Doxycycline inhibits elastin degradation and reduces metalloproteinase activity in a model of aneurysmal disease

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Purpose: **Abdominal aortic aneurysms are characterized by degradation of the extracellular matrix, with a reduction in the elastin concentration of the arterial media. These changes are mediated by increased levels of endogenous metalloproteinases (MMPs) within the aorta, which provide a potential therapeutic target for pharmacologic agents aimed at reducing the growth rate of small aneurysms. In this study, the ability of doxycycline—an MMP inhibitor—to reduce matrix degradation was assessed in a previously described model of aneurysmal disease that used a brief pulse of elastase to induce MMP production and elastin degradation in arterial organ cultures.**

Methods: **Porcine aortic segments (n = 8) were preincubated in exogenous pancreatic elastase for 24 hours before culture in standard conditions for 13 days with both 1 and 10 mg/L doxycycline. Control segments were cultured both without doxycycline and without elastase. At the termination of culture, MMPs were extracted from the tissue and quantified by a combination of substrate gel enzymography and immunoblotting. The volume fractions of elastin and collagen were determined by stereologic analysis of sections stained with Miller's elastin and van Gieson's stain.**

Results: **Stereologic analysis demonstrated a significant preservation of elastin in aorta treated with doxycycline 10 mg/L ($p < 0.001$) and demonstrated that this preservation was accompanied by a significant reduction in MMP-9 activity ($p < 0.02$). Immunoblotting for tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) showed no decreased production in the doxycycline-treated groups.**

Conclusions: **Therapeutic ranges of doxycycline significantly inhibited elastin degradation and MMP-9 production within aortic organ cultures. These data suggest that doxycycline may have a potential application in reducing the growth rates of small abdominal aortic aneurysms. (J Vasc Surg 1998;27:354-61.)**

In England, aortic aneurysms account for more than 11,000 admissions per year, and unusually for a vascular disorder, the prevalence of this condition appears to be increasing.[1] Abdominal aneurysms and their management remain a significant health problem that is likely to assume greater importance with the expansion of the elderly population. At present, there is a general surgical consensus that patients who have abdominal aneurysms in excess of 5.5 cm should be recommended for elective surgical repair, which has a mortality rate approaching 5%.[2,3] At present it is uncertain whether patients with aneurysms between 4 and 5.5 cm should undergo surgery, and this is the subject of a prospective randomized trial that is expected to be reported in 1998.[4]

Current management strategies for small (<5.5 cm) aneurysms involve serial ultrasound examination, with elective surgery when growth exceeds 1 cm per year or the absolute aneurysm size reaches 5.5 cm.[5] Small aortic aneurysms expand exponentially by approximately 10% of their diameter per year,[6,7] although this rate may be significantly higher in patients who are hypertensive or who smoke.[8] Collin[9] monitored 145 patients with screen-detected aneurysms <4 cm and demonstrated that 14% required elective surgery as a result of rapid expansion or growth to a diameter exceeding 5.5 cm. In

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0741-5214/98/$5.00 + 0 **24/1/86069**

addition to the complications of elective surgical repair, patients with small aneurysms also have a finite risk of rupture. Cronenwett et al.[10] demonstrated that 9% of patients with small AAAs required emergency surgery during a 3-year study, whereas Katz et al.[11] demonstrated an annual rupture rate of 3.3% for patients with aneurysms <5 cm. With the advent of community-based or hospital-based screening programs, the number of small aneurysms presenting to vascular surgeons is likely to increase. Clearly, a therapeutic strategy is required to limit both the expansion and rupture rate of small abdominal aneurysms and to offer an effective treatment to patients with this condition.

Consequent to this requirement, the biochemical and molecular mechanisms of aneurysm formation have begun to attract more attention, and these studies have suggested novel therapeutic avenues. The characteristic feature of early abdominal aneurysms is the segmental depletion of elastin. The loss of elastin from the arterial wall has been related to a generalized enhancement of systemic and localized proteolytic capacity; increased levels of neutrophil elastase[12] and tissue matrix metalloproteinases (MMPs)[13] have been reported in patients who have AAAs. The MMPs are a family of zinc-dependent enzymes that have the capability to degrade all components of the extracellular matrix. These enzymes are secreted by mesenchymal cells of the arterial wall and infiltrating white cells. The enzymes are divided on the basis of their substrate specificity into the collagenases (MMP-1), the gelatinases (MMP-2 and MMP-9), and the stromelysins (MMP-3).[14] Recent evidence has suggested that elevated levels of MMPs may be the crucial determinant of aneurysm formation and growth, as all members of the elastolytic and collagenolytic MMPs are demonstrable in aneurysm tissue at elevated levels.[15-22]

These enzymes therefore provide a potential target for pharmacologic therapy aimed at preventing aortic wall matrix degradation and aneurysm growth. The aim of this study was to investigate the use of doxycycline, a nonspecific MMP inhibitor, in a previously described organ culture model of aneurysmal disease.[23] The model used porcine aortic segments that were cultured in sterile conditions. The segments were exposed to a brief pulse of exogenous elastase to initiate matrix degradation and endogenous MMP production. The arterial organ culture model facilitated the study of isolated cellular interactions and allowed the investigation of various doses of doxycycline within the system.

