Matrix Metalloproteinase-9 (MMP-9), MMP-2, and Serum Elastase Activity Are Associated With Systolic Hypertension and Arterial Stiffness

Yasmin, Sharon Wallace, Carmel M. McEniery, Zahid Dakham, Pawan Pusalkar, Kaisa Maki-Petaja, Mike J. Ashby, John R. Cockcroft, Ian B. Wilkinson

Background—Arterial stiffness is an independent determinant of cardiovascular risk, and arterial stiffening is the predominant abnormality in systolic hypertension. Elastin is the main elastic component of the arterial wall and can be degraded by a number of enzymes, including matrix metalloproteinase-9 (MMP-9) and MMP-2. We hypothesized that elastase activity would be related to arterial stiffness and tested this using isolated systolic hypertension (ISH) as a model of stiffening and separately in a large cohort of healthy individuals.

Methods and Results—A total of 116 subjects with ISH and 114 matched controls, as well as 447 individuals free from cardiovascular disease were studied. Aortic and brachial pulse wave velocity (PWV) and augmentation index were determined. Blood pressure, lipids, C-reactive protein, MMP-9, MMP-2, serum elastase activity (SEA), and tissue-specific inhibitor 2 of metalloproteinases were measured. Aortic and brachial PWV, MMP-9, MMP-2, and SEA levels were increased in ISH subjects compared with controls (P < 0.001). MMP-9 levels correlated linearly and significantly with aortic (r = 0.45; P < 0.001) and brachial PWV (r = 0.22; P = 0.002), even after adjustments for confounding variables. In the younger, healthy subjects, MMP-9 and SEA were also independently associated with aortic PWV.

Conclusions—Aortic stiffness is related to MMP-9 levels and SEA, not only in ISH, but also in younger, apparently healthy individuals. This suggests that elastases including MMP-9 may be involved in the process of arterial stiffening and development of ISH. (Arterioscler Thromb Vasc Biol. 2005;25:372-378.)

Key Words: MMP-9 □ MMP-2 □ elastase activity □ pulse wave velocity □ augmentation index □ elastin

Although originally considered as inert conduits, the large arteries are now recognized to play an important role in buffering the cyclic changes in blood pressure attributable to intermittent ventricular ejection.1 Stiffening of the large arteries has a number of adverse hemodynamic consequences, including widening of the pulse pressure and alterations in shear stress, which promote vascular and cardiac remodeling, and ultimately increase cardiovascular risk.1 Indeed, aortic pulse wave velocity (PWV), a measure of distensibility, predicts mortality in patients with end-stage renal failure, hypertension, diabetes, and in older individuals, independently of confounding factors.2–4 However, the mechanisms responsible for arterial stiffening remain incompletely understood.

The main physiological determinants of large artery stiffness are structural elements within the arterial wall, smooth muscle tone, and mean arterial pressure (MAP).1 Among the various structural proteins, elastin provides the main “elastic” element of arteries. Although elastin was once considered inert, a number of cells within the arterial wall are now known to synthesize elastin de novo, even in adults. Elastin is also susceptible to degradation, and the amino acid composition of arterial elastin changes with age.5 Together, this suggests that arterial elastin is actually turned over, albeit with a long half life. Interestingly, a number of enzymes such as serine proteases and several members of the matrix metalloproteinases (MMPs), including MMP-9 and MMP-2, are able to break down elastin.

Increased total serum elastase activity (SEA) is associated with atherosclerosis and an increased risk of cardiovascular disease (CVD).7 More specifically, MMP-9 and MMP-2 play an important role in vascular remodeling.8–10 Increased MMP-9 and MMP-2 activity is associated with destruction of the elastic laminae of arteries, and aneurysm formation in animals11 and humans.12 Moreover, functional variants in the promoter region of the MMP-9 gene are associated with the
severity of CVD and systemic arterial stiffness in patients with known CVD. More recently, plasma MMP-9 levels have been identified as a novel predictor of cardiovascular risk in patients with coronary artery disease and stroke. However, the role of MMP-9 and MMP-2 in arterial stiffening per se has not been investigated previously. We hypothesized that serum MMP-9 and MMP-2 levels, and SEA would be independently related to aortic stiffness in humans. The aim was to investigate the relationship between arterial stiffness and MMP-9, MMP-2, and SEA levels using 2 different approaches: first, in subjects with isolated systolic hypertension (ISH), as a clinically relevant model of arterial stiffening, and, second, in a large cohort of younger, healthy individuals free from the potential confounding influence of CVD.

