Basic Science Research

The Nitric Oxide Donor DETA-NONOate Decreases Matrix Metalloproteinase-9 Expression and Activity in Rat Aortic Smooth Muscle and Abdominal Aortic Explants

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Our objective was to examine the role of an exogenous nitric oxide (NO) donor, DETA-NONOate (DETA), on matrix metalloproteinase (MMP)-9, MMP-2, and tissue inhibitor of matrix metalloproteinases (TIMP)-1 expression and activity in interleukin (IL)-1β-induced rat aortic smooth muscle cells (RA-SMCs) and rat aortic explants (RAEs). RA-SMCs were incubated with IL-1β (2 ng/ml), an inflammatory cytokine known to induce MMP-9 expression, and increasing concentrations of DETA (0, 1.0, 10, 100 μM; n = 3/group) for 48 hr. RAEs were incubated with IL-1β (2 ng/mL) and increasing concentrations of DETA (0, 5.0, 50, 100, and 500 μM; n = 3/group) for 48 hr. Media were collected and assayed for NO_x by the Griess reaction and MMP-9 activity by zymography. Messenger RNA (mRNA) was extracted from cells and analyzed for MMP-9, MMP-2, and TIMP-1 expression levels by quantitative real-time reverse-transcriptase polymerase chain reaction. All statistical analyses were performed by analysis of variance. In RA-SMCs and RAEs, DETA administration resulted in a dose-dependent increase in media NO_x concentration (RA-SCM $p < 0.01$, RAE $p < 0.01$) and a concurrent decrease in both MMP-9 expression (RASMC $p = 0.01$, RAE $p = 0.01$) and activity (RASMC $p = 0.04$, RAE $p = 0.006$). There were no significant differences seen in MMP-2 and TIMP-1 expression or activity in response to DETA exposure. DETA decreased IL-1β-induced MMP-9 expression and activity in both RA-SMCs and RAEs in a dose-dependent fashion. In addition, DETA administration had no effect on MMP-2 or TIMP-1 expression or activity in vitro. These data suggest that NO donors may be beneficial in decreasing MMP-9 levels and might serve to inhibit MMP-9-dependent vessel wall remodeling seen during abdominal aortic aneurysm formation.

INTRODUCTION

Nitric oxide (NO) is known to mediate many physiological processes in vascular tissue. NO inhibits vascular smooth muscle cell proliferation and migration, neointima formation after vessel injury, and extracellular matrix turnover.[1-5] Collectively, these events support the tenet that NO is a major mediator of vessel wall remodeling.[6] However, the interaction between NO and matrix metalloproteinases (MMPs), a family of endopeptidases involved in the degradation of extracellular matrix proteins, remains poorly understood. This interaction may be important given observations

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suggesting the central importance of MMPs in the development of abdominal aortic aneurysms (AAAs).[7-17] MMP-9, a 92 kDa gelatinase, has been shown to be critical in the destructive process that accompanies aneurysm development, as evidenced by the inhibition of aneurysm formation following elastase perfusion in mice with a targeted disruption of the MMP-9 gene.[14,18] Levels of MMP-2 and tissue inhibitor of metalloprotienases-1 (TIMP-1) also may contribute to aneurysm pathogenesis. A balance between MMPs and TIMP may in fact control the pathological remodeling involved in aneurysm formation.[19]

Previous studies have demonstrated that increasing concentrations of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) increases MMP-9 expression and activity in a dose-dependent manner in rat aortic smooth muscle cells (RA-SMCs) and rat aortic explants (RAEs).[1,6] Furthermore, studies have shown that mice lacking the inducible NO synthase (iNOS) gene appear to develop experimental aortic aneurysms in an accelerated fashion.[20] Based on these data, we hypothesized that increased NO levels would conversely serve to downregulate MMP-9. The objective of this study was to elucidate the ability of the NO donor DETA-NONOate (DETA) to reduce MMP-9 expression and activity in rat aortic tissue and smooth muscle cells.

