

Mechanism of inhibition of matrix metalloproteinase-2 expression by doxycycline in human aortic smooth muscle cells

Jian Liu, MD, Wanfen Xiong, PhD, Lisa Baca-Regen, PharmD, Hideaki Nagase, MD, and B. Timothy Baxter, MD, *Omaha, Neb*

Degradation of the extracellular matrix components elastin and collagen has been implicated in vascular diseases, including abdominal aortic aneurysm (AAA) and atherosclerotic plaque rupture. Increased expression of matrix metalloproteinases (MMPs) is involved in these disease processes. Our previous studies have demonstrated that MMP-2 derived from mesenchymal cells is required for aneurysm development in a murine model. Doxycycline is a nonspecific inhibitor of MMPs. In the present study, the mechanisms of the inhibitory effects of doxycycline on MMP-2 expression from cultured human aortic smooth muscle cells (SMCs) and human aortic aneurysm tissue explants were studied. Doxycycline inhibited MMP-2 expression from cultured SMCs in a concentration-dependent manner (5–40 $\mu\text{g}/\text{mL}$; inhibitory concentration of 50%, 6.5 $\mu\text{g}/\text{mL}$). At normal therapeutic serum concentration (5 $\mu\text{g}/\text{mL}$) doxycycline significantly reduced MMP-2 production from SMCs (37%; $P < .05$), which were stimulated with conditioned media from macrophage or lymphocyte co-culture simulating the inflammatory milieu of AAA tissue. This correlated with a decrease in MMP-2 mRNA half-life, from 49 hours to 28 hours, which suggests that doxycycline inhibits SMC MMP-2 production in part by reducing MMP-2 mRNA stability. When AAA tissue was cultured for 10 days with doxycycline at concentrations of 2.5 to 40 $\mu\text{g}/\text{mL}$, the media exhibited a concentration-dependent decrease in both active and latent forms of MMP-2 and MMP-9. Doxycycline at a concentration of 5 $\mu\text{g}/\text{mL}$ reduced active and latent MMP-2 secreted from cultured AAA tissue by 50% and 30%, respectively ($P < .05$). These study findings demonstrate that doxycycline at standard therapeutic serum concentrations inhibits MMP-2 expression from cultured human aortic SMCs and AAA tissue explants. Inasmuch as MMP activity contributes to extracellular matrix degradation in AAAs and atherosclerotic plaque, doxycycline may have potential value in treating these diseases. (*J Vasc Surg* 2003;38:1376–83.)

Clinical Relevance: Numerous studies have made it clear that proteolytic degradation of aortic structural proteins is responsible for initiation and expansion of abdominal aortic aneurysm (AAA). Elastin and collagen degradation in aneurysm tissue is caused, in part, by MMP-2 produced by mesenchymal cells of the aortic media. The present study shows that the antibiotic doxycycline can inhibit MMP-2 in cell culture and in human aneurysm tissue *ex vivo*. This lends further support to the concept that doxycycline could inhibit AAA growth. Presently there is no medical treatment to inhibit AAA growth, and the only effective option to protect against rupture is repair. A medical option, if it existed, could lead to screening, detection, and medical treatment of small aneurysms.

Abdominal aortic aneurysm (AAA) is common in the US elderly population. Its most significant complication, rupture of the aorta, is responsible for more than 15,000 deaths annually in the United States.¹ The only proven treatment is mechanical repair of large aneurysms. Destruction of extracellular matrix components of the artery, including elastin and fibrillar collagen, is a consistent feature of AAA,^{2–4} and is associated with dense inflammatory infiltration of the media and adventitia, consisting of macrophages and lymphocytes.^{5,6}

The matrix metalloproteinases (MMPs), a family of zinc endopeptidases, are responsible for degradation of

extracellular matrix in AAAs.^{3,7,8} Among MMPs identified in AAA tissue, MMP-2 (gelatinase A) is able to degrade both major macromolecules of aortic extracellular matrix, elastin and fibrillar collagen.^{9,10} MMP-2 is predominantly expressed by mesenchymal cells, that is, smooth muscle cells (SMCs) and fibroblasts.¹¹ Increased MMP-2 protein and messenger RNA levels have been reported in AAAs, which suggests a role in pathogenesis or progression of aneurysms.^{12–14} Our data demonstrate that MMP-2 from mesenchymal cells is required for aneurysm formation.¹⁵ Of importance, MMP-2 expression appears to be increased when mesenchymal cells are surrounded by inflammatory cells.^{14,16} We have confirmed with *in vitro* studies that MMP-2 expression in aortic SMCs is increased when cells are incubated with media conditioned by exposure to macrophages and lymphocytes.¹⁷

Drugs that block MMP expression from vascular mesenchymal cells could have therapeutic potential in treating AAA. Independent of its antimicrobial properties, doxycycline, a derivative of tetracycline, is a nonspecific MMP inhibitor.¹⁸ Doxycycline inhibits MMP-2 secretion from

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Reprint requests: B. Timothy Baxter, MD, University of Nebraska Medical Center, 8111 Dodge St, Suite 220, Omaha, NE 68114 (e-mail: btbaxter@unmc.edu).

