Elevation in Plasma MMP-9 Following Carotid Endarterectomy is Associated with Particulate Cerebral Embolisation

K. J. Molloy,1* M. M. Thompson,2 E. C. Schwalbe,1 P. R. F. Bell,1 A. R. Naylor1 and I. M. Loftus1

Department of1 Surgery, University of Leicester, Leicester, and 2Vascular Surgery and Cardiovascular Research Group, St George’s Hospital Medical School, London, UK

Objectives. To study plasma MMP-9 levels before and after carotid endarterectomy (CEA).

Design. Observational study.

Methods. Pre-operative (morning of surgery) and post-operative (48 h) plasma samples were obtained from 75 consecutive patients undergoing CEA. MMP-9 concentrations were quantified using ELISA. Transcranial Doppler monitoring was performed on each patient to detect particulate embolisation during the dissection phase of the CEA, until the application of carotid clamps.

Results. The median post-operative plasma MMP-9 level of emboli-positive patients was significantly higher than their median pre-operative value (14.9 ng/ml vs. 8.8 ng/ml; \(p = 0.038\)). However, no significant difference was seen in the plasma MMP-9 level of emboli-negative patients (7.7 ng/ml vs. 7.1 ng/ml; \(p = 0.364\)). A greater rise was seen in the median plasma MMP-9 levels of those patients suffering \(>2\) emboli (from 3.4 to 19.3 ng/ml; \(p = 0.041\)) than those patients suffering \(1\) or \(2\) emboli (from 10.1 to 12.8 ng/ml; \(p = 0.340\)).

Conclusions. Plasma MMP-9 only rises after CEA in patients with evidence of embolisation. This increase is more pronounced in those with high numbers of emboli. These data suggest that the increase in MMP-9 is due to cerebral damage caused by embolisation.

Key Words: Atherosclerosis; Carotid artery plaque; Embolisation; Metalloproteinases; Plasma.

Introduction

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes responsible for degradation of the extracellular matrix.1 The MMPs play an integral part in acute disruption of the atherosclerotic plaque,2,3 which precedes stroke and myocardial infarction.4 Of particular interest is MMP-9, which exhibits increased levels and activity in unstable coronary5 and carotid6 plaques. MMP activity is tightly regulated in vivo and the major inhibitor of MMP-9 is tissue inhibitor of metalloproteinase-1 (TIMP-1).

Patients with significant carotid stenoses in whom spontaneous cerebral embolisation is detected exhibit raised levels of plasma MMP-9.7 The source of this MMP-9 is unknown. It may reflect a systemic manifestation of increased local synthesis within the carotid plaque, (Fig. 1) and possibly multiple other plaques throughout the vascular tree. Alternatively, plasma MMP-9 may arise from circulating neutrophils and monocytes (Fig. 2)—there is mounting evidence to suggest a causal role for systemic inflammation in plaque destabilisation.8 Finally, it may represent inflammation secondary to cerebral damage caused by embolisation from the plaque (Fig. 3).

The aim of this study was to quantify plasma MMP-9 and TIMP-1 levels before and after carotid endarterectomy (CEA), and further define their relationship with cerebral embolisation.

Materials and methods

Patients

The local research ethics committee approved the study and all patients gave informed written consent. Pre-operatively, a detailed history was obtained from each patient. The occurrence and timing (in relation to...
CEA) of carotid territory symptoms (transient ischaemic attack, amaurosis fugax, central retinal artery occlusion or stroke) were noted. Patients suffering no carotid territory symptoms within 6 months of surgery were classified as asymptomatic. Demographic details and risk factors for atherosclerosis were recorded. The degree of carotid stenosis was assessed 24 h pre-operatively by duplex ultrasound.

Peripheral blood samples were obtained from 75 patients admitted to a single vascular unit for CEA at the following two time-points—pre-operatively (on the morning of surgery) and post-operatively (on the morning of the second post-operative day). Samples were collected in tubes containing EDTA and were centrifuged at 2500 rpm for 10 min at 4°C. Plasma was then decanted into vials, which were ‘snap-frozen’ in liquid nitrogen and stored at −80°C. MMP-9 and TIMP-1 concentrations were quantified by ELISA (Amersham, UK).

**Cerebral embolisation and operative procedure**

Transcranial Doppler (TCD) monitoring of the middle cerebral artery was performed on each patient during two periods—a 30 min period 24 h prior to CEA, and during the dissection phase of the procedure. A standard technique was employed by each of the four Consultant Surgeons operating on patients enrolled in this study. This involved dissection of the common carotid artery proximal to the carotid bulb, followed by the internal carotid artery distal to the plaque. This took place prior to clamping of the carotid artery but involved minimal dissection of the diseased segment. For the purpose of this study, recording was discontinued upon clamping to ensure all recorded emboli were due to dislodgement of particulate matter from the plaque, rather than air or procedure-related thrombus.