MATERIALS AND METHODS

Cell Culture

All experiments were performed with the approval of the University of Michigan Committee on Laboratory Animal Medicine. RA-SMCs were derived from the abdominal aortas of young, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) by an explant method previously described.[1] Briefly, medial tissue was cut into 2 mm^2 pieces and plated in tissue culture dishes. Culture media included Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), 100 units/mL penicillin, and 100 μg/mL streptomycin. Media and antibiotics were from GIBCO (Rockville, MD). Cultures were maintained at 37°C in a humidified, 5% CO_2 atmosphere for 4-7 days. After removing the explant, the remaining cells were dispersed by treatment with Dispase (Collaborative Research, Bedford, MA), centrifuged, and resuspended in complete medium, which was placed into 75 cm^2 culture flasks. Reagents were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Experimental conditions were carried out with confluent RA-SMCs in serum-free medium supplemented with antibiotics. Confluent monolayers of RA-SMCs were costimulated in T-75 plates with interleukin (IL)-1β, an inflammatory cytokine known to induce MMP expression, at a physiological concentration of 2 ng/mL and increasing concentrations DETA (0, 1, 10, 100 μM; $n = 3$/group). After 24 hr incubation, RA-SMCs were lysed using TRIzol (Life Technologies, Rockville, MD) and total cellular RNA was collected and purified to messenger RNA (mRNA) to quantify MMP-9, MMP-2, iNOS, and TIMP-1 expression. After 48 hr incubation, RA-SMC conditioned medium was collected for measurements of NO_x, MMP, and TIMP activity. The remaining RA-SMCs were solubilized in 0.1% sodium dodecyl sulfate (SDS), and total cellular protein was determined by a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL).

Trypan blue dye exclusion assay was used to measure cell viability. Confluent RA-SMCs were removed from the culture flask by trypsinization and centrifuged for 5 min at 1,100 rpm. The supernatant was discarded and the pellet resuspended in cold phosphate-buffered saline (PBS). Subsequently, 100 μl of suspension was transferred to a 1.5 mL Eppendorf tube, and 100 μL of 0.4% trypan blue (Sigma) was added to the suspension. Next, 10 μL of this was transferred to each side of a hemocytometer slide. Total numbers of cells and of stained cells were determined. Viable cells were noted as unstained cells.

Aortic Tissue Explants

Sprague-Dawley rats, weighing 300-350 g, were anesthetized by inhalation of isoflurane (1-2%). Through a midline abdominal incision, the infrarenal aorta was exposed and the animals were killed. Aortic tissue was excised, and explants were supported by conventional means in tissue culture. Each specimen was washed with PBS in a tissue culture hood. Aortas were bisected longitudinally to remove clotted blood from the lumen. Segments were placed into separate wells of a 24-well tissue culture plate. Aortic tissue was maintained in DMEM with 100 units/mL penicillin and 100 μg/mL streptomycin. All reagents were purchased from Sigma, unless otherwise indicated. Tissue culture media and antibiotics were obtained from GIBCO. Tissues were incubated at 37°C in humidified, 5% CO_2 atmosphere. After 24 hr, the tissue was washed with PBS, and subsequently the medium for the experimental condition was added.

Experimental conditions were carried out in serum-free media with 2 ng/mL IL-1β and increasing concentrations of DETA (0, 5, 50, 100, and 500 μm; *n* = 3/group). After 72 hr incubation, aortas were lysed using TRIzol and total cellular RNA was collected and purifed to mRNA to quantify MMP, iNOS, and TIMP-1 expression (*n* = 3). The experimental medium was collected for measurements of NO_x, MMP, and TIMP activity. For aortas in which NO_x content and MMP activity assays were done, aortas were solubilized in 0.1% SDS and total cellular protein was determined by BCA protein assay.