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cultured epithelial cells¹⁹; however, the mechanism of this inhibition is not understood. Petrinc et al²⁰ and Pyo et al²¹ showed that doxycycline inhibits aneurysm formation in an elastase-induced rat model of AAA. Both MMP-2 and MMP-9 expression appear to be reduced in this model.²⁰ Our previous findings demonstrate that standard doxycycline doses inhibit AAA growth in a mouse model and that this occurs with decreased MMP activity.²² Although these animal studies suggest that doxycycline could have a role in treatment of AAA, they raise several important questions. Will doxycycline inhibit MMP-2 production by human aortic SMCs or in human AAA tissue? What is the mechanism of this inhibition? Can MMP-2 inhibition be achieved within the standard therapeutic dose range for doxycycline? The purpose of the present study was to investigate the effects and mechanisms of doxycycline on MMP-2 expression in cultured aortic SMCs and human AAA tissue explants.

METHODS

Cell culture and treatment. Human SMC culture was established from normal infrarenal aorta obtained from transplant donors, as described.²² SMCs between the second and fourth passage were used for the experiments. Monocytes and lymphocytes (a generous gift from Dr Howard E. Gendelman, Department of Pathology and Microbiology, University of Nebraska Medical Center) were separated and purified with countercurrent centrifugal elutriation of peripheral blood mononuclear cells from leukaphoresis in healthy donors. The purity (>97%) of the monocytes was confirmed with the criteria for cell anatomy on Wright-stained cytosmears, and with granular peroxidase and nonspecific esterase staining. The monocytes (3×10^6) were cultured for 7 days with Dulbecco modified Eagle medium (DMEM; Gibco BRL, Gaithersburg, Md) containing 10% human serum (Sigma, St Louis, Mo) and 2- $\mu\text{g}/\text{mL}$ purified recombinant human macrophage colony-stimulating factor (Genetics Institute, Cambridge, Mass), to enable differentiation into macrophages. Peripheral lymphocytes were activated with phytohemagglutinin (1 $\mu\text{g}/\text{mL}$; Sigma) for 24 hours. Co-culture was carried out by incubation of 3.0×10^7 macrophages and 1.8×10^8 activated lymphocytes in 30 mL of serum-free M-199 medium. The conditioned medium was collected after 24 hours. Gelatin-binding proteins, including MMP-2 and MMP-9 present in the conditioned medium of co-cultured inflammatory cells, were removed with gelatin-sepharose (Pharmacia Biotech, Uppsala, Sweden) before the medium was applied to SMCs. Zymography was performed on the conditioned medium after the gelatin-sepharose column to confirm the absence of MMP-2 and MMP-9. SMCs in serum-free M-199 or SMCs activated with the conditioned medium of macrophage-lymphocyte co-culture were then treated with doxycycline (Sigma) at concentrations of 5 to 40 $\mu\text{g}/\text{mL}$ for 24 hours. The SMC medium was harvested, and total RNA was extracted from the cells.

AAA tissue culture and treatment. Infrarenal aortic tissues were obtained at surgical repair of AAA. Informed

consent was obtained for tissue collection in accordance with a protocol approved by the Institutional Review Board and Research Committee of the University of Nebraska Medical Center and the Omaha Veterans Administration Medical Center. The AAA tissue was cut into pieces 10 mm in diameter and incubated in a 12-well plate with serum-free M-199 medium. Doxycycline at concentrations of 2.5 to 40 $\mu\text{g}/\text{mL}$ was added to the culture. The culture medium was changed every 24 hours. The tissue culture medium was collected at day 10 for MMP analysis.

Gelatin zymography. MMP-2 and MMP-9 concentrations in culture media (10 μL per lane) were measured with gelatin zymography, which was conducted with sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) containing gelatin (0.8 mg/mL), as described.¹⁵ Conditioned media from human macrophages and fibroblasts served as a positive control for MMP-9 and MMP-2, respectively. Gelatinolytic activities were quantified with densitometry (Molecular Dynamics, Piscataway, NJ).