METHODS

Organ culture. Porcine thoracic aortas were kindly provided by Dawkins International Ltd. (Nuneaton, U.K.). Thoracic as opposed to abdominal aortas were used, as numerous sections were obtained from each aorta and this proved easier using thoracic tissue. Porcine thoracic aorta has a predictable structure containing numerous elastic lamellae.[24] Under sterile conditions, fat and loose adventitial tissue were removed. One square centimeter segments of aorta were excised and pinned, intimal surface uppermost, onto a polyester gauze support resting on sylgard resin (Dow Corning, Seneffe, Belgium) in the base of a 6 cm Petri dish (Fisons Loughborough, U.K.). Samples were denuded of endothelium and cultured for 14 days in standard medium (7 ml) containing 5% fetal calf serum (Sera Lab, Crawley, U.K.), which was changed after 24 hours and then every 48 hours.[23,24]

Experimental design. Porcine thoracic aortas were divided into six sections. One sample was harvested fresh. One was cultured for 14 days without exposure to elastase or doxycycline. Four samples were preincubated in culture medium supplemented with porcine pancreatic elastase (100 units/ml; Calbiochem, Nottingham, U.K.) for 24 hours. After this period, one sample was harvested and the three remaining aortic sections were washed thoroughly to remove all traces of exogenous elastase and cultured for a further 13 days in standard culture medium or culture medium supplemented by two concentrations of doxycycline (1 mg/L and 10 mg/L). The experiment was replicated in eight separate aortas.

Exposure to a brief pulse of elastase was necessary in this experimental design because previous studies had demonstrated that incubation with elastase for 24 hours initiated a time-dependent elastin degradation and MMP production in aortic organ cultures, even though exogenous elastase was not detectable by casin zymography at 3 days. Interestingly, culture of aortic tissue in standard conditions was associated with MMP induction, but did not result in elastin degradation in the absence of exogenous elastase.[23]

Histologic analysis. After fixation in formalin, samples were dehydrated in 99% industrial methylated spirit (Sigma, Poole, U.K.), transferred into xylene (Sigma) for 4 hours, and embedded in paraffin wax. Sections (4 μm) were stained with both hematoxylin and eosin and Miller's elastin and van Gieson's stain,[25] which gave a good balance in stain-

ing for elastin (black), collagen (pink), and smooth muscle cells (yellow).

Stereologic tissue analysis. The volume fractions of elastin, collagen, and smooth muscle cells in the extracellular matrix were determined by stereologic analysis as described previously.[26,27] Aortic sections, stained with Miller's elastin and van Gieson's stain, were viewed at 400× magnification using an Olympus microscope incorporating an eyepiece graticule with a 100-point test grid (Graticules Ltd., Kent, U.K.). One hundred test points were then analyzed, with test points hitting black indicating elastin, points hitting yellow indicating smooth muscle cells, and points hitting pink indicating collagen. After analysis, the relative volume fraction of each component was calculated. To maintain a constant frame of reference throughout all experiments, the adventitial aspect of the media was point counted, with eight randomly chosen fields quantified for each sample. Stereologic tissue analysis reflects the ability of the tissue to take up histologic stain, and thus measures of collagen, elastin, and smooth muscle cells reflect changes in the relative concentrations of each of these elements rather than changes in protein content. This form of tissue analysis will therefore demonstrate an inverse relationship between collagen and elastin in the extracellular matrix. Previous experiments have determined the interobserver limits of agreement for this technique in our institution.[23]

Gel enzymography. Metalloproteinases were extracted from frozen tissue using the method of Vine and Powell[15] as previously described.[23] Tissue was thawed over ice, diced into 1 mm^3 pieces, and homogenized in buffer. The homogenate was centrifuged and dialyzed, and the protein concentration was standardized for each sample to 0.9 mg/ml with phosphate-buffered saline solution.