Measurement of MMP and TIMP expression

mRNA was isolated by treatment of RA-SMCs and RAEs with TRIzol reagent and reverse-transcribed by incubating with oligo-(dT) primer (Invitrogen Life Technologies, Grand Island, NY) and Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) at 94°C for 3 min followed by 40°C for 70 min. The resultant complementary DNA (cDNA) was amplified by Taq polymerase (Promega, Madison, WI) in the SmartCycler quantitative polymerase chain reaction (PCR) system (Cepheid, Sunnyvale, CA). SYBR Intercalating Dye (Roche, Indianapolis, IN) was used to monitor levels of cDNA amplification of each gene. SmartCycler quantitation will be presented as cycle threshold (C_t). MMP-2, MMP-9, TIMP-1, and β-actin primer sequences were derived either from published literature sources or using Primer Premier Software (Premier Biosoft International, Palo Alto, CA) based on primary mRNA sequences from GenBank. The sequences for primers are as follows: β-actin sense 5′-ATGGGTCAGAAGGATTCCTATGTG-3′, β-actin antisense 5′-CTTCATGAGGTAGTCAGTCAGGTC-3′, MMP-9 sense 5′-TCTCAAGGAGGTCGGTAT-3′, MMP-9 antisense 5′-TCGGGGCAATAAGAAAGG-3′, MMP-2 sense 5′-CATCGCTGCACCATCGCC-CATCATC-3′, MMP-2 antisense 5′-CCCAGGGTC-CACAGCTCATCATCATCA-3′, TIMP-1 sense 5′-AATGCCACAGGTTTCCGGTTC-3′, TIMP-1 antisense 5′-ACACCCCACAGCCAGCACTAT-3′.

Results were normalized using the housekeeping gene β-actin. For quantification of mRNA levels, ΔC_t values were calculated by the following formula: $\Delta C_t = C_{t\ \text{target gene}} - C_{t\ \beta\text{-actin}}$. Expression of the target gene in ratio to β-actin expression was calculated by the following formula: target gene/β-actin = $2^{-(\Delta Ct)}$. PCRs were repeated and analyzed in triplicate for each of the experimental groups.

Substrate Gel Zymography and Reverse Zymography

MMP distribution of treated RA-SMCs or treated RAEs was determined by zymography using reagents from Novex (San Diego, CA) and the experimental medium. Gelatin substrate zymograms were prepared using precast 10% SDS-polyacrylamide gels containing 1 mg/mL of gelatin. Experimental samples of equal volume were diluted into 2 × Tris-glycine SDS sample buffer and separated electrophoretically under nonreducing conditions. Proteins were renatured in 2.7% Triton X-100 and the gels incubated for varying time periods in order to best quantify the desired MMP band at 37°C in 50 mM Tris-HCl containing 5 mM $CaCl_2$ and 0.2% Brij 35. Following staining with Coomassie blue R-250 and destaining with 10% acetic acid, gelatinase activity was evident as a clear band against a dark blue background. RA-SMCs demonstrated pro-MMP-9 activity, whereas RAEs produced both pro-MMP-9 and active MMP-9.

Reverse zymography was performed to measure TIMP-1 activity. Zymogram substrate was prepared using 2 mg/mL porcine gelatin, 0.25 M Tris-HCl (pH 8.8), 0.125% SDS, 0.5 μL/mL TEMED, 0.4 mg/mL ammonium persulfate, 15% (w/v) acrylamide, 0.4% bisacrylamide, and 200 ng/mL of progelatinase A (Oncogene, Boston, MA). A standard 4% polyacrylamide stacking gel was used. Experimental samples of equal volumes were diluted into 2 × Tris-glycine SDS sample buffer and separated electrophoretically under nonreducing conditions. Proteins were renatured in three changes of 2.7% Triton X-100 for 60 min each. Gels were incubated for 17 hr at 37°C in 50 mM Tris-HCl, 5 mM $CaCl_2$, and 0.2% Brij 35. Gelatinase inhibitory activity was noted as a blue band on a clear background following staining with Coomassie blue R-250 and destaining with 10% acetic acid.

The relative molecular weight of each band was determined by comparison of the bands against MMP-2 (72 kDa), MMP-9 (92 kDa), and TIMP-1 (29 kDa) standards (Oncogene). Semiquantitative measurements of MMP activity were performed by densitometry and normalized to total cellular protein. MMP activity was quantified by densitometry. All gel images were acquired using a FOTO/Analyst charge-coupled device camera (Fotodyne, Hartland, WI). Band strength was quantified using GEL-Pro Analyzer software, version 3.1 (Media Cybernetics, Silver Spring, MD).

