leq 0.05$) of that synthesized by untreated cells. We did not see significant changes in MMP-13 protein produced by nonlesional chondrocytes in response to doxycycline (Figure 3). After

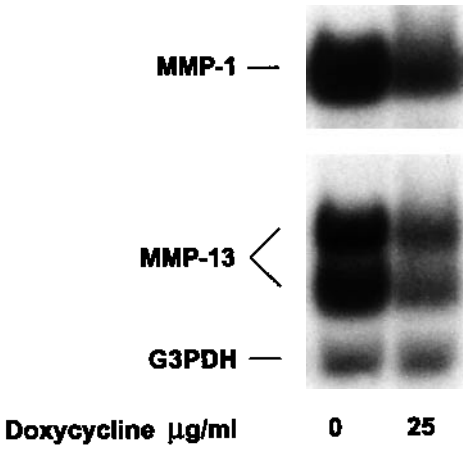

Figure 1. Northern blot analyses of the effect of doxycycline on MMP-1 and MMP-13 mRNA levels in OA chondrocytes. Chondrocytes were isolated from cartilage of OA patients undergoing implant surgery for total knee replacement (n = 7) and cultured 24 h in the presence or absence of 25 μg/ml doxycycline. Doxycycline significantly inhibited both MMP-1 and MMP-13 mRNA levels. Data are from a representative sample of lesional chondrocytes.

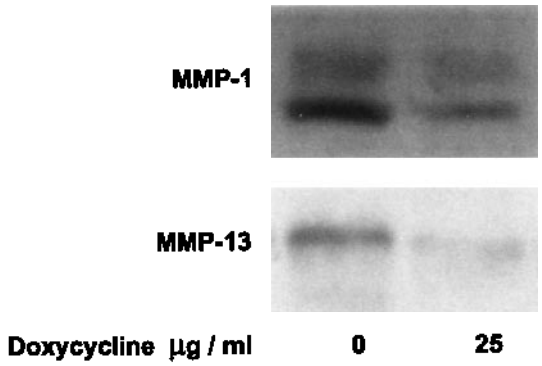

Figure 2. Western blot analyses of the effect of doxycycline on MMP-1 and MMP-13 protein levels in OA chondrocytes. After 24 h of OA chondrocyte cell culture (n = 7) in the presence or absence of 25 μg/ml doxycycline, supernatants were collected and analyzed. Doxycycline significantly inhibited both MMP-1 and MMP-13 protein levels in OA chondrocytes isolated from the area around the OA lesion.

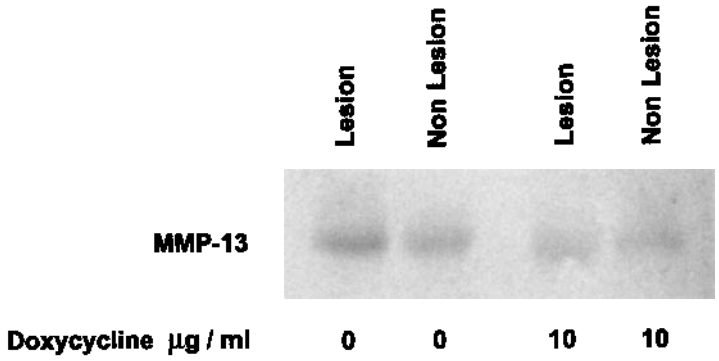

Figure 3. The effect of doxycycline on MMP-13 protein levels in supernatants of cultured OA chondrocytes isolated from either the cartilage around the OA lesion or grossly normal (nonlesion) cartilage from the same patient (n = 4) and cultured in the presence or absence of 10 μg/ml doxycycline. After 24 h supernatants were collected and examined by Western blot analysis: 10 μg/ml doxycycline inhibited MMP-13 protein levels in OA chondrocytes isolated from the area around the OA lesion. At the same time doxycycline had no significant effect on constitutive production of MMP-13 by nonlesion OA chondrocytes.

treatment with doxycycline, the level of MMP-13 protein produced by lesional chondrocytes was similar to that produced by nonlesional chondrocytes. A similar pattern of response was observed for MMP-1 (data not shown).

