Role of Matrix Metalloproteinases in Early Hypertensive Vascular Remodeling

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Abstract—Hypertension is associated with vascular remodeling characterized by rearrangement of extracellular matrix proteins. To evaluate how matrix metalloproteinase (MMP)-9 contributes to the progression of hypertensive vascular disease in vivo, wild-type (wt) or MMP-9−/− mice were treated with angiotensin II (Ang II; 1 μg/kg per minute, by minipump) plus a 5% NaCl diet during 10 days. Baseline blood pressure was equivalent in wt and knockout mice, but Ang II treatment increased systolic blood pressure to a greater extent (P<0.05) in MMP-9−/− mice (94±6 to 134±6 mm Hg; P<0.001) than in wt animals (93±4 to 114±6 mm Hg; P<0.01). In wt mice, Ang II treatment increased the carotid artery pressure–diameter relationship significantly, and maximal diameter reached 981±19 μm (P<0.01 versus sham; 891±10 μm). In contrast, in MMP-9−/− mice, carotid artery compliance was actually reduced after Ang II (P<0.05), and maximal diameter only reached 878±13 μm. Ang II treatment induced MMP-2 and increased carotid media thickness equally in both phenotypes. However, MMP-9 induction and in situ gelatinase activity were only enhanced in Ang II-treated wt mice, and vessels from these mice also produced more collagen I breakdown products than their MMP-9−/− counterparts (P<0.05). Inversely, staining for collagen IV was particularly enhanced in vessels from MMP-9−/− mice treated with Ang II. These results demonstrate the following: (1) the onset of Ang II–induced hypertension is accompanied by increased MMP-9 activity in conductance vessels; (2) absence of MMP-9 activity results in vessel stiffness and increased pulse pressure; and (3) MMP-9 activation is associated with a beneficial role early on in hypertension by preserving vessel compliance and alleviating blood pressure increase. (Hypertension. 2007;50:212-218.)

Key Words: angiotensin ■ metalloproteinases ■ remodeling ■ hypertension ■ collagen

Blood pressure is a major determinant of vessel wall structure and composition. Any change in strain will generate counteracting radial and tangential forces that drive transformations in the vessel wall to accommodate the new pressure setting and restore basal levels of tensile stress.1 It therefore follows that a key characteristic of hypertensive conductance arteries is increased wall thickness accompanied by enhanced rigidity. In hypertensive patients, this is manifest by elevated pulse wave velocity, an independent predictor of mortality in patients with end-stage renal failure, hypertension, and diabetes, as well as in older individuals.2 Nevertheless, using an ex vivo model of carotid artery, we have shown recently that early vascular remodeling in the hypertensive context is actually associated with increased conductance vessel distensibility rather than rigidity.3 In fact, results from our own laboratory1 and others4–8 have shown that exposing arteries or vascular cells to stretch induces the release of matrix metalloproteinases (MMPs). This perplexing observation led us to hypothesize that increased compliance may be an early compensatory mechanism allowing vessels to expand in the face of newly elevated pressure and that this may alleviate the initial rise in blood pressure in a prohypertensive hormonal environment.

To further our understanding of the role of early increased distensibility on the hypertensive remodeling response, we used wild-type (wt) mice or mice deficient in MMP-9 (−/−) to investigate how a hypertensive 10-day treatment with angiotensin II (Ang II) affects artery structure and mechanical properties in vivo. We also determined how MMPs influence the rise in blood pressure in this context.

Methods
Alzet osmotic minipumps (model 2004) were filled with saline solution set to deliver 1 μg/kg per minute of Ang II (Sigma) or saline solution alone and placed in 0.9% NaCl solution for 48 hours at 37°C. Male MMP-9−/− mice and wt C57bl/6 mice aged 8 weeks (Charles River) were then anesthetized with an IP injection of 100 μL/100 g Avertin 2.5% and fitted with the minipumps delivering either Ang II or saline solution (sham). Osmotic pumps were
subcutaneously placed in the mice for 10 days. Sham animals received a standard laboratory diet and water ad libitum. Ang II-treated mice were placed on a diet containing 5% NaCl. Experiments were performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and were approved by the local ethics committee.