Methods

Subjects
All subjects were studied as part of an ongoing, community-based investigation into the factors influencing arterial stiffness. Individuals were selected at random from local general practice lists by letter of invitation (the overall response rate was 85%). Subjects with diabetes, hypercholesterolemia (total cholesterol ≥6.5 mmol/L), renal disease (defined as a clinical history, creatinine ≥150 μmol/L, or an active urinary sediment), a history of CVD (defined as a clinical history or evidence at examination), known inflammatory conditions, malignancy, or a recent history of infection were excluded from the present analyses, as were subjects receiving any medication. Approval was obtained from the Local Research Ethics Committee and written informed consent obtained from each participant.

Study 1
A total of 116 never-treated subjects with ISH, defined as blood pressure ≥140 and <90 mm Hg on ≥3 occasions, and no previous history of diastolic or systolic diastolic hypertension, were studied, together with 114 age- and gender-matched controls. Blood pressure, PWV, and biochemical markers including lipids, glucose, C-reactive protein (CRP), SEA, MMP-9 and MMP-2, and tissue-specific inhibitor 2 of metalloproteinases (TIMP-2) were measured. Reported smoking status was also noted.

Study 2
A total of 447 younger, healthy individuals, untreated and with no evidence of symptomatic CVD, was recruited. Blood pressure, PWV, and biochemical markers including lipids, glucose, CRP, SEA, and MMP-9 were assessed. Reported smoking status was also noted.

Clinical Measurements
All studies were conducted in a quiet, temperature-controlled room. After 20 minutes of supine rest, peripheral blood pressure was recorded in the brachial artery of the dominant arm using a validated oscillometric technique (HEM-705CP; Omron Corp.). Radial artery waveforms were obtained with a high-fidelity micromanometer (SPC-301; Millar Instruments) from the wrist, and from this, a corresponding central waveform was generated using a validated transfer function (Sphygmocor; AtCor Medical) as described previously in detail. Augmentation index (Alx), a composite measure of systemic arterial stiffness and wave reflection, and heart rate were determined using the integral software. Aortic PWV was measured using the same device by sequentially recording ECG-gated carotid and femoral artery waveforms and brachial PWV from carotid and radial arteries. All measurements were made in duplicate and mean values used in the subsequent analysis.

Laboratory Measurements
A total of 20 mL of blood was drawn and the serum separated and stored at –80°C. Total cholesterol, triglycerides, glucose, and CRP were determined using standard methodology. Commercially available sandwich ELISA assays (Oncogene Research Products) were used to determine levels of MMP-2, MMP-9, and TIMP-2. The lower limit of detection was 0.1 ng/mL for MMP-2 and MMP-9, and 3.0 ng/mL for TIMP-2. TIMP-2 was measured because it inhibits MMP-2 and MMP-9. The coefficient of variation was <8% for all assays. Initial experiments of the assay indicated inhibition of elastases with EDTA plasma, hence, all the assays were measured using serum samples.

SEA was determined using a synthetic substrate, succinyl tria- nine paranitroanilide, according to a modified colorimetric assay. Standard curves were prepared with crystalline pancreatic elastase type IV (Sigma). The absorbance of the assay was read at 0 hours and 24 hours after incubation at 37°C (titertech MR5000) at 405 nm. Results were expressed as μmol/mL. All samples were analyzed in duplicate. The coefficient of variation was <10% in study 1 and study 2.

Data Analysis
Data were analyzed using SPSS software. Logarithmic transformations were performed for distributions that were significantly skewed, and these variables were used in the subsequent analysis. Student t test was used to compare group differences. Correlation and stepwise regression analyses were performed to investigate the relationship between arterial stiffness and other parameters. All values represent means and SDs. For skewed variables, data are presented as medians and interquartile range, and Mann–Whitney test was used for group comparisons. A P value of <0.05 was considered significant.

