Eur J Vasc Endovasc Surg **35**, 580–584 (2008)
doi:10.1016/j.ejvs.2007.12.004, available online at http://www.sciencedirect.com on ScienceDirect

Elevated Plasma MMP1 and MMP9 are Associated with Abdominal Aortic Aneurysm Rupture

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Background. *The role of matrix metalloproteinases (MMPs) in abdominal aortic aneurysm (AAA) formation is well established. However the changes in plasma MMP levels with AAA rupture have not been reported. The aim of this study was to determine circulating levels of MMPs in non-ruptured and ruptured AAA immediately prior to open repair.*
Methods. *Concentrations of MMPs and their endogenous tissue inhibitors (TIMPs) were quantified using ELISA in pre-operative plasma samples from non-ruptured and ruptured AAA.*
Results. *MMP1 and MMP9 were elevated in the plasma of ruptured AAA versus non-ruptured AAA. A four-fold elevation in pre-operative plasma MMP9 was associated with non-survival at 30 days from rupture surgery compared with those surviving for greater than 30 days.*
Conclusion. *In conclusion, these findings support the role of MMPs in AAA pathogenesis. Elevation of MMP9 was associated with ruptured aneurysm related 30-day mortality and may represent a survival indicator in this group.*

Keywords: Abdominal aortic aneurysm; Matrix metalloproteinase; Rupture.

Introduction

The principal of abdominal aortic aneurysm (AAA) treatment is the exclusion of the aneurysmal vessel from the circulation either by open surgical repair or more recently by endovascular means. The commitment to elective surgery is not without risk and 30-day mortality following elective aneurysm repair ranges from 2 to 6%.[1,2] The mortality associated with AAA rupture is unacceptably high with post-operative mortality around 40%.[3] Although the risk of aneurysm rupture increases exponentially with vessel diameter, rupture can occur in small aneurysms and the accurate prediction of impending rupture has proved elusive.

The role of matrix metalloproteinases (MMPs) in aneurysm development and rupture is well described.[4] However, a clear role for plasma MMPs in disease prediction has not been established. The plasma concentrations of various MMPs have been reported to predict the natural history of small AAAs.[5] Furthermore, falling circulatory levels of MMPs may indicate successful AAA exclusion after endovascular repair while persistently high levels may indicate an endoleak.[6] The elevation of specific MMPs within the vessel wall of a ruptured AAA has been observed[7] but a change in plasma MMP levels at the time of rupture has not been described.

This study was the first to quantify plasma MMP and the endogenous tissue inhibitor of MMPs (TIMP1) in blood samples taken immediately prior to the repair of elective and ruptured AAA. The primary aim of this study was to determine if circulating levels of MMPs and TIMPs reflected the clinical state of an AAA, namely stable versus ruptured AAA. The secondary aim of this study was to observe changes in MMP and TIMP levels associated with 30-day mortality in rupture patients.

Methods

Study design

Two patient groups were studied, 52 patients with non-ruptured AAA undergoing elective repair, and 16 patients with ruptured AAA undergoing emergency surgery. Local research ethics committee approval for

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1078–5884/000580 + 05 $34.00/0

the study was obtained and written consent was taken from all patients. The diameter of each ruptured AAA was measured intra-operatively. The maximum external diameter of each non-ruptured AAA was determined from a pre-operative computed tomogram.

Patient demographic information included age, gender, smoking history (current or ex-smoker of less than 10 years versus non-smoker or ex-smoker of greater than 10 years), presence of a cardiovascular event (documented myocardial infarction, cerebrovascular or peripheral vascular disease, angina requiring medication), hypertension (requiring medication), and diabetes (requiring medication or dietary modification). Cardiovascular medication was also recorded (statin, beta-blocker, calcium channel-blocker, acetylcholine-esterase inhibitor and non-steroidal anti-inflammatory). Outcome data included 30-day all-cause mortality in the rupture cohort.

Sample collection

Blood samples were obtained immediately pre-operative, by venepuncture, to tubes containing sodium ethylenediamine tetra-acetate. Separation of cellular and plasma components was achieved with centrifugation (3000 g for 20 mins); the plasma was decanted and stored at $-80\,^{\circ}C$.