**Arterial Pressure Measurement**

Mice were anesthetized with an IP injection of pentobarbital (50 mg/kg) and placed on a servocontrolled heating table that maintained body temperature at 37°C. A tracheotomy was performed, and a tracheal catheter (phycocerythin 60) was inserted to facilitate breathing. The right femoral vein was cannulated with a pulled phycocerythin-10 catheter for continuous infusion of BSA (2.5%; 10 μL/min) and isosmotic saline solution (0.9% NaCl; 10 μL/min). This infusion rate was selected to ensure body volume homeostasis. Additional doses of pentobarbital were given intravenously as required. The right femoral artery was cannulated with a tapered phycocerythin-100 catheter connected to a pressure transducer for continuous monitoring of systolic, diastolic, and mean arterial pressures.

**Organ Culture**

Organ culture of the carotid arteries was carried out as described previously. Arterial segments were kept at an intraluminal pressure of 80 mm Hg or 150 mm Hg for 72 hours. Some segments were treated with Ang II (hypertensin; 10^{-7} mol/L; Ciba). At the end of the 3-day period, 2 mL of the culture medium was used to stimulate extracellular-regulated kinase 1/2 activity in smooth muscle cells in culture to confirm that hypertensin remained active.

**Pressure–Diameter Analysis**

Carotid arteries or mesenteric arteries were mounted into a video-monitored perfusion system (Living Systems Instrumentation), as described previously. Pressure was raised by 25-mm increments from 25 to 200 mm Hg, and corresponding diameter changes were measured by video microscopy. To account for hysteresis, 2 cycles of preconditioning were applied before data acquisition. Only passive diameter was measured in carotid arteries, because in these vessels, passive and active diameters overlap, whereas in mesenteric arteries, active diameter was measured first, followed by passive diameter after a change in the Kreb’s solution. Compliance (C) was calculated as: $C=(A_0-A)/\Delta P$, where $A_0$ and $A_1$ are vessel lumen areas before and after pressure increment, and $\Delta P$ is the change in pressure (25 mm Hg). $\beta$ Index was measured as $\beta=ln(Ps/Pd)/(Ds-Dd)/Ds$, where $Ps$ and $Pd$ are the in vivo systolic and diastolic pressures, and $Ds$ and $Dd$ are the carotid artery diameters at the corresponding pressures.

**Tissue Extraction**

Vessel segments were ground in ice-cold lysis buffer containing 20 mmol/L of Tris-HCl (pH 7.5), 5 mmol/L of EGTA, 150 mmol/L of NaCl, 20 mmol/L of glycophosphate, 10 mmol/L of NaF, 1 mmol/L of sodium orthovanadate, 1% Triton X-100, 0.1% Tween 20, and protease inhibitors (Boehringer Mannheim). Detergent-soluble fractions were retained, and protein concentrations were equalized according to the Bradford protein assay (Bio-Rad).

**Zymography**

Zymography using gelatin-containing gels was performed as described previously. Gelatinolytic activity was visualized as clear bands of lysis against a dark background. For in situ zymography, a fluorogenic gelatin substrate (QD gelatin, Molecular Probes) was used as described previously. Proteolytic activity was detected as green fluorescence (530 nm), and results were quantified using Histolab software (Microvision Instruments) and expressed as percentage of surface showing positive fluorescence.
not increase the pressure–diameter relationship in carotid arteries from MMP-9-/- mice but was instead associated with reduced vessel compliance ($P<0.05$). Further assessment of vessel rigidity using $\beta$ index calculation confirmed that, at in vivo pressure values, carotid arteries from Ang II–treated MMP-9-/- mice were significantly ($P<0.05$) stiffer ($\beta=5.08\pm0.39$) than those of untreated MMP-9-/- mice ($\beta=3.88\pm0.20$) or treated and untreated wt mice ($\beta=3.49\pm0.37$ and $3.85\pm0.48$, respectively). In comparison, neither passive (Figure 3) nor active (data not shown) mesenteric artery compliance was altered by Ang II treatment in either phenotype. Moreover, using organ culture of mouse carotid arteries, we found that Ang II alone neither modified the pressure–diameter relationship in vessels maintained at 80 mm Hg nor accentuated the increase in distensibility in vessels at 150 mm Hg (data not shown).