Western blot analysis. Media from tissue culture (70 μL per lane) were subjected to 10% SDS-PAGE under reducing conditions, and the proteins were transferred to 0.45 $\mu\text{mol}/\text{L}$ nitrocellulose membranes (BioRad, Hercules, Calif). The membrane was immunoblotted with mouse anti-human MMP-2 primary antibody (Oncogene, Cambridge, Mass), and the secondary sheep anti-mouse immunoglobulin G was conjugated with horseradish peroxidase (Amersham Corp, Arlington Heights, Ill). The specific MMP-2 bands were detected with the electrochemiluminescent system (Amersham) and quantified with densitometry.

MMP-2 mRNA half-life determination. The half-life of MMP-2 mRNA in SMCs was measured in serum-free M-199 control cultures and cultures containing 10 $\mu\text{g}/\text{mL}$ of doxycycline. After doxycycline treatment for 24 hours, actinomycin D (5 $\mu\text{g}/\text{mL}$; Sigma) was added to the cultures. Then the total RNA was extracted from SMCs at 0, 4, 8, 12, 24, and 48 hours after addition of actinomycin D. MMP-2 mRNA was analyzed with dot-blot hybridization. Total RNA (5 μg) was mixed with 20 μL of 100% formamide, 7 μL of 37% formaldehyde, and 2 μL of 20 \times saline-sodium citrate (SSC), and denatured by heating at 65°C for 15 minutes. The samples were placed on ice, and 80 μL of 20 \times SSC were added to each sample. The samples were then blotted onto Nytran membrane (Schleicher and Schuell, Keene, NH) and washed with 1 mL of 10 \times SSC, and the RNA was immobilized on the membrane with ultraviolet illumination. Hybridization with phosphate 32-labeled MMP-2 complementary DNA probe, washing, and autoradiography of dot-blot membrane were performed.

Northern blot analysis. Total RNA was extracted with TRIzol reagent (Gibco BRL), and 2 μg of RNA were electrophoresed on a 1.4% agarose/2.2-mmol/L formaldehyde gel. RNA was transferred onto Nytran membranes with capillary elution. The membranes were hybridized with ³²P random-labeled cDNA probes overnight at 55°C in a solution containing 50% formamide, 5 \times SSC, 5 \times

Denhardt solution, 1% SDS, and 250- μ g/mL sheared salmon sperm DNA. The membranes were washed three times in $2\times$ SSC at room temperature, followed by three 30-minute incubations in $0.1\times$ SSC and 0.5% SDS at 65°C . The hybridized signals were visualized with autoradiography with Kodak x-ray film, and were quantified with densitometry.

Cell proliferation assay. Equal numbers of SMCs (3000 cells per well) were cultured in 96-well plates with serum-free M-199 and various concentrations (5-40 μ g/mL) of doxycycline for 24 hours. The cell numbers were then evaluated with the 3-(4,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay with a Cell Titer 96 kit (Promega, Madison, Wis), which measures conversion of tetrazolium salt into blue formazan by viable cells. Absorbance of soluble MTT formazan products was measured at 490 nm.

Statistical analysis. The results are presented as mean \pm SEM. Differences between groups were determined with analysis of variance. $P < .05$ was considered significant. Results of mRNA half-life were compared with the Student t test for equality of slopes. Correlation was determined with liner regression, and expressed as correlation coefficient.

RESULTS

Doxycycline inhibits SMC MMP-2 production. To assess the effect of doxycycline on expression of MMP-2 by SMCs, we performed gelatin zymography and Western blot analysis. Only the latent form of MMP-2 was detected in both assays (Fig 1, A, B). When stimulated with co-cultured macrophage or lymphocyte conditioned medium, SMCs produced two to three times more MMP-2 than unstimulated cells did (Fig 1, C). Doxycycline inhibited MMP-2 secretion from both conditioned media stimulated and unstimulated SMCs in a concentration-sensitive response (Fig 1, A). In conditioned medium stimulated cells, the MMP-2 concentration was decreased by 37% with 5 μ g/mL of doxycycline, by 80% with 10 μ g/mL, and by 97% with 20 μ g/mL of doxycycline. Inhibition of MMP-2 production by doxycycline was more substantial when the cells were incubated with co-cultured macrophage or lymphocyte conditioned medium (Fig 1, C). Inasmuch as serum doxycycline levels measured in volunteers given a standard dose (2-3 mg/kg/d) of doxycycline were in the range of 2 to 6 μ g/mL,²² we specifically compared inhibition of MMP-2 production in SMCs incubated with and without conditioned media at 5 μ g/mL of doxycycline. At a concentration of 5 μ g/mL, doxycycline significantly decreased MMP-2 production in SMCs incubated with macrophage or lymphocyte co-cultured condition media ($P < .05$); however, decrease in unstimulated SMCs was not statistically significant (Fig 1, D). These observations suggest that doxycycline has a selective effect in inhibiting MMP-2 overexpression.