**Statistical analysis**

The discrete variables (clinical characteristics, medication use, symptomatology) are presented as actual numbers (and percentages) and compared using Fisher’s exact test. The continuous variable of age showed normal distribution. It is presented as a mean value (and total range) and compared using the parametric unpaired t-test. The other continuous variables (severity of carotid stenosis, MMP-9 and TIMP-1 concentration) did not show normal distributions. They are presented as median values (and
interquartile ranges) and compared using the non-parametric Mann–Whitney \( U \)-test. Significance is assumed at the \( p < 0.05 \) level.

**Results**

Five patients were excluded from the study due to lack of a TCD ‘window’ (\( n = 3 \)) or patient refusal of the post-operative blood sample (\( n = 2 \)). No patient suffered a post-operative stroke.

Only four emboli (in three patients) were recorded during the pre-operative 30 min period, and all three of these patients also demonstrated dissection phase embolisation. These groups were therefore analysed together as ‘emboli-positive’, as the small number of patients undergoing pre-operative embolisation precluded meaningful analysis at this time-point.

Of the 70 patients analysed, a total of 49 emboli were detected in 15 patients during the dissection phase. Symptomatic patients, and those with tighter carotid stenoses, were more likely to be emboli-positive (Table 1). There was a trend towards decreased statin use in the emboli-positive patients, though this did not reach significance. The groups were otherwise comparable.

For the 15 emboli-positive patients, the median post-operative plasma MMP-9 level was significantly higher than the median pre-operative value (14.9 ng/ml vs. 8.8 ng/ml; \( p = 0.038 \)). However, no significant change was seen in the plasma MMP-9 level of the 55 emboli-negative patients (7.7 ng/ml vs. 7.1 ng/ml; \( p = 0.364 \)) (Fig. 4).

Comparison of pre-operative MMP-9 levels showed no difference between emboli-positive and emboli-negative groups. However, the post-operative values were significantly higher in the emboli-positive group (Table 2).

There were no changes in TIMP-1 concentrations between the pre-operative and post-operative samples in either the emboli-positive or emboli-negative groups, nor when comparing the two groups at the pre-operative and post-operative stages (Table 3).

On sub-analysis of the emboli-positive group, a significantly greater rise in plasma MMP-9 levels was demonstrated in the six patients with more than two emboli detected (Fig. 5).

**Discussion**

We have previously demonstrated increased plasma MMP-9 levels in patients awaiting carotid endarterectomy in whom spontaneous particulate embolisation was detected by transcranial Doppler.\(^7\) In that study, no difference in the level of TIMP-1 was observed, suggesting a shift in the balance of circulating enzymes towards proteolysis—a process which is normally under tight control. What was unclear from this previous study was the origin of the increase in systemic MMP-9.

The intraplaque level, expression and activity of MMP-9 are significantly higher in unstable carotid plaques (based on symptoms, embolisation and histology).\(^6\) The plasma level of MMP-9 may reflect this localised increase in activity, or may perhaps be a sign of a generalised increase in tandem with a systemic inflammatory response promoting plaque degradation. Alternatively, it may be due to cerebral ischaemia caused by particulate embolisation from the plaque.

The present study demonstrated a significant rise in plasma MMP-9 after surgery in patients with evidence

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Table 1. Comparison of patient characteristics between emboli groups