**Ang II Induces MMP Activity**

Gelatinase assays were undertaken to elucidate whether MMP activation could account for the changes in the press-
sure–diameter relationships observed in carotid arteries from Ang II-treated mice. Using immunohistochemical staining, we found that expression levels of MMP-3, MMP-8, and tissue inhibitor of metalloproteinase-1 were equivalent in all of the animals irrespective of treatment or phenotype (data not shown). However, in-gel zymography (Figure 4) revealed that, in both wt and MMP-9−/− mice, Ang II triggered a strong increase in pro-MMP-2 and MMP-2 gelatinase activity (P<0.05 versus sham). In addition, vessels from wt mice given Ang II displayed a significant MMP-9 activity (P<0.05 versus sham). Nevertheless, the carotid artery in situ gelatinolytic response was markedly different between animal subtypes (Figure 5). In wt mice, Ang II treatment induced a >2-fold increase in gelatinase activity, observed throughout the vessel wall, whereas in MMP-9−/− mice, no such response was observed. The discrepancy between low total MMP activity detected in situ (Figure 5) and apparent high MMP-2 activity detected in gel (Figure 4) in hypertensive MMP-9−/− mice could be explained by mismatch between the 2 zymographic techniques, the former revealing only active enzymes, the latter revealing both active and inactive enzymes. Alternatively, activation of multiple gelatinase subtypes (including MMP-2, MMP-9, MMP-3, and MMP-8) may be necessary for optimal in situ gelatinase detection, and high MMP-2 activity alone may not have been sufficient to achieve this.

MMP activity was also evaluated in the supernatant of mouse aortas. Aortas obtained from mice treated with vehicle or Ang II during 10 days were maintained in culture medium for 18 hours. In-gel zymography patterns mirrored observations made in vessel lysates: Ang II treatment was associated with greater MMP-2 activity in wt and MMP-9−/− animals, and MMP-9 activity was also enhanced in wt mice (Figure 6). However, when type I collagen breakdown was assessed, significant differences were again noted between wt and MMP-9−/− mice. The release of collagen I telopeptide was greater (P<0.05) in aortas of Ang II-treated wt mice than in vessels of Ang II-treated MMP-9−/− mice (Figure 6).

Vascular Remodeling Associated With Ang II Treatment

Despite the increase in type I collagen breakdown observed in Ang II-treated animals, particularly in wt mice, staining for type I collagen did not vary between conditions (data not shown). On the other hand, type IV collagen staining was particularly strong in carotid arteries from Ang II-treated mice, being significantly enhanced in arteries from both wt and MMP-9−/− mice (P<0.001; Figure 7). Moreover, although collagen IV levels did not differ between untreated wt

Figure 4. Carotid artery gelatinase activity is enhanced by 10-day Ang II treatment. A, In-gel zymographic activities of latent (pro–MMP-2; top band) and active MMP-2 (bottom band) were upregulated in arteries of all of the mice exposed to the hypertensive treatment. Vessels of wt mice also displayed enhanced MMP-9 activity. Lytic bands corresponding with MMP activity in wt (B) and MMP-9−/− mice (C) are quantified and presented as arbitrary units (AU). Results are mean±SEM of n=4. *P<0.05, **P<0.01, and ***P<0.001 Ang II vs sham.

Figure 5. A, In situ gelatinase activity, detected by enhanced fluorescence of a fluorogenic substrate, is enhanced only in carotid arteries of Ang II–treated wt mice. In MMP-9−/− animals, 10-day Ang II treatment is not associated with elevated carotid artery in situ gelatinase activity. Autofluorescent elastic laminae appear in blue. B, Quantification of percentage of vessel surface area covered by bright green fluorescence. Data are mean±SEM of n=4. ***P<0.001 Ang II vs sham. §§§P<0.001 MMP-9−/− vs wt.
and MMP-9−/− mice, collagen accumulation after Ang II was 2-fold greater in MMP-9−/− mice compared with untreated shams or wt mice. A, Brown staining corresponding with type IV collagen is markedly enhanced in the endothelial and medial layers of carotid arteries from Ang II–treated MMP-9−/− mice. B, Quantification of vessel wall staining for type IV collagen, presented as arbitrary units (AU). Data are mean±SEM of n=4. **P<0.01 Ang II vs sham; §§§P<0.001 MMP-9−/− vs wt.