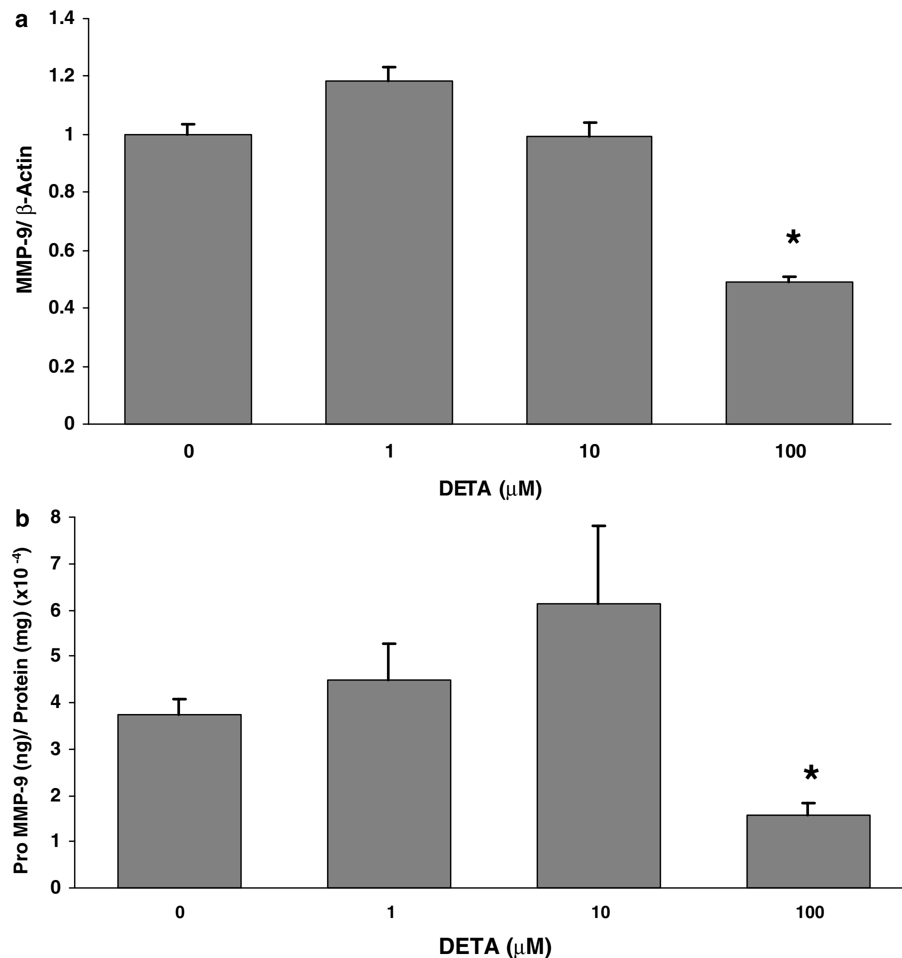

Fig. 1. **a** In RA-SMCs, MMP-9/β-actin mRNA expression by real-time PCR demonstrated a significant dose-dependent decrease in response to increasing doses of DETA (*$p = 0.01$). **b** In RA-SMCs, pro-MMP-9 activity, as measured by zymography, decreases with increasing doses of DETA (*$p = 0.04$).

RESULTS

DETA Administration Increases NO Levels and Decreases MMP-9 Expression and Activity in RA-SMCs

DETA caused an increase in NO_x levels of 72% in the 100 μM DETA group compared to IL-1β alone ($p < 0.01$, figure not shown). DETA alone without costimulation with IL-1β caused no change in MMP-2, MMP-9, or TIMP-1 expression and activity. Conversely, cell cultures costimulated with IL-1β and increasing concentrations of DETA demonstrated a decrease in MMP expression of 49% in the 100 μM DETA-treated group in comparison to IL-1β alone ($p = 0.01$, Fig. 1a). There was a concurrent 58% decrease in pro-MMP-9 activity in the 100 μM DETA group in comparison to IL-1β alone as measured by gel substrate zymography ($p = 0.04$, Fig. 1b). There were no significant differences in either MMP-2 or TIMP-1 expression or activity (data not shown). No statistically significant differences in cell viability were noted among any of the treatment groups.