Substrate gels were prepared by incorporating gelatin (1 mg/ml; Sigma) into a 10% SDS-polyacrylamide gel. Fifteen microliters of standardized tissue extract plus an equal volume of nonreducing sample buffer were loaded onto the gel. Electrophoresis was performed at 60 mA for 4 hours at 4° C with the Mini-Protean II system (Bio-Rad, Hemel Hempsted, U.K.), after which the gel was washed three times with 2.5% Triton X-100 (Sigma), incubated in buffer for 18 hours, and finally stained with Coomassie blue R250. The molecular weight of each band was estimated by comparison with the positions of known molecular weight standards (Bio-Rad). The relative density of each lytic band was determined from negative photographic images of gels with a Pharmacia LKB Imagemaster scanning densitometer (Pharmacia LKB, St. Albans, Hertfordshire) and expressed as a product of the optical density and area of the band. The protein concentration used in this analysis had been previously determined to be within the linear range for densitometric quantification (data not shown). To allow for variation between zymographic gels, all paired samples were run on the same gel, and paired statistics were used in analysis. No comparison was made between different gels.

Immunoblotting. Tissue extracts were fractionated on a 10% SDS-polyacrylamide and transferred to a nitrocellulose membrane (Hybond ECL, Amersham, U.K.) in the Mini-transblott apparatus (Bio-Rad) as previously described.[23] Mouse monoclonal antibodies specific for MMP-2, MMP-9, and the tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 (Oncogene Science, Paris) were used to identify MMPs and TIMPs within the samples. These antihuman antibodies had been demonstrated to cross-react with porcine proteins in a prior study.[23]

Statistical analysis. Median values and interquartile ranges for the volume fractions of elastin, collagen, and smooth muscle cells were calculated for all sections. These were then compared using nonpaired, nonparametric analysis (Mann-Whitney U test). The densitometric analysis of MMPs were compared using the nonparametric, paired, one-tailed Wilcoxon test.

RESULTS

Stereologic analysis. The histologic appearance of four paired aortic segments are illustrated in Fig. 1. There was no reduction in the elastin concentration in the sections of aorta cultured in standard conditions for 14 days. Exposure to a 24 hour pulse of elastase-induced (100 u/ml) matrix degradation in a time-dependent manner as described previously,[23] resulting in significant elastin depletion at 14 days.

Stereologic analysis confirmed that there was a significant preservation of elastin in the elastase-exposed aortic sections cultured in standard medium supplemented by doxycycline (10 mg/L), when compared with the sections not treated with doxycycline ($p < 0.001$; W = 28.0, 95% confidence interval, 36.00 to 9.9). There was also a trend to elastin preservation in the sections treated with a dose of 1 mg/L, although this was not significant ($p = 0.09$; W = 39.0; 95% confidence interval, 12.5 to –0.62). The percentage elastin concentration for all aortic