Doxycycline does not affect cultured aortic SMC viability. It was important to demonstrate that the effects of doxycycline on MMP activity are not the result of drug

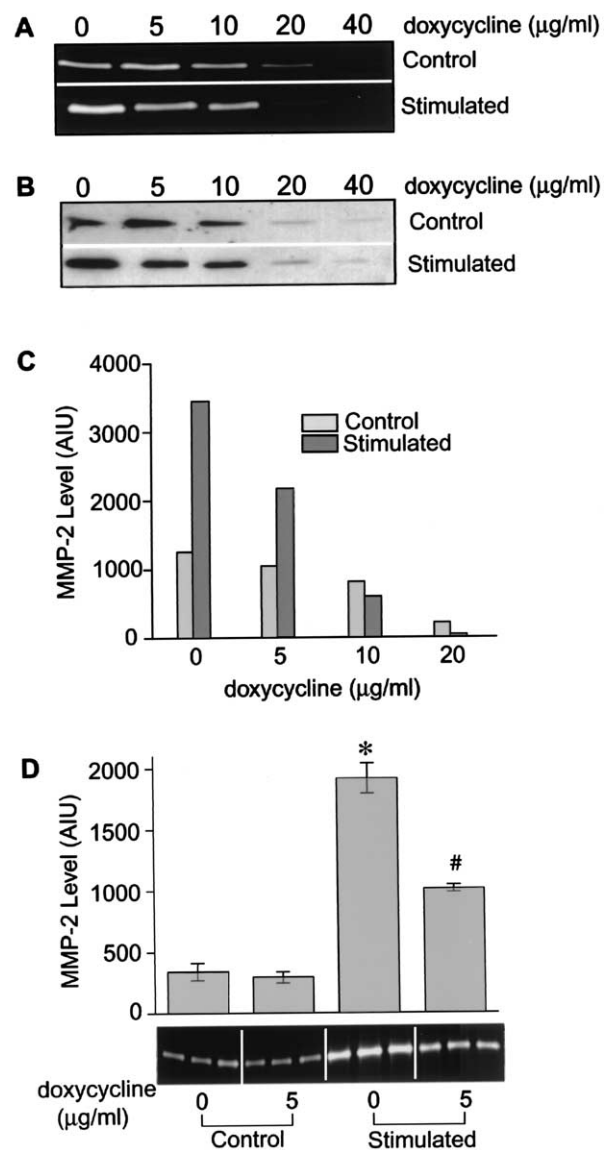


Fig 1. Effect of doxycycline on matrix metalloproteinase-2 (MMP-2) production from cultured aortic smooth muscle cells (SMCs). Aortic SMCs cultured in either serum-free M-199 (*Control*) or macrophage or lymphocyte co-culture medium (*Stimulated*) were treated with doxycycline at indicated concentrations for 24 hours. MMP-2 concentration in cell culture medium at indicated doses of doxycycline were determined with gelatin zymography (A) and Western blot analysis (B). C, MMP-2 band intensity was determined with quantitative densitometry. Quantitative values in arbitrary integrator units represent mean obtained from three independent experiments. D, Inhibition of MMP-2 production from SMCs treated without (*Control*) or with (*Stimulated*) co-cultured macrophage or lymphocyte media in response to 5 μ g/mL of doxycycline. Gelatin zymography (*lower panel*) represents SMCs from three separate plates. * $P < .05$ compared with control SMCs; # $P < .05$ compared with medium-treated SMCs without doxycycline.

cytotoxicity. This was addressed by determining cell numbers in the presence of doxycycline. The number of cells at

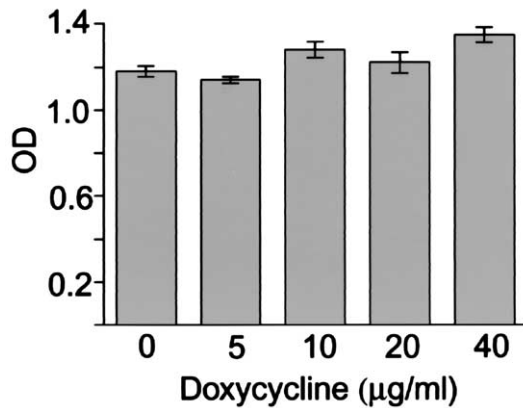


Fig 2. Effect of doxycycline on proliferation of cultured aortic smooth muscles (SMCs). SMCs were treated with indicated dose of doxycycline in serum-free M-199 medium for 24 hours. Cell numbers were determined by measuring conversion of tetrazolium salt into blur formazan in viable cells. Values are mean \pm SE of triplicate measurements. OD, Optic density.

indicated concentrations of doxycycline was tested with the MTT reduction assay (Fig 2). There was no significant change in cell number when SMCs were treated with doxycycline at 5 to 40 $\mu\text{g}/\text{mL}$ for 24 hours. This suggests that the inhibitory effect of doxycycline on MMP activity is not due to cytotoxicity of this drug.