DETA Administration Increases NO and Decreases MMP-9 Expression and Activity in RAEs

NO_x levels increased in a dose-dependent manner in relation to DETA exposure. In RAEs treated with 500 μM DETA, the NO_x concentration increased 502% compared to IL-1β alone ($p < 0.01$, figure not shown). MMP-9 expression demonstrated a dose-dependent decrease ($p = 0.01$, Fig. 2a), with the 100 μM and 500 μM DETA-treated groups experiencing 70% and 78% decreases, respectively.

Similarly, there was a significant decrease in total pro-MMP-9 activity in both the 100 μM and 500 μM DETA-treated groups (69% and 72%, respectively, compared to the control group). Pro-MMP-9 activity decreased in a dose-dependent relationship to exogenous NO administration ($p = 0.006$, Fig. 2b). Active-MMP-9 also decreased in response to DETA exposure ($p = 0.015$, Fig. 2c), with no active MMP-9 being detectable in the 500 μM DETA-treated group. There was no significant difference in either MMP-2 or TIMP-1 expression or activity (data not shown).

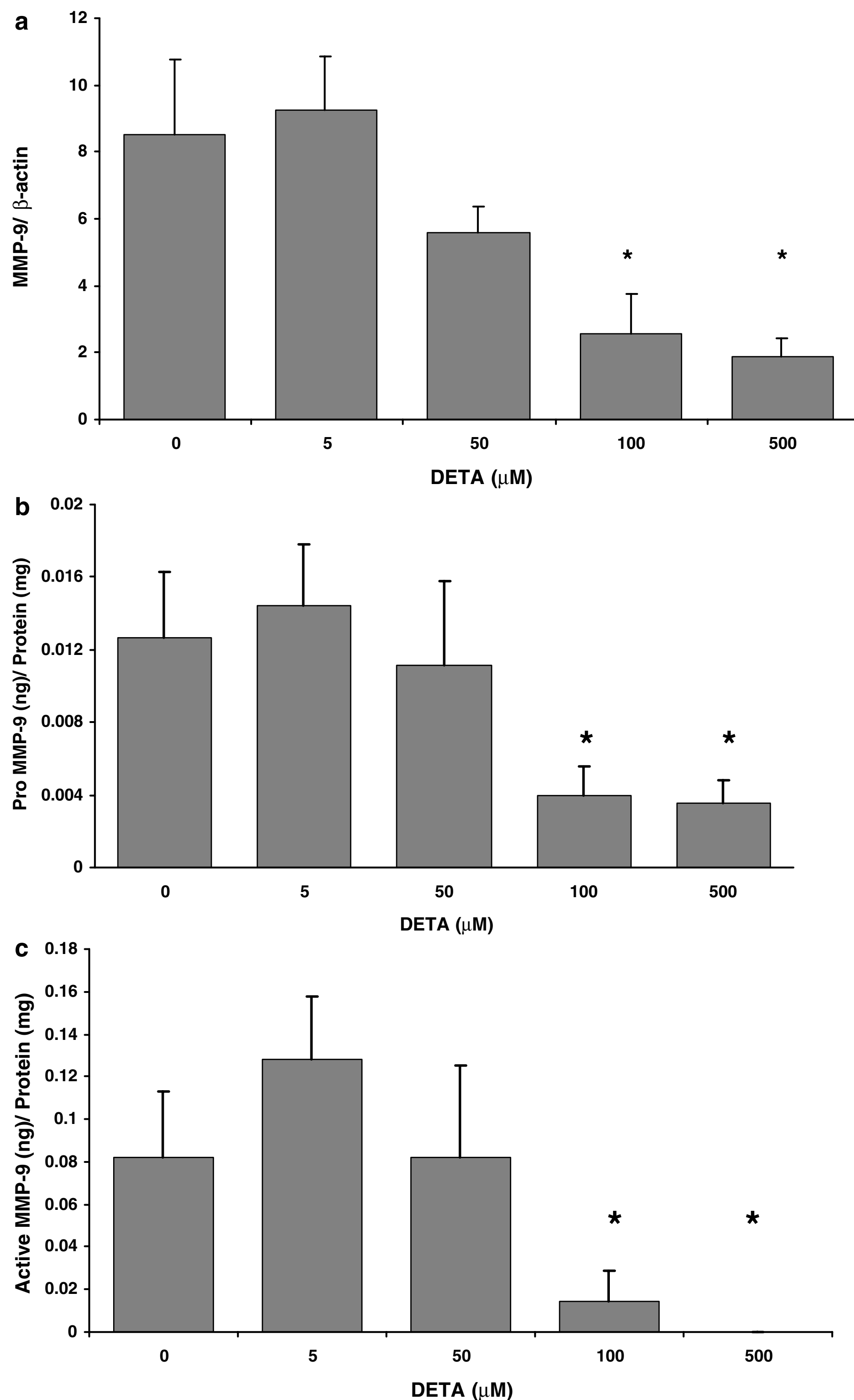

Fig. 2. **a** In RAEs, MMP-9/β-actin mRNA expression by real-time PCR demonstrated a significant dose-dependent decrease in expression in response to increasing doses of DETA (*$p = 0.01$). **b** In RAEs, pro-MMP-9 activity, as measured by zymography, decreases in a dose-dependent manner with increasing doses of DETA (*$p = 0.006$). **c** In RAEs, cleaved or active MMP-9 activity, as measured by zymography, decreases with increasing doses of DETA. Cleaved MMP-9 is not detectable in the 500 μM-treated group (*$p = 0.015$).

DISCUSSION

This study demonstrates that exposure to exogenous NO, using the NO donor DETA, decreases both MMP-9 expression and activity in a dose-dependent manner in both RA-SMCs and RAEs. Importantly, expression and activity for MMP-2 and TIMP-1 did not change in response to increasing DETA exposure. These data suggest that MMP-9 in RA-SMCs and RAEs is selectively inhibited by increased local bioavailable NO in the presence of physiological IL-1β levels.[21]

