Matrix Metalloproteinase Expression After Human Cardioembolic Stroke
Temporal Profile and Relation to Neurological Impairment

Joan Montaner, MD; José Alvarez-Sabín, MD; Carlos Molina, MD; Ana Anglés, MD; Sonia Abilleira, MD; Juan Arenillas; Miguel Angel González; Jasone Monasterio, MD

Background and Purpose—Uncontrolled expression of matrix metalloproteinases (MMPs) can result in tissue injury and inflammation. In animal models of cerebral ischemia, the expression of MMP-2 and MMP-9 was significantly increased. However, their role in human stroke in vivo remains unknown. Therefore, we sought to determine the temporal profile of MMP expression in patients with acute ischemic stroke and to investigate its relationship to stroke severity, location of arterial occlusion, and total infarct volume.

Methods—Serial MMP-2 and MMP-9 determinations were made in 39 patients with cardioembolic strokes that involved the middle cerebral artery territory by means of enzyme-linked immunosorbent assay. Blood samples, transcranial Doppler recordings, and National Institutes of Health Stroke Scale (NIHSS) scores were obtained at baseline and at 12, 24, and 48 hours after stroke onset. Infarct volume was measured with CT scanning at 48 hours.

Results—No correlation was found between MMP-2 and NIHSS score at any time point, although a close relation appeared between mean MMP-9 and final NIHSS score ($r=0.486$, $P=0.002$). MMP-9 value was the only factor associated with the final NIHSS score in the multiple logistic regression model (OR 4.54, 95% CI 1.5 to 13.75). A cut-point of MMP-9 $142.18 \text{ ng/mL}$ had a positive predictive value of 94.4% to assess a patient’s NIHSS ($<8$ or $\geq8$) by the end of the study.

Final MMP-2 and MMP-9 levels were significantly lower when recanalization occurred ($528\pm144.3$ versus $681.4\pm239.2 \text{ ng/mL}$, $P=0.031$ for MMP-2; $110.2\pm100.9$ versus $244.8\pm130 \text{ ng/mL}$, $P=0.004$ for MMP-9). A positive correlation was found between mean MMP-9 and infarct volume ($r=0.385$, $P=0.022$).

Conclusions—MMPs are involved in the acute phase of human ischemic stroke. MMP-9 levels are associated with neurological deficit, middle cerebral artery occlusion, and infarct volume. (Stroke. 2001;32:1759-1766.)

Key Words: cerebral ischemia • matrix metalloproteinases • stroke, cardioembolic

MMPs are a family of zinc-binding proteolytic enzymes that normally remodel the extracellular matrix and pathologically attack substrates as part of the neuroinflammatory response. MMP-2 (72 kDa, gelatinase A) and MMP-9 (92 kDa, gelatinase B) specifically attack type IV collagen, laminin, and fibronectin, which are the major components of the basal lamina around cerebral blood vessels. Proenzyme activation and enzyme activities are tightly regulated by tissue inhibitors of MMPs (TIMPs) and interactions with surrounding extracellular matrix molecules.

MMPs participate in many physiological tissue remodeling processes, including embryological and bone remodeling, wound healing, angiogenesis, ovulation, and implantation. However, uncontrolled expression of MMPs can result in tissue destruction and inflammation.

Recently, abnormal MMP activity has been implicated in cerebral ischemia. In animal models, MMP-2 and MMP-9 expression has been shown to increase early after ischemic onset. Treatment with MMP inhibitors and MMP-neutralizing antibodies has also been shown to reduce both vasogenic edema formation and infarct size in a rat model of focal cerebral ischemia. In addition, reduced ischemic lesion volumes have been observed in MMP-9 knockout mice compared with wild-type littermates. These results suggest that the MMP family participates in the pathophysiology of cerebral ischemia.

Because this hypothesis has not been tested for humans in vivo, we sought to determine the temporal profile of MMP expression in patients with acute ischemic stroke and to investigate its relationship with stroke severity, location of arterial occlusion, and total infarct volume.
Subjects and Methods

Study Population

From June 1999 to March 2000, we prospectively studied consecutive patients with an acute stroke. A total of 110 patients evaluated within the first 12 hours of stroke onset were included in the study. A detailed history of vascular risk factors was obtained from each patient. To identify potential mechanism of cerebral infarction, a set of diagnostic tests was performed that included ECG, chest radiography, carotid ultrasonography, complete blood count and leucocyte differential, and blood biochemistry in all patients; many also underwent special coagulation testing, transthoracic echocardiography, and Holter monitoring. With this information and the neuroimaging and transcranial Doppler (TCD) data, previously defined etiological subgroups were determined. Eighty-four (76.4%) patients had a nonlacunar stroke that involved the vascular territory corresponding to the middle cerebral artery (MCA). Of these, 44 (40%) patients were considered to have had a cardioembolic stroke. Most of these patients had atrial fibrillation.

We excluded patients with a known inflammatory or malignant disease (n=3), as well as patients with an inadequate transcranial window for TCD (n=2). A total of 39 patients (51.3% male, mean age 74±15 years) were included in the definitive analysis.