Results

Study 1
The baseline characteristics of the subjects are shown in Table 1. There were no significant differences between the 2 groups for age (range 58 to 83 years), gender, body mass index, lipid fractions (cholesterol, triglycerides), and glucose levels. As expected, systolic, diastolic, and mean pressures were significantly higher in ISH subjects compared with controls. Although aortic and brachial PWV were significantly higher in ISH hypertensives, Alx did not differ. Moreover, only aortic PWV was significantly higher in the ISH group after correcting for MAP (mean difference 1.5 m/s; P=0.001). SEA, MMP-2, and MMP-9 levels were all significantly higher in the hypertensives compared with controls, but TIMP-2 levels did not differ.

Aortic PWV was significantly correlated with MMP-9 (Figure), MMP-2, and SEA (Table 2). Similar associations were observed for brachial PWV (Table 2). As expected, MMP-9 correlated significantly and positively with SEA (r=0.26; P=0.001; data not shown).

Because arterial stiffness is influenced by a number of factors such as age, MAP, and cardiovascular risk factors, we performed a stepwise regression analysis on the whole data set. MMP-9 levels, age, and MAP were independently associated with aortic PWV (19%). Substitution of SBP for MAP in this model did not significantly alter the results or the factors predicting PWV (data not shown). In a separate regression analysis, MMP-2 and SEA also associated independently with aortic PWV (data not shown).
Study 2
The baseline characteristics of the subjects are shown in Table 4. The mean age of the group was 42 years (range 19 to 80 years). As expected, the average systolic, diastolic, and mean blood pressures were in the normal range. Similarly, the mean and median values of the serum markers were also within the normal range.

Serum MMP-9 levels and SEA were significantly and positively correlated with aortic PWV and MAP (Table 2). Using a stepwise regression analysis, MMP-9 was found to be an independent predictor of aortic PWV (Table 3). A similar relationship was observed when systolic blood pressure was included in the model rather than MAP (data not shown). Furthermore, a similar independent association was also found between SEA and aortic PWV (data not shown).

Discussion
The current study examined, for the first time, the relationship between serum elastases and arterial stiffness in subjects with ISH, a condition characterized by increased arterial stiffness, and also in healthy controls. The main findings were that MMP-9 and MMP-2 levels, and SEA were increased in subjects with ISH compared with controls and correlated independently with aortic PWV. Moreover, even in young, healthy, normotensive individuals MMP-9 levels were an independent predictor of aortic stiffness. In addition, we confirmed our previous observations by demonstrating a

![Graph showing relationship between aortic PWV and serum MMP-9 levels. The correlation coefficient for the whole group was 0.45 (P<0.001). ISH subjects are indicated by ▲; matched controls, ●.](image)
significant independent relationship between inflammation and aortic PWV and an association between MMP-9 and CRP. These data suggest a role of elastases, including MMP-9, in large artery stiffening.

Stiffening of the large arteries is associated with a widening of pulse pressure and an increased cardiovascular risk. The large arteries contain a number of different structural proteins, but elastin, which accounts for 40% dry weight, provides the main elastic element. However, elastin is susceptible to chemical attack and is degraded by a number of enzymes, including MMP-9, MMP-2, and various serine proteases. Previous studies have demonstrated increased SEA and particularly MMP-9 and MMP-2 levels in patients with CVD. MMP-9 is thought to be involved in destruction of the arterial media and plaque growth. Moreover, targeted deletion of the MMP-9 attenuates collagen accumulation and enhanced expression of other MMPs after myocardial infarction, suggesting that MMP-9 plays a prominent role in extracellular matrix remodeling.