Doxycycline reduces MMP-2 mRNA stability in SMCs. A decrease in steady-state levels of MMP-2 mRNA was observed at Northern blot analysis after SMCs were treated with doxycycline at therapeutic concentrations (5 and 10 $\mu\text{g}/\text{mL}$) for 24 hours (Fig 3, A). This finding was observed on three separate Northern blots from different plates. This did not represent a general decrease in cellular mRNA, because glyceraldehyde-3-phosphate dehydrogenase mRNA was unchanged. At concentrations of doxycycline greater than 10 $\mu\text{g}/\text{mL}$, no consistent downregulation of mRNA was observed. To determine whether doxycycline can regulate expression of MMP-2, the nuclear run-on transcription assay was performed. Doxycycline did not appear to change SMC MMP-2 mRNA transcription rate (data not shown). To investigate whether doxycycline can affect transcription of MMP-2 in SMCs, stability of MMP-2 mRNA in SMCs was measured with dot-blot hybridization. After addition of the gene transcription inhibitor actinomycin D, decrease in concentration of MMP-2 mRNA was measured with and without doxycycline treatment. In control SMCs, the half-life of MMP-2 mRNA was estimated to be about 49 hours, consistent with published observations.²³ After doxycycline treatment, MMP-2 mRNA half-life was reduced to about 28 hours. Furthermore, the slopes of mRNA half-life data were significantly different between control and doxycycline-treated SMCs ($P = .002$; Fig 3, B), indicating that increased degradation or reduced stability of MMP-2 mRNA contributed to some of the observed doxycycline inhibition of MMP-2 production in SMCs.

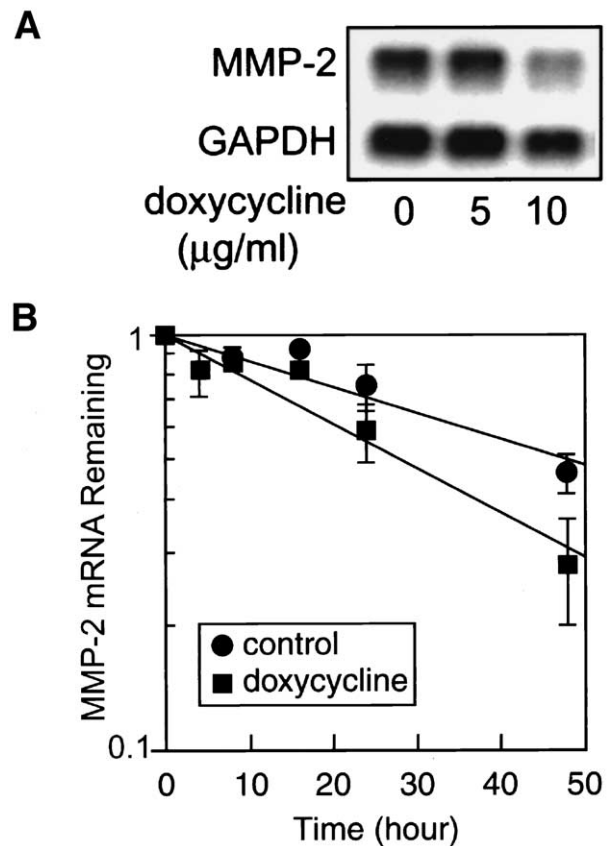


Fig 3. Effect of doxycycline on steady-state level and stability of matrix metalloproteinase-2 (MMP-2) mRNA from smooth muscle cells. **A**, Aortic SMCs were treated with doxycycline at concentrations of 5 and 10 $\mu\text{g}/\text{mL}$ for 24 hours. Total RNA was extracted from the cells, and MMP-2 and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA was determined with Northern blot analysis. **B**, Confluent cultures of human SMCs were incubated in presence or absence of 20 $\mu\text{g}/\text{mL}$ of doxycycline. After 24 hours, actinomycin D (5 $\mu\text{g}/\text{mL}$) was added (0 h), and cultures were further incubated for indicated times. Each value represents results from three separate plates. Total RNA was extracted, dot blotted onto Nytran membrane, and probed with [³²P]deoxycytidine triphosphate-labeled MMP-2 complementary DNA fragment. Dot blots were quantified with densitometry, and data were semilogarithmic plotted as mean \pm SE ($n = 5$). Best-fit curves were generated and used to estimate messenger RNA half-life.