AAAs are characterized by structural alterations of the aortic wall resulting, in part, from the degradation of collagen and elastin.[18] An inflammatory infiltrate and overexpression of MMPs are important factors involved in the pathological vascular remodeling accompanying AAA development.[6,16,18] MMP-9 has been shown to be especially critical in this process.[16,17] Previous in vitro studies have suggested that NO, a ubiquitous free radical, modulates MMP-9 expression and its corresponding physiological effects. In this study, addition of 100 μM and 500 μM DETA resulted in supraphysiological NO levels of twofold and sixfold, respectively, in comparison to IL-1β alone. It has also been shown that endothelial NOS gene transfer into RA-SMCs inhibits both MMP-2 and MMP-9 activity and limits SMC migration.[22] Knipp et al.[23] demonstrated that selective iNOS inhibition in IL-1β-induced rat SMCs decreases MMP-9 expression through a nuclear factor κB (NF-κB)-dependent mechanism.

Studies in human tissue further support an associative role for NO in the formation of AAAs. Paik and Tilson[24] were the first to suggest a role for NO in AAAs. Knipp and colleagues[25] demonstrated that patients with AAAs, compared to well-matched patients with peripheral vascular occlusive disease and normal controls, sustain decreased endothelium- and non-endothelium-dependent relaxation in areas remote from the AAA (i.e., brachial artery). This occurred paradoxically in the setting of increased circulating levels of NO. Troxler and others[26] documented increased circulating levels of nitrotyrosine in patients undergoing AAA repair. Importantly, following AAA repair, circulating nitrotyrosine returned to control levels, suggesting that the aneurysm served as the source of increased NO. Finally, data from other vascular beds (e.g., cerebral) have also verified altered NOS in association with an increased risk of human aneurysms.[27,28]

Attempting to sort out the exact role of NO using experimental aneurysm models has been both confusing and problematic. A study using DNA array technology in the elastase- perfused rodent aortic aneurysm model clearly documented a role for iNOS by demonstrating that this gene was upregulated compared to controls 52- and 5.2-fold on postoperative days 2 and 7, respectively.[29] Lee and others[20] extended this observation by documenting accelerated aneurysm formation in $iNOS^{-/-}$ mice but only in females. In contrast, other in vivo studies in the rodent elastase model using both nonselective[30] and selective[31] iNOS inhibitors demonstrated that iNOS inhibition limits aortic expansion. This decrease in AAA size occurred in the setting of documented increases in arterial blood pressure (i.e., decreases in NO concentrations).