### TABLE 2. Correlations Between Hemodynamic Measurements and Serum Markers

<table>
<thead>
<tr>
<th>Studies</th>
<th>MMP-9</th>
<th>MMP-2</th>
<th>SEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic PWV</td>
<td>Study 1</td>
<td>0.45</td>
<td>0.21 (0.006)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.18 (0.001)</td>
<td>—</td>
</tr>
<tr>
<td>Brachial PWV</td>
<td>Study 1</td>
<td>0.22 (0.002)</td>
<td>0.04 (NS)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.11 (0.03)</td>
<td>—</td>
</tr>
<tr>
<td>A1x</td>
<td>Study 1</td>
<td>0.13 (0.04)</td>
<td>0.10 (NS)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.06 (NS)</td>
<td>—</td>
</tr>
<tr>
<td>MAP</td>
<td>Study 1</td>
<td>0.23 (0.002)</td>
<td>0.21 (0.007)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.10 (0.03)</td>
<td>—</td>
</tr>
<tr>
<td>SBP</td>
<td>Study 1</td>
<td>0.38 (0.001)</td>
<td>0.20 (0.008)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.13 (0.009)</td>
<td>—</td>
</tr>
<tr>
<td>DBP</td>
<td>Study 1</td>
<td>0.21 (0.001)</td>
<td>0.18 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Study 2</td>
<td>0.08 (NS)</td>
<td>—</td>
</tr>
</tbody>
</table>

SEAP indicates systolic blood pressure; DBP, diastolic blood pressure.

### TABLE 3. Multiple Regression Analyses for Aortic PWV

<table>
<thead>
<tr>
<th>Study</th>
<th>Regression Coefficient</th>
<th>Significance</th>
<th>$R^2$ Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMP-9</td>
<td>&lt;0.001</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>&lt;0.001</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>&lt;0.001</td>
<td>4</td>
</tr>
<tr>
<td>Study 2</td>
<td>$R^2$ value=0.36; $P=0.0001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>&lt;0.001</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>MMP-9</td>
<td>&lt;0.001</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>&lt;0.001</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>LnCRP</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Male gender</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td>$R^2$ value=0.60; $P=0.0001$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MMP-9 levels also predict cardiovascular risk in patients with CVD and functional variants in MMP-9 gene are associated with severity of vascular disease and large artery stiffness in patients with CVD. However, the role of elastases in essential hypertension remains controversial because increased and decreased MMP-1, MMP-2, and MMP-9 levels have been reported. Moreover, the role of elastases in arterial stiffening per se and in ISH has not been investigated previously. Therefore, the aim of the present study was to investigate the relationship between serum elastases and aortic stiffness using ISH as a model of large artery stiffening.

### TABLE 4. Subject Characteristics in Study 2

<table>
<thead>
<tr>
<th>Mean±SD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42±18</td>
</tr>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>121±12</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>74±9</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>108±13</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>75±10</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>90±9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>67±11</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.0±14.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8±1.0</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.5±1.1</td>
</tr>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>124.5±50.4</td>
</tr>
<tr>
<td>A1x (%)</td>
<td>14±17</td>
</tr>
<tr>
<td>Brachial PWV (m/s)</td>
<td>7.6±1.3</td>
</tr>
<tr>
<td>Aortic PWV (m/s)</td>
<td>6.9±1.7</td>
</tr>
<tr>
<td>Glucose (mmol/L)*</td>
<td>4.7 (4.3–5.2)</td>
</tr>
<tr>
<td>SEA (µmol/mL)*</td>
<td>24.5 (15.5–40.4)</td>
</tr>
<tr>
<td>CRP (mg/L)*</td>
<td>1.6 (1.0–3.5)</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

*Values presented as medians and interquartile range, and group comparisons made by Mann–Whitney test.
but also in a much larger cohort of younger, healthy subjects free from the potential confounding influence of CVD or cardiovascular risk factors.