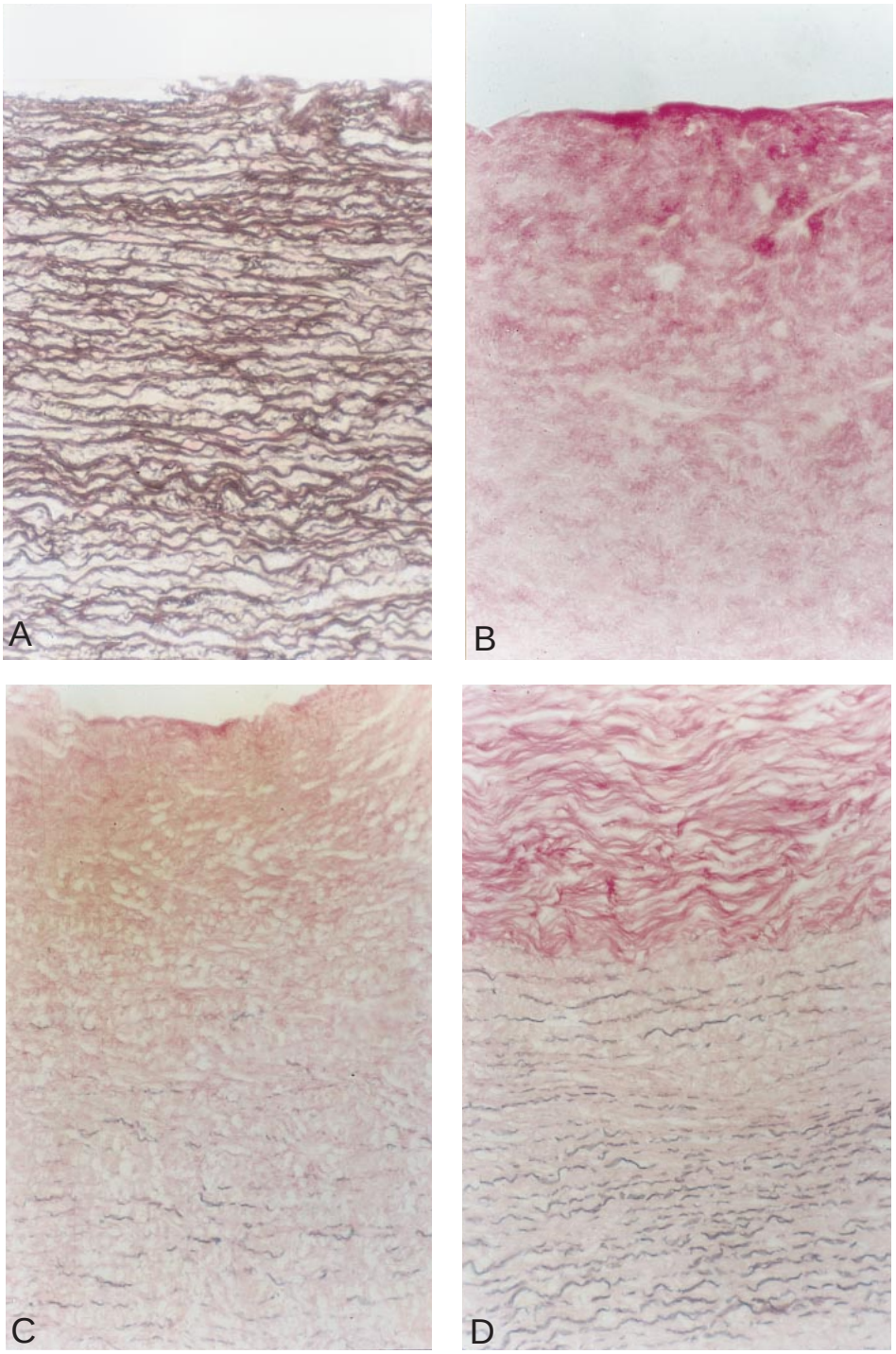

Fig. 1. Four histologic sections from the same aorta stained with Miller's elastin and van Gieson's stain (original magnification, 400×). Cultured control **(A)** and three sections exposed to a pulse of elastase, one subsequently cultured in standard medium **(B),** one in standard medium supplemented by doxycycline 1 mg/L **(C),** and one in standard medium supplemented by doxycycline 10 mg/L **(D).** Adventitial collagen **(D)** is noted to be particularly well preserved, although the significance of this is unknown.

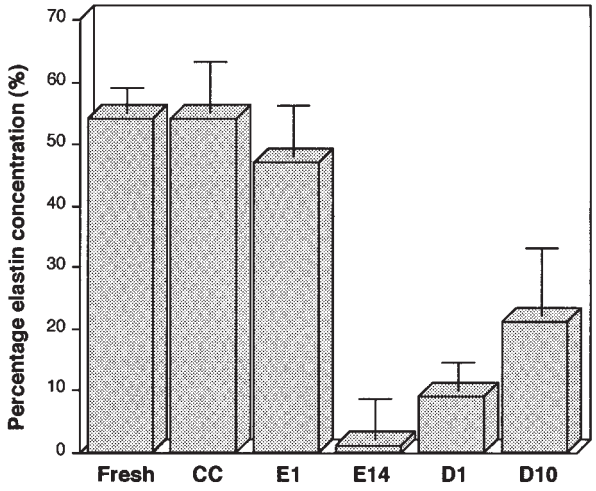

Fig. 2. Graph plots percentage elastin concentration (median values and Q3) for fresh aorta *(Fresh),* cultured control *(CC),* elastase exposed after 24 hours *(E1),* elastase exposed after 14 days *(E14),* doxycycline-treated 1 mg/L *(D1),* and doxycycline-treated 10 mg/L *(D10).*

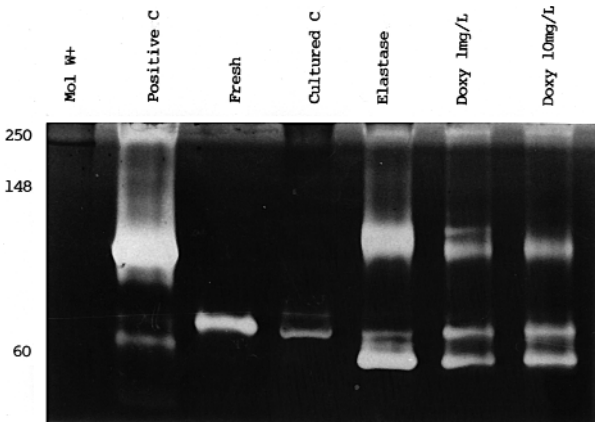

Fig. 3. Gelatin zymogram shows metalloproteinase activity for all sections of one aorta and demonstrates reduction in MMP-9 activity in doxycycline-treated segments. *Positive C,* HT 1080 fibrosarcoma cell line, which produces large quantities of MMP-2 and MMP-9. *Fresh,* Freshly harvested, noncultured aortic sample. *Cultured C,* Aorta cultured without exposure to elastase for 14 days. *Elastase,* Aorta exposed to elastase for 24 hours and then cultured in standard conditions for a further 13 days. *Doxy 1 mg/l* and *Doxy 10 mg/l,* Aortas exposed to elastase for 24 hours and then cultured with doxycycline for 13 days.

segments is illustrated in Fig. 2. The reduction in elastin concentration after exposure to elastase was accompanied by a parallel increase in collagen concentration, which obviously reflects the type of stereologic volume fraction analysis used.