Effect of doxycycline on cytokine mRNA expression in OA chondrocytes. To determine the inhibitory effect of doxycycline on collagenase production by lesional chondrocytes, we investigated the effects of doxycycline on autocrine cytokine production. Analysis by RNase protection assay revealed that human OA lesional chondrocytes, treated with 10 μg/ml doxycycline for 24 h, produce less mRNA for cytokines such as IL-1α and IL-12 ($p \leq 0.05$). A similar trend was observed for IL-1ß, IL-6, and IFN-γ mRNA ($p \geq 0.05$). At the same time TGF-ß3 and IFN-ß mRNA levels were significantly upregulated compared to nontreated cells. IL-1Ra mRNA levels were also increased. The doxycycline treatment did not affect the level of the TGF-ß1 mRNA (Figure 4, Table 1).

Effect of doxycycline on expression of mRNA for cytokine receptors in OA chondrocytes. To further characterize the role of doxycycline in collagenase inhibition, we analyzed the expression of mRNA for cytokine receptors. We found that treatment of human OA chondrocytes with 10 μg/ml doxycycline for 24 h resulted in upregulation of mRNA for IL-1 RI, IL-1 RII, TGF-ß RI, and TGF-ß RII receptors ($p \leq 0.05$). No statistically significant effect was seen on TNF RI, TNF RII, and IL-6 receptor mRNA expression (Figure 5; Table 2).