While the present in vitro and ex vivo results are the first to document that increased NO decreases MMP-9 expression and activity, the exact relationship between NO and MMP-9 remains complicated. More experiments are needed to determine the exact mechanism(s) responsible for the paradoxical effects of NO on MMP-9. Earlier experiments from our laboratory suggest a role for NF-κB and activator protein-1.[23] Another study demonstrated that NO may inhibit MMP-9 expression and activity via inhibition of the extracellular signal-regulated kinase (ERK) pathway in RA-SMCs.[32] However, further studies are necessary to fully elucidate the interaction between NO and MMP-9. For example, it will be important to confirm if NO acts directly on MMP-9 or if it works through the increased generation of reactive oxygen species, such as peroxynitrite. Finally, delivery of NO to in vivo tissue is very problematic for a number of reasons, including NO's extremely short half-life. Attempts (data not shown) were made to translate the current in vitro and ex vivo findings using NO donors to the rodent aortic elastase perfusion model. Unfortunately, despite using multiple dosing schedules, multiple concentrations of the drug, and various delivery methods (intra-arterial and intraperitoneal), we were unable to reliably inhibit AAA formation in the rodent aneurysm model. Given these difficulties in limiting aneurysmal growth in a rodent model, it is uncertain whether NO and MMP have a clinically significant interaction in human AAA development. Despite our inability to document an in vivo effect on AAA formation in the rodent, understanding the interactions between complex enzymes such as the MMPs and NO synthases involved in AAA pathology may help to promote pharmacological treatments for limiting AAA growth.

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REFERENCES

1. Eagleton MJ, Peterson DA, Sullivan MD, et al. Nitric oxide inhibition increases aortic wall matrix metalloproteinase-9 expression. J Surg Res 2002;104:15.