Gelatinolytic activity. Gelatin enzymography confirmed a time-dependent increase in MMP activity within elastase-treated cultures compared with the control samples. Fresh aortic tissue demonstrated lytic bands at 70 kd, whereas elastase-treated samples demonstrated a progressive increase in gelatinolytic activity at 70 kd (doublet) and appearance of proteolytic bands at 90 kd and 250 kd.

Immunoblotting with specific monoclonal antibodies demonstrated immunoreactivity of a 70 kd doublet with MMP-2 antibody and a 90 and 250 kd protein reacting with an antibody to MMP-9 (data not shown).

A representative zymogram of four paired aortic sections is depicted in Fig. 3. Densitometric analysis of the aortic segments treated with doxycycline demonstrated that these samples had significantly less MMP-9 activity when compared with those cultured in standard medium after elastase exposure (p = 0.04, W = 33.0; and p = 0.02, W = 35.0 for doxycycline concentrations of 1 mg/L and 10 mg/L, respectively). MMP-9 activity as a percentage of the cultured control is illustrated in Fig. 4. The reduction in MMP-9 activity was accompanied by a lesser reduction in MMP-2 activity, although this was not significant (p = 0.45, WI = 9.0; and p = 0.08, WI = 25.0 for doxycycline concentrations of 1 mg/L and 10 mg/L, respectively).

Immunoblotting for the endogenous MMP inhibitors, the tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), demonstrated no obvious difference in activity in the doxycycline-treated segments (Fig. 5).

DISCUSSION

The management of small aneurysms is currently one of the critical problems in vascular surgery, and it is clearly desirable that pharmacologic treatments are developed to retard the expansion rate of such aneurysms. Previous experimental studies have suggested that β-blockers may inhibit aneurysm growth by lowering blood pressure and promoting collagen and elastin cross-linkage.[28-30] Interest in these agents was further stimulated by a report from Gadowski et al.,[31] who demonstrated that the expansion rate of large aneurysms was lower in a cohort of patients who took β-blockers. Unfortunately, this was an uncontrolled, nonrandomized study, and recent data from the MRC small aneurysm study has suggested that β-blockade has no effect of aneurysm growth.[32] Although the role of β-blockers in aneurysms is defined by a random-

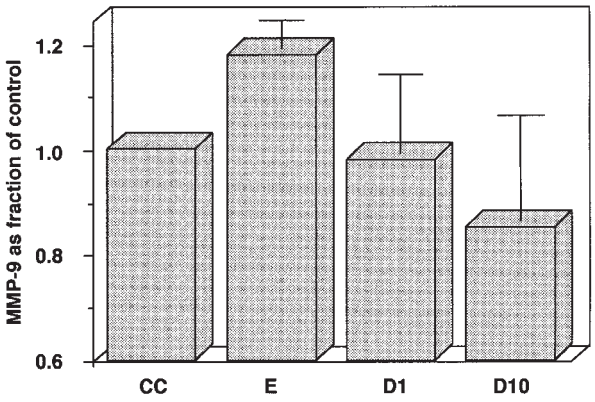

Fig. 4. Graph plots MMP-9 activity (median values and Q3) as a fraction of cultured control *(CC),* confirming that elastase exposure induced MMP-9 activity *(E)* that was progressively inhibited by an increasing dose of doxycycline at 1 mg/L and 10 mg/L (*D1* and *D10*).

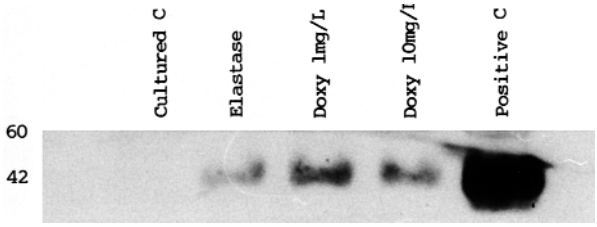

Fig. 5. Immunoblot demonstrates TIMP-1 immunoreactivity. *Cultured C,* Aorta cultured without exposure to elastase for 14 days. *Elastase,* Aorta exposed to elastase for 24 hours and then cultured in standard conditions for a further 13 days. *Doxy 1 mg/l* and *Doxy 10 mg/l,* Aortas exposed to elastase for 24 hours and then cultured with doxycycline for 13 days. *Positive C,* HT 1080 fibrosarcoma cell line, which produces large quantities of MMP-2 and MMP-9.

ized trial,[33] the necessity for alternative strategies has suggested that potential therapeutic agents should be specifically targeted to pathophysiologic processes within the aneurysm wall.