MMP quantification

Enzyme-linked immunosorbent assay kits (Amersham Pharmacia Biotech, Buckinghamshire, UK) were used to quantify the following from each plasma sample; MMP1, MMP2, MMP3, MMP9 and TIMP1. All samples were run in duplicate, and an average obtained. The final concentration of each MMP and TIMP was expressed as nanograms of target protein per millilitre of plasma.

Statistical analysis

Statistical analysis used GraphPad Prism 5. Discrete variables were presented as numbers and percentages and compared using Fisher's exact test. The continuous variable of age was normally distributed, presented as a mean (and standard error) and compared using the independent t-test. Other continuous variables were non-normally distributed, reported as a median and interquartile range (AAA diameter, MMP and TIMP levels) and compared using the Mann-Whitney U-test. Correlations used the Spearman's test, statistical significance was assumed at the $p < 0.02$ level.

Results

Patient demographics

The clinical features of the non-ruptured and ruptured AAA patient cohorts are described in Table 1. Median AAA diameter was greater in ruptured than non-ruptured AAA. There were no other differences in the characteristics of the study cohorts.

Plasma MMP and TIMP concentrations: ruptured AAA versus non-ruptured AAA

Table 2 details plasma levels if MMPs and TIMPs from ruptured and non-ruptured AAA. There were no statistical differences in the concentrations of MMP2, MMP3 or TIMP1. In contrast, the concentrations of MMP1 and MMP9 were significantly elevated in the plasma of ruptured AAA compared with non-ruptured AAA (MMP1, 20.2 ng/ml [16.1–28.7] vs. 8.9 ng/ml [5.6–15.7], $p < 0.0001$; MMP9, 59.1 ng/ml [20.8–123.7] vs. 17.5 ng/ml [10.3–34.2], $p = 0.006$).

Table 1. Characteristics of patients with non-ruptured and ruptured abdominal aortic aneurysms (AAA)

Patient Characteristics	Non-Ruptured AAA ($n = 52$)	Ruptured AAA ($n = 16$)	p-value
Age	72 (+/−1.0) yrs	70 (+/−1.2) yrs	0.289*
AAA size	6.0 (5.7–6.5)	10 (7.5–11)	<0.001†
Gender Distribution	46 males (88%)	13 males (81%)	1.0
Cardiovascular Event	18 (35%)	3 (19%)	0.229
Hypertension	26 (50%)	7 (44%)	0.778
Smoking History	42 (81%)	12 (75%)	0.726
Diabetes	5 (9.6%)	0 (0%)	0.330
Statin	12 (23%)	1 (6%)	0.273
Beta-Blocker	11 (21%)	1 (6%)	0.268
Ca^{2+} Channel Blocker	12 (23%)	5 (31%)	0.523
ACEI	14 (27%)	1 (6%)	0.094
NSAID	20 (38%)	3 (19%)	0.248

(Comparison used independent t-test (*) with group mean and standard error in years, Mann-Whitney U-test (†) with group median and interquartile range in cm, and Fisher's Exact Test; acetylcholinesterase inhibitor (ACEI), non-steroidal anti-inflammatory (NSAID), $p < 0.02$).

Table 2. Comparison of plasma matrix metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) concentrations in non-ruptured abdominal aortic aneurysms (AAA) and ruptured AAA

Enzyme Group	Non-Ruptured AAA ($n = 52$)	Ruptured AAA ($n = 16$)	p-value
MMP-1	8.90 (5.57–15.69)	20.18 (16.09–28.68)	<0.001*
MMP-2	42.14 (28.71–68.69)	51.58 (42.23–68.44)	0.325
MMP-3	9.43 (6.99–13.54)	8.36 (5.91–11.44)	0.268
MMP-9	17.54 (10.30–34.24)	59.11 (20.82–123.70)	0.006*
TIMP-1	460.80 (199.80–611.00)	229.50 (192.60–492.20)	0.456

(Concentrations shown as median and interquartile range, units ng/ml, comparison used Mann Whitney-U test, $p < 0.02$, * = significance).