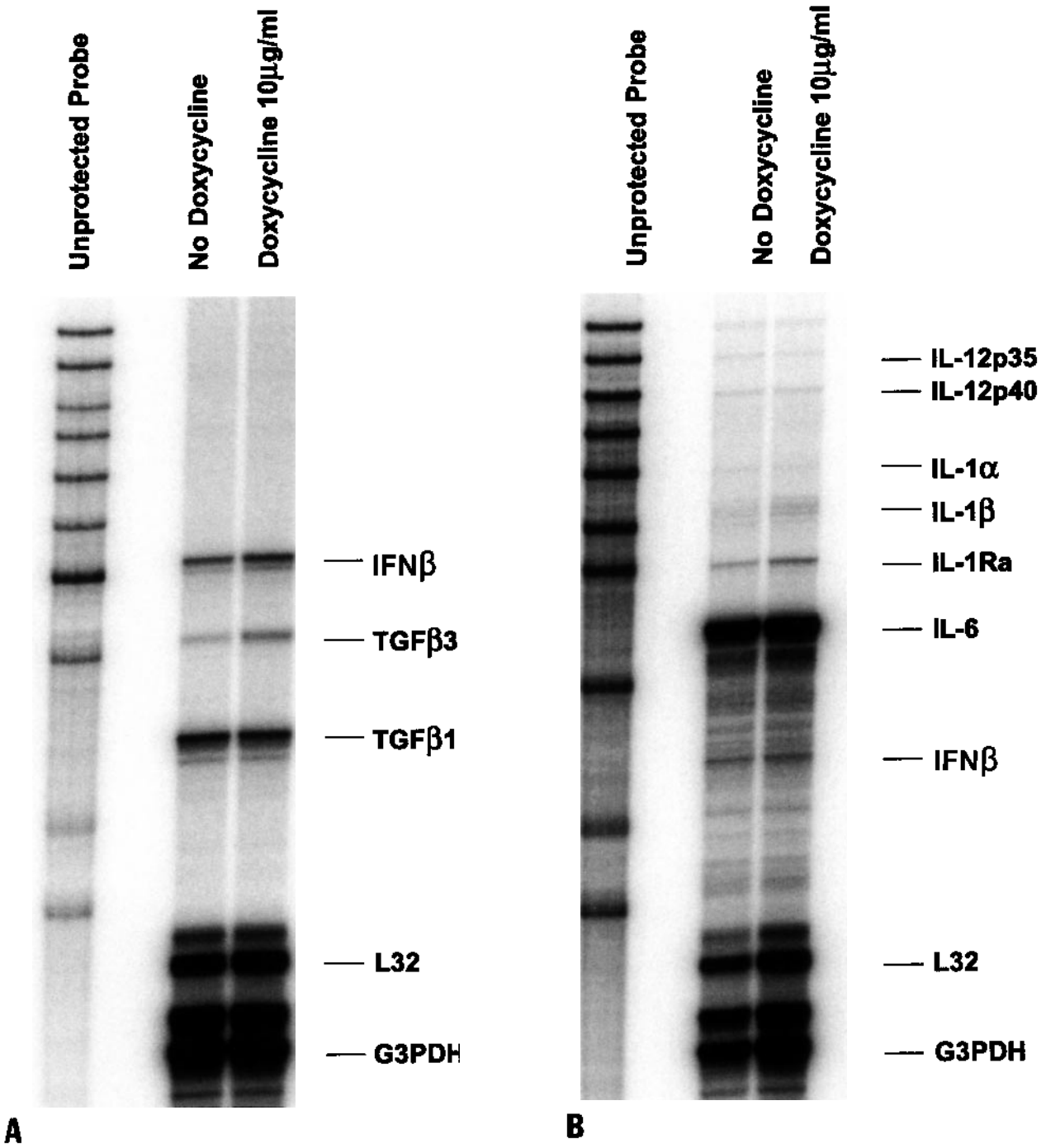

Figure 4. The effect of doxycycline on cytokine mRNA expression in OA chondrocytes. A. Measurement by RNase protection assay shows OA chondrocytes constitutively produce mRNA for IFN-ß, TGF-ß1, TGF-ß3. Treatment of OA chondrocytes with 10 μg/ml doxycycline resulted in a 2.2-fold increase of mRNA production for TGF-ß3 and 1.5-fold for IFN-ß. TGF-ß1 mRNA level was unchanged. All data were normalized to L-32 and GAPDH levels. B. OA chondrocytes constitutively produce mRNA for IL-12, IL-1ß, IL-1Ra, IFN-γ, and IL-6. Treatment of OA chondrocytes with 10 μg/ml doxycycline resulted in 50% inhibition of mRNA production for IL-12 and 25% inhibition for IL-6 and IFN-γ. At the same time the level of IL-1Ra of mRNA increased 1.4-fold. Doxycycline treatment had no statistically significant effect on the level of IL-1ß mRNA.

DISCUSSION

A combination of mechanical and enzymatic factors is involved in the development of OA[18-21]. Because synovial inflammation has been an inconsistent finding in OA it has been hypothesized that chondrocytes play a major role in the disease process. One important way that chondrocytes are involved is in the degradation of the cartilage matrix. This is accomplished in part through the production of collage-

Table 1. Cytokine mRNA production by OA chondrocytes in response to treatment with doxycycline. Results are presented as a -fold increase compared to cytokine production by cultured, untreated OA chondrocytes (n = 9).

Cytokine	Treated*/Control Chondrocytes
IL-6	0.7
IL-1α	0.5**
IL-1ß	0.8
IFN-ß	1.5**
IFN-γ	0.7
TGF-ß1	1.0
TGF-ß3	2.2**
IL-1Ra	1.4**
IL-12	0.5**

*mRNA was measured by RNase protection assay. **p ≤ 0.05.

Table 2. Cytokine receptor mRNA production by OA chondrocytes in response to treatment with doxycycline. Results are presented as a -fold increase compared to cytokine production by cultured, untreated OA chondrocytes (n = 9).