Doxycycline decreases MMP-2 and MMP-9 production in AAA. To further test the efficacy and kinetic features of doxycycline in AAA, we used explant cultures from AAA tissue treated with doxycycline. At gelatin zymography, both the latent and active forms of MMP-2 and MMP-9 were observed in conditioned media-cultured AAA tissue (Fig 4, A). Doxycycline at concentrations of 2.5 to 40 $\mu\text{g}/\text{mL}$ exhibited concentration-dependent inhibition of both latent and active MMP-2 and MMP-9 in AAA tissue media collected at day 10 of tissue culture (Fig 4). Linear regression analysis demonstrated a significant correlation between increased doxycycline concentrations in tis-

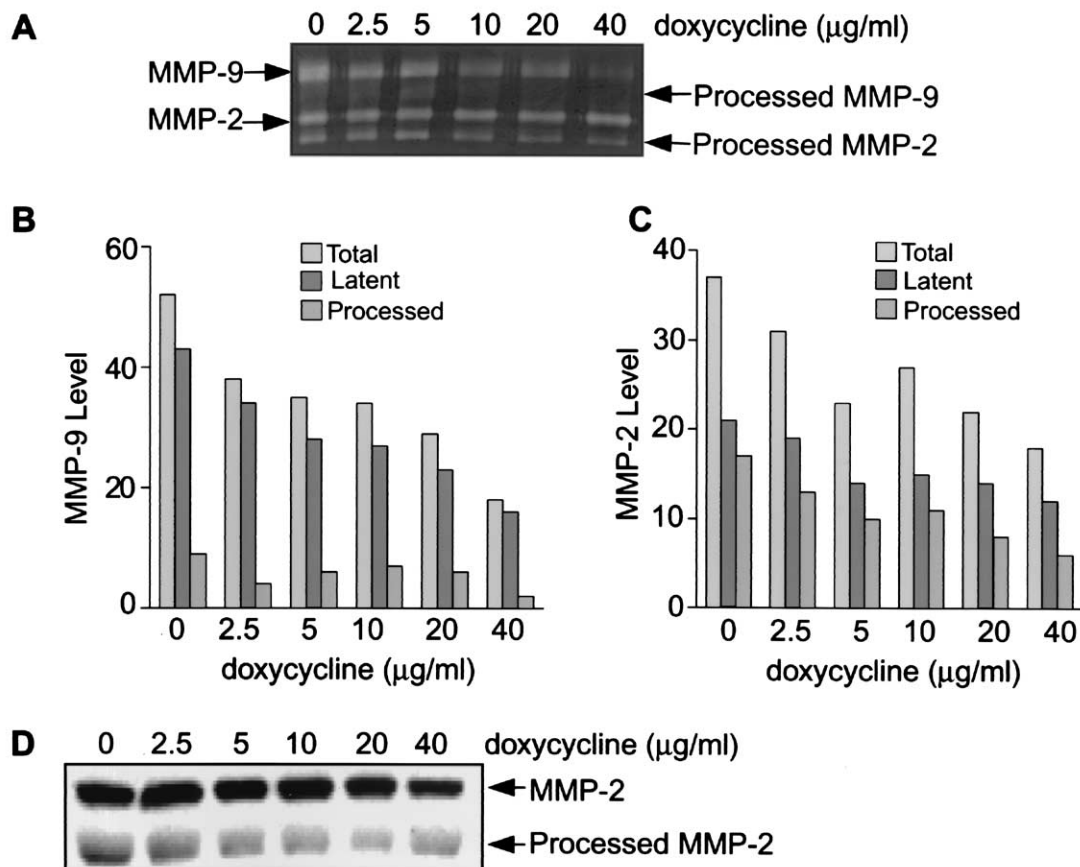


Fig 4. Doxycycline-inhibited matrix metalloproteinase (MMP)-9 and MMP-2 expression from cultured human abdominal aortic aneurysm (AAA) tissue. Human AAA tissue was cultured in serum-free M-199 medium and treated without or with doxycycline at 2.5 to 40 $\mu\text{g}/\text{mL}$ for 10 days. Media were collected, and MMP-2 and MMP-9 concentration was examined with gelatin zymography and Western blot analysis. **A**, Representative gelatin zymography shows both MMP-2 and MMP-9 concentrations in media of AAA tissue culture treated at indicated dose of doxycycline. MMP-9 (**B**) and MMP-2 (**C**) activity was quantified with densitometry. Values in arbitrary integrator units were reported. **D**, Western blot analysis of MMP-2 concentration in culture media of AAA tissue treated with indicated concentration of doxycycline.