Elevated plasma MMP9 concentrations in ruptured AAA: association with 30-day mortality

Comparison of MMP and TIMP levels in ruptured AAA with respect to 30-day mortality (Table 3) demonstrated significantly higher levels of MMP9 immediately pre-operatively in the plasma of non-survivors at 30 days from rupture surgery compared to survivors beyond 30 days (131.9 ng/ml [75.5–191.8] versus 32.2 ng/ml [5.9–129.5], $p = 0.017$). Furthermore, MMP1 was higher and TIMP1 lower in non-survivors of rupture though neither comparison reached significance.

Plasma MMP and TIMP concentrations: correlation with aneurysm size

There were no significant correlations between AAA diameter and enzyme concentrations within the non-ruptured and ruptured cohorts (Table 4). The correlation

Table 3. Comparison of plasma matrix metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) concentrations in ruptured abdominal aortic aneurysms (AAA) surviving greater than 30 days versus non-survivors at 30 days

Enzyme Group	Ruptured AAA non-survivors at 30 days ($n = 5$)	Ruptured AAA survivors beyond 30 days ($n = 11$)	p-value
MMP-1	26.46 (17.76–50.93)	17.76 (5.43–55.65)	0.079
MMP-2	52.43 (43.39–70.19)	51.12 (26.76–72.75)	0.428
MMP-3	9.61 (4.78–30.25)	7.77 (2.13–73.85)	0.777
MMP-9	131.90 (75.49–191.80)	32.23 (5.86–129.5)	0.017*
TIMP-1	189.91 (82.81–310.12)	232.84 (187.24–7458.63)	0.113

(Concentrations shown as median and interquartile range, units ng/ml, comparison used Mann–Whitney-U test, $p < 0.02$, * = significance).

Table 4. Correlation of plasma matrix metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) concentrations in non-ruptured abdominal aortic aneurysms (AAA) and ruptured AAA

Enzyme Group	Correlation between AAA diameter and enzyme concentration	p-value
Non-ruptured AAA		
MMP-1	0.147 (−0.142–0.414)	0.302
MMP-2	0.126 (−0.163–0.395)	0.379
MMP-3	−0.004 (−0.287–0.280)	0.977
MMP-9	0.196 (−0.092–0.454)	0.167
TIMP-1	−0.069 (−0.346–0.218)	0.626
Ruptured AAA		
MMP-1	0.576 (0.019–0.861)	0.040
MMP-2	0.183 (−0.424–0.677)	0.549
MMP-3	0.271 (−0.346–0.724)	0.371
MMP-9	0.390 (−0.222–0.782)	0.187
TIMP-1	−0.286 (−0.732–0.331)	0.344

(Comparison used Spearman Correlation, $p < 0.02$).

of MMP1 with ruptured AAA diameter approached significance but importantly the correlation with non-ruptured AAA diameter was non-significant. The non-significance of these correlations indicated that elevated plasma MMP1 and MMP9 were associated with AAA rupture rather than AAA diameter.

Discussion

Observational studies support the association between elevated matrix metalloproteinase expression at vascular tissue level and the presence of aneurysmal aortic dilatation and rupture[4,7] Gene knockout models confirm the critical role of MMP9 in the genesis of the abdominal aortic aneurysm. The MMP9 deficient genotype is unable to develop an aneurysmal phenotype following chemical stimulus. Macrophage transfection of the MMP9 gene results in restoration of MMP9 protein expression and subsequently the development of the aneurysmal phenotype.[8]

Circulating MMP levels in aneurysm patients are less comprehensively understood however, elevation of plasma MMP levels in the presence of an abdominal aortic aneurysm is described. Plasma levels of MMP9 are higher in AAA patients than in normal or athero-occlusive controls.[9,10] Indeed plasma MMP9 is significantly associated with the size and expansion of smaller AAA, of 3–5 cm in diameter.[5] Interestingly, plasma MMP-9 and MMP-3 levels fall markedly after successful open and endovascular repair,[10] but remained significantly elevated in a subgroup of patients with endoleak.[6]

Our study observed the significant elevation of plasma MMP1 and MMP9 in ruptured AAA relative to a cohort of stable elective AAA, in blood samples taken immediately prior to open repair. The changes in plasma MMP1 and MMP9 levels were not

associated with a reciprocal elevation in TIMP1. Interestingly, within the cohort of ruptured AAA, patients who deceased within or at 30 days following surgery had pre-operative plasma MMP9 levels 4-fold higher than those who survived beyond 30 days.