Cytokine Receptor	Treated*/Control Chondrocytes
IL-1 RI	2.0**
IL-1 RII	1.9**
TNF RI	1.0
TNF RII	1.0
IL-6 R	1.0
TGF-ß RI	2.0**
TGF-ß RII	1.7**

*mRNA was measured by RNase protection assay. **p ≤ 0.05.

nases. The presence of collagenases in normal cartilage, their significant upregulation during OA, and their activity against type II collagen support a role for these enzymes in collagen degradation and cartilage damage that characterize OA[16,22-26].

Tetracyclines have been reported to directly inhibit the activity of some, but not all MMP, including collagenases[6–11] and gelatinases[27,28]. Doxycycline, a member of the tetracycline family, has been shown to decrease collagenase and gelatinase protein levels and activities in connective tissues proper, in cartilage, and bone tissues in canine OA and thus matrix degradation[1,10,12,29]. It is difficult to understand how inhibition of enzyme activity would change the protein levels. Our data suggest that doxycycline may act at many different levels to decrease MMP-1 and MMP-13 transcription. This may explain the earlier data for decreased collagenase protein.

Doxycycline and chemically modified tetracyclines have been used as MMP inhibitors in the treatment of periodontal

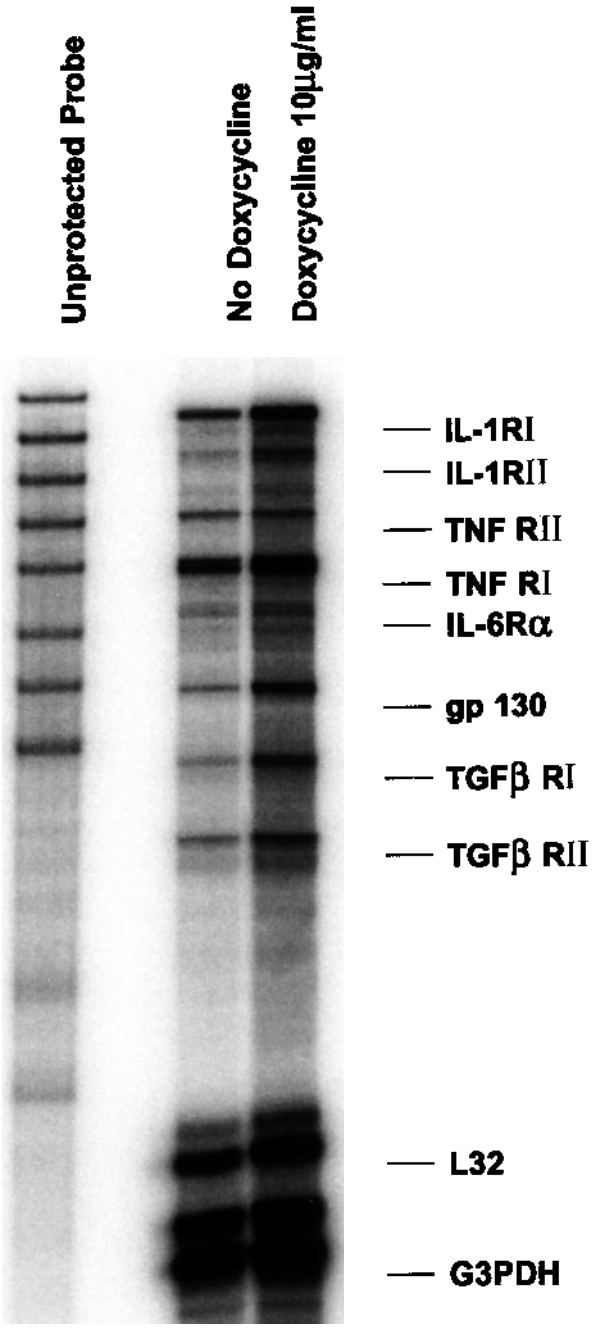

Figure 5. The effect of doxycycline on cytokine receptor mRNA expression in OA chondrocytes. Measurement by RNase protection assay shows OA chondrocytes constitutively produce mRNA for IL-1 RI, IL-1 RII, TNF RI, TNF RII, IL-6 R, TGF-ß RI, and TGF-ß RII. Treatment of OA chondrocytes with 10 μg/ml doxycycline resulted in upregulation of IL-1 RI, IL-1 RII, TGF-ß RI, and TGF-ß RII. No statistically significant effect was seen on TNF RI, TNF RII, and IL-6 mRNA expression. All data were normalized to L-32 and GAPDH levels.