sue culture and reduction of MMP-2 ($r = 0.91$, $P < .05$) and MMP-9 ($r = 0.82$, $P < .05$) activity in AAA tissue (Fig 4, B, C). Reduction of MMP-2 concentration in the medium of AAA tissue explants treated with doxycycline was corroborated with decreased MMP-2 protein content measured with Western blot analysis (Fig 4, D). The magnitude of the decrease in MMP-2 activity from AAA tissues treated with 5 $\mu\text{g}/\text{mL}$ of doxycycline was further quantified. At 5 $\mu\text{g}/\text{mL}$, doxycycline significantly inhibited latent (30%) and active (50%) MMP-2 from cultured AAA tissue (Fig 5).

DISCUSSION

Destruction of extracellular matrix components and marked local inflammatory cell infiltration are striking histologic features of human AAA tissue.^{5,24-26} Increased local production of MMPs has been implicated in aortic dilatation and AAA formation.^{4,7,11,14,27-30} It has also been suggested that MMPs are important in weakening of

the atherosclerotic plaques in atherosclerosis disease,^{8,31-33} which leads to plaque rupture and acute myocardial infarction.³⁴ Drugs with ability to block MMP overexpression could have therapeutic potential in treating these common and lethal diseases. Tetracyclines have been used in the treatment of several chronic inflammatory diseases associated with matrix destruction, such as rheumatoid arthritis and periodontal disease.^{18,35,36} Their efficacy is presumed related to inhibiting local MMP production.¹⁸ Inhibition of MMPs with doxycycline,²⁰ hydroxamate inhibitors such as BB-94,³⁷ and local overexpression of tissue inhibitor of MMP³⁸ reduce elastin destruction and aortic dilation in various animal models of aneurysm. Among these therapeutic approaches, doxycycline is the most attractive for use in patients with AAA, because of extensive clinical experience with this drug, and its relative safety profile.³⁹

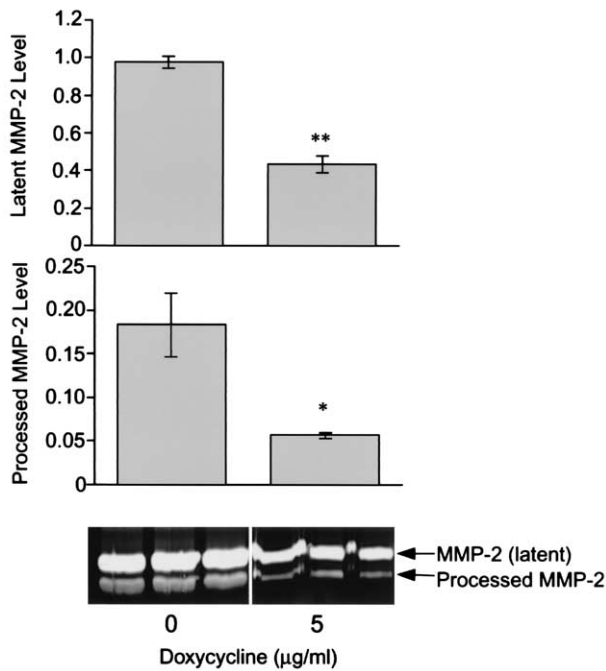


Fig 5. Effect of therapeutic dose of doxycycline on matrix metalloproteinase-2 (MMP-2) expression from cultured AAA tissue. Explanted AAA tissue was treated with 5 $\mu\text{g}/\text{mL}$ of doxycycline for 10 days. MMP-2 concentration in tissue culture medium was determined with gelatin zymography, and quantified with densitometry. Values represent mean \pm SE of three different patient samples run in triplicate. * $P < .05$; ** $P < .01$. Gelatin zymography (lower panel) represents a patient sample run in triplicate.