Unlike tissue MMP levels, the correlation of plasma levels of MMP's with aneurysm diameter has not been widely reported. A weakly negative correlation between tissue levels of MMP9 and increasing AAA diameter has been suggested.[7,11] In our study, plasma MMP levels did not correlate significantly with AAA diameter. Although plasma MMP1 levels did approach significance in the ruptured group, it is important to note the correlations for both MMP1 and MMP9 with non-ruptured AAA diameter were non-significant. Therefore, the observed difference in aneurysm size between non-ruptured and ruptured aneurysms did not confound our analysis.

In the stable aneurysmal state, plasma MMPs probably originate directly from the site of vascular abnormality i.e. directly from the aneurysm wall and/or the lining thrombus. This is supported by the significant fall of circulating MMP levels following exclusion of the aneurysmal sac from the systemic circulation.[10,12] Within the aneurysmal wall various candidate cells are proposed as the source of MMP expression. These including native mesenchymal cells (fibroblasts and smooth muscle cells) and infiltrating inflammatory cells.[7,13,14]

This study did not establish whether the elevation of plasma MMP1 and MMP9, seen in ruptured AAA, was part of a gradual phenomenon, which predated the rupture event, or a rapid change occurring after the point of vessel rupture. A gradual pre-rupture elevation of plasma MMP levels may suggest the source of increased MMP production was the aneurysm itself. An acute post-rupture elevation in plasma MMP1 and MMP9 levels could also result from an increase in MMP production from the site of aneurysm rupture or originate from the systemic inflammatory changes that ensue following aneurysm rupture.

Previous work indicates that MMP9 is elevated in ruptured AAA however, upregulation is only observed at the site of aneurysm rupture and not globally in the ruptured vessel.[7] Of note, increased circulating MMP9 is observed following other acute cardiovascular events including carotid plaque instability, acute coronary syndrome and myocardial infarction.[15,16] During the acute stage of a myocardial infarct, the release of MMP9 is considered to originate mainly from unstable plaque and the myocardium.[16]

Alternatively, since a systemic inflammatory response normally follows aneurysm rupture, it is likely that release from cells within the systemic inflammatory cascade accounts for some if not all of the circulating MMP9 and MMP1 after the rupture event. The elevation of circulating MMP9 is described in other critically ill patients.[17,18] Hoffam *et al.*, described higher circulating MMP9 levels in septic patients relative to non-septic patients, yet MMP2 levels were similar.[17] Yassen *et al.*, reported in a smaller study, the elevation of MMP9 in critically ill patients (both septic and non-septic) relative to normal controls.[18]

Our observation of the pre-operative four-fold elevation in plasma MMP9 in non-surviving patients versus surviving rupture patients at 30 days, suggested the process following aneurysm rupture was more aggressive in the non-survivors. Elevation of MMP9 in non-surviving patients following a physiological insult has been reported though not exclusively in ruptured aneurysm patients. Nakamura *et al.* observed a greater elevation of MMP9 at plasma protein and monocyte mRNA levels in non-surviving versus surviving septic patients.[19]

We failed to observe significant differences in TIMP1 concentrations in stable versus ruptured aneurysms or 30-day survivors versus non-survivors. However, other reports indicated that TIMP1 was elevated in non-survivors of sepsis compared with survivors and TIMP1 was suggested as a survival indicator in this cohort of patients.[17] However, the equivalence in our findings mirrors the findings from other aneurysm studies reporting tissue levels in stable versus ruptured AAA[7,11] and equivalence of circulating levels despite expansion.[5] This sustains the hypothesis that elevation of MMP levels and the failure to mount a reciprocal TIMP response results in increased proteolytic activity.

In conclusion, this study supports the central role of MMPs in AAA pathogenesis. Elevated plasma MMP9 is associated with the presence of an aneurysm and the expansion of small aneurysms.[5] Here we demonstrate the further elevation of plasma MMP9 and MMP1 immediately following aneurysm rupture. Though the study cannot indicate if these are acute or chronic changes, MMP9 is significantly higher in non-survivors than survivors of aneurysm rupture and may represent a survival indicator in this patient group.

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Accepted 11 December 2007
Available online 15 January 2008