Many of the features observed during aneurysm development and expansion may be related to an overproduction of MMPs within the arterial wall. Particularly important in this respect are the gelatinolytic enzymes MMP-2 and MMP-9. Recently, Freestone et al.[34] investigated the enzyme profile in aneurysms of differing diameters and demonstrated that the elastolytic MMP-2 was the dominant MMP in small aneurysms, whereas MMP-9 was preferentially elevated in larger vessels. This investigation suggested that MMP-2 might play a role in aneurysm genesis, whereas MMP-9 may be more important in expansion of larger aneurysms. Sakalihasan et al.[35] confirmed these observations by describing high levels of activated MMP-9 in aneurysmal tissue, whereas Newman et al.[36] have previously localized MMP-9 activity to the mononuclear infiltrate in the aneurysm wall.

An etiologic role in aneurysm genesis for the elastolytic MMPs, and MMP-2 in particular, has been suggested by two contemporary studies that demonstrated that smooth muscle cells cultured from the abdominal aneurysm wall expressed higher levels of MMP-2 than cells from control tissue culture.[37,38] These results suggested that a primary elastolytic insult from high levels of MMP-2 may initiate aneurysm formation and growth. Clearly, these findings require extensive further investigation into the control of MMP-2 production, as traditionally MMP-2 is considered as a relatively nonresponsive gene with different promoters to the majority of the MMP family.[14]

Theoretically, elastin degradation will release elastin-derived peptides, which further stimulate MMP production and induce leukocyte infiltration into the tissue.[12,39] These changes may then initiate a cascade leading to elastin degradation, leukocyte infiltration, and aneurysm formation. Recent in vitro investigations from our department have confirmed that an initial wave of elastin degradation may induce aneurysmal type changes in arterial tissue,[23,40] and these sequelae have also been observed in the elastase-infusion animal model of aneurysmal disease.[41-43] Evidence from the above studies has suggested that the elastolytic MMP-2 and MMP-9 may be intimately involved in aneurysm pathogenesis and growth, and these enzymes provide an attractive target for pharmacologic agents aimed at reducing small aneurysm expansion and rupture.

One agent that may fulfill the criteria for use in small aneurysms is doxycycline. Doxycycline belongs to the family of tetracyclines and chemically modified tetracyclines. These groups of antibiotics have proven long-term safety and efficacy in the treatment of acne vulgaris. The drugs are frequently used in relatively low doses for many months or years and have good side-effect profiles.[44-46] Doxycycline nonselectively inhibits MMPs by binding to the active zinc sites[47] and also by binding to an inactive calcium site, which causes conformational change[48] and loss of enzymatic activity. Secondary mechanisms of inhibition have also been proposed, which include a reduction in activation,[49] decreased gene expression,[50] and stabilization of specific and nonspecific inhibition.[51]

The present study has shown that doxycycline significantly reduces elastin degradation and MMP-9 activity in an in vitro model of aneurysmal disease and also has an insignificant effect to reduce the production of MMP-2. The findings from our in vitro study are similar to those from Petrinec et al.,[50] who investigated the therapeutic potential of doxycycline in the elastase infusion rodent model. The authors observed preservation of aortic medial elastin with doxycycline administration and suggested that this was as a result of reduced MMP-9 production by the infiltrating inflammatory cells. We have not looked specifically at the role of leukocyte infiltration in the present study, although it would be possible to incorporate a macrophage-rich infiltrate into this model.[23]

Doxycycline provides a potentially attractive pharmacotherapeutic agent for the long-term treatment of patients who have small AAAs, with the aim of inhibiting or reducing aneurysm growth rate. The doses of doxycycline used in the present study are achievable with oral administration. A loading dose of 200 mg followed by 100 mg/day gives a serum level of 3.5 mg/L, with a tissue/serum concentration of greater than one.[52] The therapeutic potential of this family of drugs in aneurysmal disease deserves further consideration.

We thank Pfizer Limited (Sandwich, Kent, U.K.) for kindly donating the doxycycline used in this project, and Dawkins International Ltd. for provision of tissue samples.