In the present study, doxycycline at concentrations of 5 to 40 $\mu\text{g}/\text{mL}$ directly inhibited MMP-2 production from cultured human aortic SMCs and explanted AAA tissue. MMP-2 is expressed by medial SMCs and adventitial fibroblasts in the aorta.^{11,14} In addition to its potent elastolytic activity, MMP-2 also degrades intact fibrillar collagen and gelatin.⁹ Increased MMP-2 protein and mRNA in AAAs has been reported,¹²⁻¹⁴ indicating that MMP-2 may have a critical role in aneurysm formation and dilation. Increased expression of MMP-2 in AAAs was also suggested by immunohistochemistry.^{11,14} These inflammatory cells contribute directly to local production of MMP-9 and MMP-12.^{7,15,40} With *in vitro* experimental conditions designed to simulate the inflammatory milieu of AAA tissue, Grange et al¹⁷ found that media conditioned from co-cultured macrophages and phytohemagglutinin-activated lymphocytes could upregulate MMP-2 expression from cultured human aortic SMCs. This observation is consistent with increased expression of MMP-2 from aortic mesenchymal cells surrounded by infiltrated inflammatory cells¹⁴ and our current observation of increased MMP-2 expression from SMC cultured in the presence of macrophage or lymphocyte conditioned media. Inasmuch as cells were not counted after 24-hour exposure to conditioned media, it is possible, but unlikely, that the increased MMP-2 levels

reflect significant SMC proliferation. Interestingly, we found that doxycycline has greater inhibitory effect when MMP-2 production is stimulated by macrophage- or lymphocyte-conditioned media. We emphasize a dose of 5 $\mu\text{g}/\text{mL}$ because we found that serum doxycycline levels measured in volunteers given a standard dose (2-3 mg/kg/d) ranged from 2 to 6 $\mu\text{g}/\text{mL}$.²² Doxycycline at 5 $\mu\text{g}/\text{mL}$ resulted in a significant 37% decrease in MMP-2 secretion from SMCs cultured in macrophage or lymphocyte conditioned media. The decrease in MMP-2 production was only 15% when cells were cultured in serum-free M-199 media. This suggests that doxycycline exhibits a selective benefit by inhibiting MMP overproduction. Doxycycline did not demonstrate toxic effects on cultured SMCs at 5 to 40 $\mu\text{g}/\text{mL}$ after 24 hours, as determined by the MTT assay, which estimates cell numbers.

An important finding is that doxycycline directly inhibits both latent and active MMP-2 secretion from explanted human AAA tissue. We also noted decreased secretion of MMP-9. With treatment of explant for 10 days, we did not observe cytotoxicity, but did not directly measure cell viability at the end of treatment. These findings corroborate the animal work of Petrincic et al,²⁰ who found that doxycycline at very high doses inhibits development in an elastase-induced rat AAA model in association with decreased MMP-2 and MMP-9 production. Boyle et al⁴¹ showed that doxycycline inhibited progressive elastin degradation in porcine aorta explants. This correlates with decreased expression of MMP-2 and MMP-9. Doxycycline inhibits murine AAA in associated with decreased MMP production. Our finding that doxycycline at normal serum concentrations inhibits MMP-2 and MMP-9 expression from explanted human AAA tissue is consistent with observations of Curci et al,⁴² who found that a short preoperative course of doxycycline decreased AAA tissue MMP levels. These studies suggest that doxycycline may be effective in clinical trials designed to inhibit growth of small AAAs.

The mechanism of inhibition of MMP production by tetracyclines has not been clearly defined. This ability of doxycycline is functionally independent of its antibiotic properties.¹⁸ Direct inhibition of MMPs by binding to metal ions such as Ca^{2+} and Zn^{2+} is seen at concentrations of doxycycline well above expected serum concentrations (>50 $\mu\text{g}/\text{mL}$).⁴³ Uitto et al¹⁹ reported that doxycycline downregulates MMP-2 mRNA in cultured epithelial cells. Jonat et al⁴⁴ reported downregulation of MMP-3 gene transcription with tetracycline from skin fibroblasts. We investigated whether doxycycline alters transcriptional regulation of MMP-2, and found that this does not appear to be so. However, we observed significantly decreased MMP-2 mRNA half-life and steady-state of MMP-2 mRNA when SMCs were treated with doxycycline. To our knowledge, ours is the first report of regulation of MMP-2 production involving posttranscriptional destabilization of MMP-2 mRNA. This effect may be beneficial in treating AAA.

In summary, at normal serum concentrations doxycycline can directly inhibit MMP-2 expression from cultured

SMCs and MMP-2 and MMP-9 production in human AAA. Downregulation of MMP-2 mRNA in SMCs with doxycycline is mediated, at least in part, by decreasing mRNA stability. Increased MMP expression in AAAs, especially MMP-2 and MMP-9, appears to be critical for aortic dilation and AAA formation, and may also be pivotal in atherosclerotic plaque rupture. Inasmuch as doxycycline has an excellent safety profile for long-term use, with few side effects,³⁹ it could offer a therapeutic option for inhibition of growth of small AAAs. This will have to be evaluated in carefully controlled clinical trials.

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