**Discussion**

This study demonstrates that, in vivo, the onset of hypertension is associated with MMP induction and collagen breakdown, concurrent with a rise in vessel distensibility. Interestingly, MMPs dampen the early rise in blood pressure, because Ang II treatment produces a steeper rise in blood pressure in MMP-9−/− mice than in wt animals.

It is particularly noteworthy that the absence of MMP-9 was associated with a more pronounced pulse pressure in Ang II–treated animals. This is consistent with the diminished compliance observed in carotid arteries of MMP-9−/− animals. Because vessel stiffness is an independent risk factor for mortality associated with cardiovascular disease, our results suggest that enhanced MMP activity in conductance vessels plays a beneficial role in the early stages of Ang II–induced hypertension. Moreover, the rise in blood pressure itself was more moderate in wt than in MMP-9−/− mice, implying that, in a hypertensive hormonal context, MMPs may help to restrain the development of hypertension, at least
in the short term. Our finding that compliance of the mesenteric arteries was not altered by Ang II and did not differ between wt and knockout animals suggests that the blood pressure differential between treatment and phenotype groups did not stem from such changes in resistance arteries. Although small artery hypertrophy is a hallmark of hypertension,\textsuperscript{10,11} this adaptive response occurs at a later time point.\textsuperscript{12} Hypertension-induced vessel thickening and medial hypertrophy were equivalent in all of the animals, indicating that medial mass had little influence on compliance. These results agree with the finding that arterial wall hypertrophy can be associated with opposing effects on the elastic properties of the carotid artery in patients.\textsuperscript{13} Furthermore, previous studies showed that the increase in wall mass resulting from chronic Ang II infusion failed to alter compliance in rat aortas\textsuperscript{14} and that differences in compliance between hypertensive and normotensive rat aortas having similar medial cross-sections could be attributed to differences in collagen cross-linking.\textsuperscript{15} Here we identified MMP-9 as a key element in regulating vessel compliance, influencing type I and IV collagen accumulation and degradation. In hypertensive wt animals, MMP-9 activation coincided with enhanced aortic type I collagen degradation, demonstrated by elevated CITP levels. In comparison, Ang II treatment in MMP-9\textsuperscript{-/-} mice was associated with less pronounced CITP release coupled with enhanced carotid artery accumulation of type IV collagen. Although the CITP and type IV collagen data were obtained from different conductance vessels, a limitation of the present study, our observations provide an explanation for the reduced carotid artery compliance in hypertensive MMP-9\textsuperscript{-/-} mice as opposed to the enhanced compliance found in wt mice.

Apart from 1 report detailing elevated plasma levels of CITP in subjects with untreated hypertensive compared with normotensive subjects,\textsuperscript{16} most clinical studies associate hypertension with diminished CITP and MMP activity\textsuperscript{17–19} and greater vessel type I collagen content.\textsuperscript{20} Nonetheless, based on the in vivo results presented here and our previous ex vivo findings,\textsuperscript{3} it appears that MMPs are induced within days of the onset of hypertensive conditions and participate in collagen breakdown, increasing vessel distensibility. Hence, it is possible that the balance between extracellular matrix synthesis and degradation evolves in time in hypertension and that our observations reflect very early processes in the development of hypertensive vessel disease.

Perspectives

In the early phase of essential arterial hypertension, it is generally admitted that the vessel wall is submitted to increased pressure because of abnormal peripheral resistances related to genetic, humoral, nervous, and/or structural factors. The vascular wall remodels to adapt to the mechanical environment of elevated tensile stress and to normalize this...
stress; vascular smooth muscle cells undergo hypertrophy/hyperplasia and synthesize key extracellular matrix proteins. At length, damage to the large arteries is clearly involved in the cardiovascular morbidity and mortality associated with hypertension. Our data demonstrate that the acute rise in MMP activity occurring at the onset of Ang II-induced hypertension increases conductance vessel compliance and is associated with a more modest increase in systolic blood pressure and pulse pressure, suggesting that, at least in the short term, MMP activation may exert a beneficial effect by counteracting the prohypertensive stimulus. Nevertheless, MMP activity may herald the beginning of matrix protein turnover that eventually results in vessel rigidification. Understanding how these processes work may lead to better management of hypertensive vascular disease and alleviate the associated target organ damage.

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Disclosures
None.

References
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