REFERENCES

1. Fowkes FGR, MacIntyre CCA, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. BMJ 1989;298:33-5.
2. Johnston KW, Scobie TK. Multicenter prospective study of nonruptured abdominal aortic aneurysms. I. Population and operative management. J Vasc Surg 1988;7:69-81.
3. Johnston KW, Canadian Society for Vascular Surgery Aneurysm Study Group. Nonruptured abdominal aortic aneurysm: six-year follow-up results from the multicenter prospective Canadian aneurysm study. J Vasc Surg 1994;20:163-70.
4. Powell JT, Greenhalgh RM, Ruckley CV, Fowkes FG. Prologue to a surgical trial. Lancet 1993;342:1473-4.
5. Thompson MM, Bell PRF. Aortic aneurysm update. In: Johnson CD, Taylor I, editors. Recent advances in surgery. 19th ed. New York: Churchill Livingstone, 1996:19-41.
6. Cronenwett JL, Sargent SK, Wall MH, Hawkes ML, Freeman DH, Dain BJ, et al. Variables that affect the expansion rate and outcome of small abdominal aortic aneurysms. J Vasc Surg 1990;11:260-9.
7. Wolf YG, Bernstein EF. A current perspective on the natural history of abdominal aortic aneurysms. Cardiovasc Surg 1994;2:16-22.
8. Cronenwett JL. Variables that affect the expansion rate and rupture of abdominal aortic aneurysms. Ann N Y Acad Sci 1996;800:56-67.
9. Collin J. The Oxford screening program for aortic aneurysm and screening first order male siblings of probands with abdominal aortic aneurysm. Ann N Y Acad Sci 1996;800:36-43.
10. Cronenwett JL, Murphy TF, Zelenock GB. Actuarial analysis of variables associated with rupture of small abdominal aortic aneurysms. Surgery 1985;98:472-83.
11. Katz DA, Littenberg B, Cronenwett JL. Management of small abdominal aortic aneurysms: early surgery vs watchful waiting. JAMA 1992;268:2678-86.
12. Cohen JR, Sarfati I, Danna D, Wise L. Smooth muscle cell elastase, atherosclerosis, and abdominal aortic aneurysms [discussion]. Ann Surg 1992;216:327-30.
13. Newman KM, Malon AM, Shin RD, Scholes JV, Ramey WG, Tilson MD. Matrix metalloproteinases in abdominal aortic aneurysm: characterization, purification, and their possible sources. Connect Tissue Res 1994;30:265-76.
14. Matrisian LM. The matrix degrading metalloproteinases. Bioessays 1992;14:455-63.
15. Vine N, Powell JT. Metalloproteinases in degenerative aortic disease. Clin Sci (Colch) 1991;81:233-9.
16. Newman K, Ogata Y, Malon A, Irizarry E, Gandhi R, Nagase H, et al. Identification of matrix metalloproteinase-3 (stromelysin-1) and 9 (gelatinase-B) in abdominal aortic aneurysms. Arterioscler Thromb 1994;14:1315-20.
17. Newman K, Malon A, Shin R, Scholes J, Ramey W, Tilson M. Matrix metalloproteinases in abdominal aortic aneurysm: characterisation, purification, and their possible sources. Connect Tissue Res 1994;30:265-76.
18. Brophy CM, Sumpio B, Reilly JM, Tilson MD. Electrophoretic characterisation of protease expression in aneurysmal aorta: report of a unique 80kDa elastolytic activity. Surg Res Comm 1991;10:315-21.
19. Senior R, Griffin G, Fliszar C, Shapiro S, Goldberg G, Welgus H. Human 92- and 72-kilodalton type IV collagenases are elastases. J Biol Chem 1991;266:7870-5.
20. Irizarry E, Newman KM, Gandhi RH, Nackman GB, Halpern V, Wishner S, et al. Demonstration of interstitial collagenase in abdominal aortic aneurysm disease. J Surg Res 1993;54:571-4.
21. Okada Y, Nagase H, Harris E. A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. J Biol Chem 1986;261:14245-55.
22. Woessner J. Matrix metalloproteinases and their inhibitors in connective tissue remodelling. FASEB J 1991;5:2145-54.
23. Wills A, Thompson MM, Crowther M, Brindle NP, Nasim A, Sayers RD, Bell PRF. Elastase-induced matrix degradation in arterial organ cultures: an in vitro model of aneurysmal disease. J Vasc Surg 1996;24:667-79.
24. Koo E, Gotlieb A. Neointimal formation in the porcine aortic organ culture. Lab Invest 1991;64:743-53.
25. Bancroft JD, Cook HC. Manual of histologic techniques. Edinburgh: Churchill Livingstone, 1984:39-62.
26. He CM, Roach MR. The composition and mechanical properties of abdominal aortic aneurysms. J Vasc Surg 1994;20:6-13.
27. Weibel ER. Stereological methods, volume 1. Practical methods for biological morphometry. New York: Academic Press, 1979:101-61.
28. Brophy C, Tilson JE, Tilson MD. Propranolol delays the for-