2. Numagachi K, Egashira K, Takemoto M, et al. Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. Hypertension 1995;26:957.
3. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium dependant. Science 1986;231:405.
4. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa NC. Direct evidence for the importance of endothelium derived nitric oxide in vascular remodeling. J Clin Invest 1998;101:731.
5. Tronc F, Wassef M, Esposito B, Henrion D, Glagsov S, Tedqui A. Role of NO in flow-induced remodeling of the rabbit common carotid artery. Arterioscler Thromb Vasc Biol 1996;16:1256.
6. Upchurch GR, Ford JW, Weiss SJ, et al. Nitric oxide inhibition increases metalloprotienase-9 expression by rat aortic smooth muscle cells in vitro. J Vasc Surg 2001;34:76.
7. Curci JA, Liao S, Huffman MD, Shapiro SD, Thompson RW. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. J Clin Invest 1998;102:1900.
8. Sinha I, Hannawa KK, Eliason JL, et al. Early MT-1 MMP expression following elastase exposure is associated with increased cleaved MMP-2 activity in experimental rodent aortic aneurysms. Surgery 2004;136:176-182.
9. 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.
10. Herron GS, Unemori E, Wong M, Rapp JH, Hibbs SM, Stony RJ. Connective tissue proteinases and inhibitors in abdominal aortic aneurysms: involvement of the vaso vasorum in the pathogenesis of aortic aneurysms. Arterioscler Thromb Vasc Biol 1991;11:1667.
11. McMillan WD, Patterson BK, Keen RR, Pearce WH. In situ localization and quantification of 72 kD type IV collagenase in aneurysmal, occlusive, and normal aorta. J Vasc Surg 1995;22:295.
12. McMillan WD, Patterson BK, Keen RR, Shively VP, Cipollone M, Pearce WH. In situ localization and quantification of mRNA for 92 kD type IV collagenase and its inhibitor in aneurysmal, occlusive, and normal aorta. Arterioscler Thromb Vasc Biol 1995;15:1139.
13. Newman KM, Ogata Y, Malon AM, et al. Identification of metalloproteinase-3 (stromelysin-1) and -9 (gelatinase B) in abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1994;14:1315.
14. Pyo R, Lee JK, Shipley M, et al. Targeted gene disruption of matrix metalloprotienase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest 2000;105:1641.
15. Tamarina NA, McMillan WD, Shively VP, Pearce WH. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. Surgery 1997;122. 264.
16. Thompson RW, Holmes DR, Mertens RA, et al. Production and localization of 92 kD gelatinase in abdominal aortic aneurysms: an elastolytic metalloproteinase expressed by aneurysm infiltrating macrophages. J Clin Invest 1995;96. 318.
17. Yamashita A, Noma T, Nakazawa A, et al. Enhanced expression of metalloproteinase-9 in abdominal aortic aneurysms. World J Surg 2001;25:259.
18. Longo MG, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter TB. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625.
19. Bendeck MP. Matrix metalloproteinases: are they antiatherogenic but proaneurysmal? Circ Res 2002;90:836.
20. Lee JK, Borhani M, Ennis TL, Upchurch GR, Thompson RW. Experimental abdominal aortic aneurysms in mice lacking expression of inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 2001;21:1393.
21. Davis VA, Persidskaia RN, Baca-Regen LM, Fiotti N, Halloran BG, Baxter BT. Cytokine pattern in aneurysmal and occlusive disease of the aorta. J Surg Res 2001;101:152.
22. Gurjar MV, Sharma RV, Bhalla RC. eNOS gene transfer inhibits smooth muscle cell migration and MMP-2 and MMP-9 activity. Arterioscler Thromb Vasc Biol 1999;19:2871.
23. Knipp BS, Ailawadi G, Ford JW, et al. Increased MMP-9 expression and activity by aortic smooth muscle cells after nitric oxide synthase inhibition is associated with increased nuclear factor-kappaB and activator protein-1 activity. J Surg Res 2004;116:70.
24. Paik D, Tilson MD. Neovascularization in the abdominal aortic aneurysm. Endothelial nitric oxide synthase, nitric oxide and elastolysis. Ann N Y Acad Sci 1996;800:277.
25. Knipp B, Peterson DA, Rajagopalan S, et al. Impaired vasoreactivity despite an increase in plasma nitrite in patients with abdominal aortic aneurysms. J Vasc Surg 2002;35:363.
26. Troxler M, Naseem KM, Homer-Vanniasinkam S. Increased nitrotyrosine production in patients undergoing abdominal aortic aneurysm repair. Br J Surg 2004;91:1146.
27. Khurana VG, Sohni YR, Mangrum WI, et al. Endothelial nitric oxide synthase gene polymorphisms predict susceptibility to aneurismal subarachnoid hemorrhage and cerebral vasospasm. J Cereb Blood Flow Metab 2004;24:291.
28. Khurana VG, Sohni YR, Mangrum WI, et al. Endothelial nitric oxide synthase T-786C single nucleotide polymorphism. Stroke 2003;34:2555.
29. Yajima N, Masuda M, Miyazaki M, Nakajima N, Chien S, Shyy J. Oxidative stress is involved in the development of experimental abdominal aortic aneurysm: a study of the transcription profile with complementary DNA microarray. J Vasc Surg 2002;36:279.
30. Johanning JM, Armstrong PJ, Franklin DP, Han DC, Carey DJ, Elmore JR. Nitric oxide in experimental aneurysm formation: early events and consequences of nitric oxide inhibition. Ann Vasc Surg 2002;26:65.
31. Johanning JM, Franklin DP, Han DC, Carey DJ, Elmore JR. Inhibition of inducible nitric oxide synthesis limits nitric oxide production and experimental aneurysm formation. J Vasc Surg 2001;24:579.
32. Gurjar MV, DeLeon J, Sharma RV, Bhalla RC. Mechanism of inhibition of matrix metalloproteinase-9 induction by NO in vascular smooth muscle cells. J Appl Physiol 2001;91:1380-1386.