diseases[30,31]. In periodontal cell culture in the presence of doxycycline, activity of MMP was decreased dependent upon the doxycycline concentration[28]. In addition, doxycycline was seen to suppress aortic wall connective tissue degradation in abdominal aortic aneurysms[32,33] and, as part of anti-cancer therapy, to reduce tumor growth[34]. In arthritis, downregulation in gelatinase and collagenase activity was seen in joint extracts from patients undergoing joint replacement surgery after oral administration of doxycycline prior to the surgery[12], but the specificity of the collagenase inhibition was unknown. *In vitro*, cultured cartilage explants of embryonic avian tibias were protected in the presence of doxycycline, whereas control cartilages were completely degraded[35]. In addition, doxycycline has been shown to inhibit osteoclast bone resorption[36]. Doxycycline treatment reduced collagenase and gelatinase activity and prevented proteoglycan loss in the cartilage matrix[35].

A single intravenous infusion of 200 mg doxycycline to patients with OA resulted in 8.3 μg/ml (range 5.4–11.5 μg/ml) of doxycycline in plasma after 3 h[37]. Similar levels were achieved by Sakellari and co-workers[38] after oral administration of 100 mg doxycycline. Concentration of doxycycline in the bones, tendons, and muscles after 3 h intravenous infusion of 200 mg of doxycycline varied from 0.13 to 5.19 μg/g[37]. The local concentration of doxycycline as well as other tetracyclines increased with time in articular joints (bone, tendons, and cartilage). Intravenous administration of 2 doses of 200 mg doxycycline at 12 h intervals resulted in the accumulation of doxycycline after the second administration in bone tissue in the amount of 3.95 μg/g and in articular cartilage in the amount of 10.47 μg/g[39]. In our experiments, concentrations of doxycycline potentially achievable in joints of patients with OA were used. Multiple treatments with small concentrations of doxycycline (5 μg/ml twice) inhibit collagenase production by OA chondrocytes more effectively compared to a single dose of 10 μg/ml (unpublished observation). Treatment of OA chondrocytes in cell culture with 10 and 25 μg/ml doxycycline mimics the treatment of multiple oral doxycycline administration (300–500 mg weekly) to patients with OA.

One problem in studying OA is the variation in the degree of cartilage destruction in different patients. Indeed, we found that chondrocytes isolated from around OA lesions and from normal appearing cartilage from the same patient exhibit different phenotypic characteristics[16,40]. For the present experiments with doxycycline, we chose to use chondrocytes isolated from around the OA lesions. These cells bear similar characteristics: they express high levels of collagenases and produce high amounts of proinflammatory cytokines. By separating OA cartilage into 2 categories, we eliminated significant differences between patients with OA in our study. In our experiments we observed the same trend of changes of cytokine and cytokine receptor patterns after administration of doxycycline among all patients studied.