Previous studies have demonstrated increased large artery stiffness in subjects with ISH. However, only 1 has previously reported increased aortic PWV in ISH, and that difference did not persist after correcting for differences in MAP. In the present study, aortic PWV was increased in the ISH group at ambient pressures compared with matched, normotensive controls, and this difference persisted even after adjusting for the slightly higher MAP in subjects with ISH (~17% higher). Conversely, although brachial PWV was higher in the ISH group, this difference did not persist after correcting for MAP, suggesting predominant stiffening of the central elastic arteries in ISH rather than the peripheral muscular arteries. Indeed, the brachial artery is known to stiffen much less with age than the aorta. In contrast, Alx, a surrogate measure of systemic arterial stiffness and wave reflection, did not differ significantly between the groups, although augmented pressure did. This suggests that large artery stiffening rather than enhanced wave reflection is the major abnormality in ISH, possibly because by the sixth decade, wave reflection has reached a peak, and from then on, further increases in aortic and brachial pulse pressure are driven mainly by increasing large artery stiffness. Although Mitchell et al did find a difference in AIx between these serum markers and arterial stiffening. However, further cohort studies are clearly required to address this issue and to explore the causal relationships between these serum markers and arterial stiffening.

Potential Limitations

There are a number of inhibitors of elastase activity in the serum and tissue, including TIMP-1, TIMP-2, TIMP-3, and α1 antitrypsin. In the present studies, MMP mass rather than activity was assessed. However, the levels of TIMP-2, a specific inhibitor of MMP-2 and MMP-9, did not differ between ISH subjects and controls, suggesting an actual increase in MMP activity. We also assessed total SEA using an established colorimetric assay, which confirmed increase elastase activity in subjects with ISH and a significant relationship between SEA and PWV. Although SEA measures MMP and serine elastase activity, initial experiments indicated that >90% of SEA was inhibited by EDTA, suggesting that zinc-containing MMPs make by far the largest contribution to SEA.

The cross-sectional nature of the present study also limits our ability to infer a causal relationship between elastases and arterial stiffness. Indeed, experimental models of hypertension in animals suggest that enhanced mechanical stress induced by acutely raising blood pressure increases the synthesis of elastin and collagen of arteries, and this in turn elevates the synthesis of MMP-9. However, the fact that we were able to demonstrate a relationship between aortic PWV and MMP-9 levels in younger normotensive subjects, independently of MAP, suggests that MMP-9 levels may not simply reflect increased MAP and hence vascular load. However, further studies are required to determine whether increased elastase activity and MMP-9 activity in particular lead to arterial stiffening and to explore potential mechanisms. Finally, because we deliberately excluded individuals with hypercholesterolemia and diabetes from both studies, we did not show any independent relationship between cholesterol, glucose, and arterial stiffness, as we and others have observed previously.

Summary

Large artery stiffness is an important determinant of cardiovascular risk. The present study indicates that SEA, MMP-9, and MMP-2 are increased in subjects with ISH, and that MMP-9 levels correlate independently and significantly with aortic PWV in individuals across a wide age range. This suggests that serum elastases, particularly MMP-9, may be involved in the process of arterial stiffening and the development of ISH. Therefore, inhibitors of MMP-9 may be of...
benefit in reducing arterial stiffness and thus cardiovascular risk in patients with accelerated or premature stiffening.

Acknowledgments

We are grateful to the British Heart Foundation for funding our work.

References


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In the January 2005 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, in the Brief Review entitled “Vitamin D, Shedding Light on the Development of Disease in Peripheral Arteries” by Norman and Powell (*Arterioscler Thromb Vasc Biol*, 2005;25:39–46), there was a typographical error in the section entitled “Vitamin D and Peripheral Arterial Calcification.” On page 42, in the fourth line from the bottom of the left column, “. . . antagonist (paricalcitol) improves survival. . . ” should have read “. . . agonist (paricalcitol) improves survival. . . ”

In the February 2005 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, in the article entitled “Matrix Metalloproteinase-9 (MMP-9), MMP-2, and Serum Elastase Activity Are Associated With Systolic Hypertension and Arterial Stiffness” by Yasmin et al (*Arterioscler Thromb Vasc Biol*, 2005;25:372–378), the order of authorship was listed incorrectly. The correct order of authorship is as follows: Yasmin, Carmel M. McEniery, Sharon Wallace, Zahid Dakham, Pawan Pusalkar, Kaisa Maki-Petaja, Mike J. Ashby, John R. Cockcroft, Ian B. Wilkinson. We apologize for these errors.