<table>
<thead>
<tr>
<th></th>
<th>Emboli-positive (( n = 15 ))</th>
<th>Emboli-negative (( n = 55 ))</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>68.1 (59–80)</td>
<td>71.0 (50–86)</td>
<td>0.176</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>10 (67)</td>
<td>38 (69)</td>
<td>1.000</td>
</tr>
<tr>
<td>Ever smoker (%)</td>
<td>11 (73)</td>
<td>42 (76)</td>
<td>1.000</td>
</tr>
<tr>
<td>IHD (%)</td>
<td>4 (27)</td>
<td>23 (42)</td>
<td>0.376</td>
</tr>
<tr>
<td>BP (%)</td>
<td>9 (60)</td>
<td>37 (67)</td>
<td>0.760</td>
</tr>
<tr>
<td>DM (%)</td>
<td>2 (13)</td>
<td>11 (20)</td>
<td>0.720</td>
</tr>
<tr>
<td>Aspirin use (%)</td>
<td>15 (100)</td>
<td>49 (89)</td>
<td>0.329</td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>6 (40)</td>
<td>34 (62)</td>
<td>0.152</td>
</tr>
<tr>
<td>% Stenosis (IQR)</td>
<td>85 (80–90)</td>
<td>80 (70–90)</td>
<td>0.019*</td>
</tr>
<tr>
<td>Symptomatic (%)</td>
<td>15 (100)</td>
<td>30 (55)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Ever smoker, past or present history of smoking; IHD, ischaemic heart disease (history of MI, or angina requiring medication); BP, hypertension (requiring medication); DM, diabetes mellitus (requiring long-term intervention including dietary modification); Carotid stenosis, median duplex ultrasound estimate of carotid stenosis severity (%); Emboli-positive, one or more embolus detected during pre-operative or dissection phase prior to carotid artery clamping; IQR, interquartile range; Symptomatic, carotid territory symptoms in 6 months prior to surgery. \( p \)-Values calculated using unpaired \( t \)-test for mean age, Mann–Whitney \( U \)-test for carotid stenosis and Fisher’s exact test for the rest. *Significant.
of embolisation in the dissection phase of their procedure (between the pre-operative and post-operative blood samples). Those with no emboli detected showed no change in plasma MMP-9 levels. The rise in MMP-9 was more marked in those patients with greater than two emboli detected. This suggests an association between plasma MMP-9 levels and cerebral embolisation rather than a phenomenon related to localised increased plaque production or generalised inflammation.

Human post-mortem studies have shown increased cerebral MMP-9 levels in association with an intense inflammatory reaction following stroke. Animal models of cerebral ischaemia have demonstrated an increase in cerebral MMP-9 with ischaemic injury. Further in vivo studies have shown an increase in plasma MMP-9 levels in a rat model of middle cerebral artery occlusion.

Kai et al. reported raised peripheral blood levels of MMP-9 in patients with acute coronary syndromes. Early and significant increases in circulating MMP-9 were demonstrated in patients with unstable angina/MI compared to stable angina and normal controls. The authors failed to show an association with CK or CK-MB isoforms, and therefore suggested the rise in MMP-9 was unlikely to be secondary to end-organ myocardial ischaemia. However, it should be recognised that CK and CK-MB do not correlate well with the volume of myocardial damage, and the number of patients in the study was small.

A more recent study of 1127 patients with coronary artery disease identified baseline plasma MMP-9 levels to be a novel predictor of cardiovascular mortality. The MMP-9 levels correlated with other

### Table 2. Comparison of plasma MMP-9 concentrations between emboli groups

<table>
<thead>
<tr>
<th>MMP-9 (ng/ml)</th>
<th>Pre-operative plasma</th>
<th>Post-operative plasma</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emboli-negative (n = 55)</td>
<td>7.1 (4.1–13.5)</td>
<td>7.7 (4.2–21.0)</td>
<td>0.364</td>
</tr>
<tr>
<td>Emboli-positive (n = 15)</td>
<td>8.8 (3.4–20.0)</td>
<td>14.9 (9.0–28.6)</td>
<td>0.038*</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.506</td>
<td>0.025*</td>
<td></td>
</tr>
</tbody>
</table>

MMP-9 concentrations shown as median values (and interquartile ranges) in ng/ml of plasma. p-Values calculated using Mann–Whitney U-test. *Significant.

### Table 3. Comparison of plasma TIMP-1 concentrations between emboli groups

<table>
<thead>
<tr>
<th>TIMP-1 (ng/ml)</th>
<th>Pre-operative plasma</th>
<th>Post-operative plasma</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emboli-negative (n = 55)</td>
<td>967 (875–1084)</td>
<td>1033 (840–1243)</td>
<td>0.324</td>
</tr>
<tr>
<td>Emboli-positive (n = 15)</td>
<td>1011 (884–1105)</td>
<td>1046 (795–1269)</td>
<td>0.934</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.534</td>
<td>0.937</td>
<td></td>
</tr>
</tbody>
</table>

TIMP-1 concentrations shown as median values (and interquartile ranges) in ng/ml of plasma. p-Values calculated using Mann–Whitney U-test. *Significant.
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acute phase reactants, suggesting a link with a more generalised inflammatory response.

Clearly further work is required to fully understand the reasons for, and consequences of, this rise in plasma MMP-9. An increase in systemic MMP-9 has been shown within 12 h of cardioembolic stroke, and it would be interesting to quantify the plasma MMP-9 levels earlier in the CEA post-operative period, and later following discharge to establish the time course of a return to normal levels. CT scans were not performed in our study because previous studies have reported a poor association between embolic end-organ damage and CT changes. Psychometric testing or magnetic resonance angiography may be more sensitive markers of embolic damage, and could be employed to confirm its association with the rise in MMP-9 levels.

Acknowledgements

The project was funded by The UK Stroke Association.

References


Accepted 3 February 2004