mation of aneurysms in the male blotchy mouse. J Surg Res 1988;44:687-9.
29. Slaiby JM, Ricci MA, Gadowski GR, Hendley ED, Pilcher DB. Expansion of aortic aneurysms is reduced by propranolol in a hypertensive rat model. J Vasc Surg 1994;20:178-83.
30. Leach S, Toole A, Stern A, DeNatale R, Tilson M. Effect of beta-adrenergic blockade on the growth rate of abdominal aortic aneurysms. Arch Surg 1988;123:606-9.
31. Gadowski GR, Pilcher DB, Ricci MA. Abdominal aortic aneurysm expansion rate: effect of size and beta-adrenergic blockade. J Vasc Surg 1994;19:727-31.
32. Brown L. Does β-adrenergic blockade limit the growth rate of abdominal aortic aneurysm [abstract]? Br J Surg 1996;83:566-7.
33. Ricci MA, Pilcher DB, McBride R. Design of a trial to evaluate the effect of propranolol upon abdominal aortic aneurysm expansion. Ann N Y Acad Sci 1996;800:252-3.
34. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1995;15:1145-51.
35. Sakalihasan N, Delvenne P, Nusgens BV, Limet R, Lapière CM. Activated forms of MMP_2 and MMP_9 in abdominal aortic aneurysms. J Vasc Surg 1996;24:127-33.
36. Newman KM, Jean-Claude J, Li H, Scholes JV, Ogata Y, Nagase H, Tilson MD. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg 1994;20:814-20.
37. Crowther M, Brindle NPJ, Sayers RD, Bell PRF, Thompson MM. Aneurysmal smooth muscle cells exhibit increased matrix metalloproteinase-2 production in vitro. Ann N Y Acad Sci 1996;800:42-3.
38. Patel MI, Melrose J, Ghosh P, Appelberg M. Increased synthesis of matrix metalloproteinases by aortic smooth muscle cells is implicated in the etiopathogenesis of abdominal aortic aneurysm. J Vasc Surg 1996;24:82-92.
39. Cohen JR, Mandell C, Margolis I. Altered aortic protease and antiprotease activity in patients with ruptured abdominal aortic aneurysms. Surg Gynecol Obstet 1987;164:355-8.
40. Thompson MM, Wills A, McDermott E, Crowther M, Brindle N, Bell PRF. An in vitro model of aneurysmal disease: effect of leukocyte infiltration and shear stress on MMP production within the arterial wall. Ann N Y Acad Sci 1996;800:270-3.
41. Anidjar S, Dobrin PB, Eichorst M, Graham GP, Chejfec G. Correlation of inflammatory infiltrate with the enlargement of experimental aortic aneurysms. J Vasc Surg 1992;16:139-47.
42. Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri JP, Michel JB. Elastase-induced experimental aneurysms in rats. Circulation 1990;82:973-81.
43. Dobrin PB, Baker WH, Gley WC. Elastolytic and collagenolytic studies of arteries. Arch Surg 1984;119:405-9.
44. Sauer GC. Safety of long term tetracycline therapy for acne. Arch Dermatol 1976;112:1603-5.
45. Harrison PV. A comparison of doxycycline and minocycline in the treatment of acne vulgaris. Clin Exp Dermatol 1988;13:242-4.
46. Layton AM. Phototoxic eruptions due to doxycycline: a dose-related phenomenon. Clin Exp Dermatol 1993;18:425-7.
47. Sorsa T, Ding Y, Salo T, Lauhio A, Teronen O, Ingham T, et al. Effects of tetracyclines on neutrophil, gingival and salivary collagenases: a functional and Western blot assessment with specific reference to their cellular sources in periodontal disease. Ann N Y Acad Sci 1994;732:112-31.
48. Lovejoy B, Clearsby A, Hassell AM, Luther MA, Weigl D, McGeehan G, et al. Structural analysis of the catalytic domain of human fibroblast collagenase. Ann N Y Acad Sci 1994;732:375-8.
49. Ramamurthy NS, Vernillo AT, Greenwald RA, Lee HM, Sorsa T, Golub LM, et al. Reactive oxygen species activate and tetracyclines inhibit rat osteoblast collagenase. J Bone Miner Res 1993;8:1247-53.
50. Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, Thompson RW. Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kD gelatinase. J Vasc Surg 1996;23:336-46.
51. Golub LM, Evans RT, McNamara TF, Lee HM. A nonantimicrobial tetracycline inhibits gingival matrix metalloproteinases and bone loss in porphyromonas gingivalis-induced periodontitis in rats. Ann N Y Acad Sci 1994;732:96-111.
52. Steigbigel NH, Reed CW, Finland M. Absorption and excretion of five tetracycline analogues in normal young men. Am J Med Sci 1968;255:296-312.

Submitted June 3, 1997; accepted Sep. 10, 1997.