There are several ways doxycycline can act to reduce collagenase activity in the joint. It can directly inhibit the enzymatic activity. It can prevent the production and release of the enzymes, or it can modulate the production of other molecules that regulate collagenase expression. Recently, Smith and co-workers showed that doxycycline disrupts the conformation of the hemopexin-like domain of MMP-13 and the catalytic domain of MMP-8[41]. However, the concentrations of tetracyclines required for direct enzyme inhibition are higher than are achievable *in vivo*[13]. Thus a direct inhibition effect is not adequate to explain its chondroprotective role[1]. The inhibition of nitric oxide synthase by doxycycline, described by Amin and co-workers[42], may also contribute to the chondroprotective effect of doxycycline since other inhibitors of this enzyme decrease MMP activities[43]. Our findings support the concept that doxycycline might act through one or more of the other mechanisms. It may downregulate the production of enzyme. The report that collagenase protein and activity are decreased in crevicular fluid after oral administration of doxycycline[44] may be due to the inhibition of its synthesis. As reported, this would affect MMP-13 more than MMP-8, which is carried preformed by the neutrophils. We have shown that treatment with doxycycline inhibits MMP-1 and MMP-13 mRNA and protein production in chondrocytes isolated from cartilage of patients with OA. It is effective both on chondrocytes isolated from areas of cartilage that have undergone significant damage (lesional chondrocytes) and on relatively undamaged areas (nonlesional chondrocytes).

In this study, we showed that treatment with doxycycline not only effectively inhibited the level of MMP-1 and MMP-13 mRNA and protein production in chondrocytes isolated from around the OA lesion, but also regulated autocrine production of the IL-1α, IL-1ß, IL-6, IL-12, IL-1Ra, and TGF-ß3 cytokines and IL-1 and TGF-ß cytokine receptors. Autocrine regulation of collagenases through cytokine stimulation and receptor expression is important for development of OA[40,45-48]. A network of proinflammatory cytokines such as IL-1α, IL-1ß, and IL-6 participates in the upregulation of matrix metalloproteinases in chondrocytes and surrounding synovial tissue and the resulting matrix breakdown of cartilage[10,16,21]. OA chondrocytes constitutively produce mRNA for both proinflammatory (IL-1α, IL-1ß, IL-6, TNF-α) and antiinflammatory cytokines (IL-1Ra, TGF-ß1, TGF-ß3). The balance between these 2 groups of cytokines is very important and may determine whether cartilage destruction develops. Previously, we have shown TNF-α, IL-1α, IL-1ß, and IL-6 directly enhance MMP-1 and MMP-13 enzyme production in human chondrocytes[16]. Increased levels of these cytokines are present in OA lesional chondrocytes as compared to adjacent nonlesional chondrocytes[40].

Cytokine activity can be affected by synthesis of the cytokine or by expression of its receptor. There are 2

different types of IL-1 receptors present on the chondrocyte surface, RI and RII. IL-1 RI is the primary signal transducing receptor and it is found on nearly all cells. IL-1 RII, as shown in nonchondrocytes, is not involved in transducing a signal. Increased number of receptors may amplify the magnitude of the response. While treatment with doxycycline upregulated IL-1 RI and RII, it downregulated production of IL-1α and IL-1ß, undermining the effect of increased levels of IL-1 receptors on collagenase production.

The activity of doxycycline may be to decrease proinflammatory cytokines in conjunction with an upregulation of the level of the protective cytokine TGF-ß3. We have shown that TGF-ß1 decreases MMP-1 and MMP-13 enzyme production in OA lesional chondrocytes[10] and presumably TGF-ß3 would act similarly. TGF-ß family members initiate their cellular action by binding to the TGF-ß RI and RII. Ligand binding induces heteromeric oligomerization of TGF-ß RI and RII, resulting in transphosphorylation of the RI by RII. The RI then phosphorylates intracellular target molecules[49]. In our experiments, doxycycline also increased the level of mRNA for TGF-ß RI and TGF-ß RII receptors. These data also explain and support previous observations that tetracycline analogs increase collagen synthesis, osteoblast activity, and bone formation[50-52]. The biologic response of cells to cytokines has been found to correlate with the level of receptors[53-55]. The net results of these actions would decrease MMP-1 and MMP-13 enzyme production. That doxycycline is capable of modulating cytokine levels provides evidence that it may act indirectly to modulate MMP and may explain its inhibitory properties. Thus, doxycycline regulation of collagenase production through cytokine and cytokine receptors serves to protect articular cartilage from proteolytic degradation.

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