Clinical examination was performed on admission and at 12, 24, and 48 hours of symptom onset, at the time of each MMP determination. Stroke severity was assessed by using the National Institutes of Health Stroke Scale (NIHSS). The NIHSS was used as a measure of initial (baseline) and final (48 hours) neurological deficit. Baseline blood samples, TCD recordings, and NIHSS scores were obtained in the first 6 hours in 80% of patients and between 6 and 12 hours in all others. If a patient died before the 48-hour time point, the last obtained score was used as the final NIHSS score.

We defined neurological improvement as a decrease in the stroke score by ≥4 points and neurological deterioration as either death or an increase in the stroke score by ≥4 points at 48 hours.10,11 We stratified patients according to the NIHSS score at each time point with previously used cut-points (<8, 8 to 20, and ≥20). Patients with a NIHSS score of ≥20 were considered to have a severe neurological deficit,12,13 and patients with a NIHSS score of <8 were considered to have a slight deficit.14

CT scanning was carried out in all patients on admission, and a control CT scan was performed at 48 hours to measure infarct volume (cubic centimeters), according to the formula 0.5×A×X×C, where A and B represent the largest perpendicular diameters through the hypodense area on CT scans, and C is the thickness of the infarction area.15

All patients received subcutaneous low-molecular-weight heparin as prophylaxis for deep venous thrombosis. Intra-venous heparin was not administered during the study period. No patient received tissue plasminogen activator. This study was approved by the ethics committee of the hospital, and all patients or relatives gave informed consent.

TCD Protocol

Serial TCD studies were conducted on admission and at 12, 24, and 48 hours of stroke onset. All TCD examinations were performed by an experienced neurologist. TCD measurements were performed with a Multi-Dop X/TCD (DWL Elektronische Systeme GmbH) device, with a hand-held transducer in a range-gated, pulsed-wave mode at a frequency of 2 MHz.

Blood flow velocities of the MCAs, anterior cerebral arteries (ACAs), and posterior cerebral arteries (PCAs) were recorded on both sides through the transtemporal window. Proximal MCA occlusion was defined as the absence of flow or the presence of minimal flow signal throughout the MCA at an insonation depth between 45 to 65 mm, whereas blood flow signal from the ipsilateral ACA and PCA was detected. Distal MCA division occlusion was indicated when there was a diffuse dampening of blood flow velocity in the affected MCA of >21% compared with the contralateral MCA.

During the follow-up control experiments, spontaneous recanalization was diagnosed when a previously absent blood flow signal reappeared (dampened or normal waveform) for a proximal MCA occlusion or when a previously dampened waveform came within the normal range for a distal MCA occlusion.17

MMP-9 and MMP-2 Determinations

Peripheral blood samples were drawn from each patient at study entry and at 12, 24, and 48 hours after stroke onset. From the 156 expected extractions, missing data for MMPs included 5 baseline determinations that were so close to the 12-hour time point that only 1 extraction was performed. No missing data existed for the 12-hour time point. Two missing items for the 24-hour time point and 6 missing items for the 48-hour time point corresponded mostly to patients who died or to technical problems.

EDTA tubes were used to collect the blood. Plasma was immediately separated via centrifugation at 3000 rpm for 15 minutes and stored at −80°C until analysis. MMP-2 and MMP-9 levels were determined with commercially available enzyme-linked immunosorbent assay (ELISA) (Biotrak; Amersham Pharmacia). ELISAs were performed according to the manufacturer’s instructions. Our laboratory reference values for healthy controls are 41±27.8 ng/mL for MMP-9 (n=62, 58% male, mean age 43 years, normal <97 ng/mL) and 53±101.8 ng/mL for MMP-2 (n=40, 47% male, mean age 43 years, normal 427 to 835 ng/mL). The mean intra-assay coefficients of variation were 8.9% for MMP-9 and 10.7% for MMP-2.

Statistical Analysis

Descriptive and frequency statistical analyses were performed and comparisons were made by use of the SPSS statistical package, version 9.0. Statistical significance for intergroup differences was assessed by the χ2 or Fisher’s exact test for categorical variables and the t test and ANOVA for continuous variables (a post hoc analysis was conducted by means of Tukey’s test). MMP values were normally distributed (Kolmogorov-Smirnov and P-P plot). A t test was performed for paired data with Bonferroni correction for multiple comparisons to compare MMP levels for different time points. To calculate the sensitivity and specificity for MMP values to predict neurological improvement as well as final NIHSS score, a receiver operator characteristic curve was configured. Two logistic regression analyses were performed to determine factors that could be considered as independent predictors for neurological improvement and final NIHSS score. Cutoff values for MMP-9 and MMP-2 with the highest sensitivity and specificity, according to outcome groups, were included. To study the correlation between MMPs and other continuous variables, the Pearson test was used except for NIHSS scores (Spearman test). P<0.05 was considered statistically significant.

Results

MMP-2 and MMP-9 Temporal Profile

Peak MMP-2 was observed at baseline determination (752.5±210.9 ng/mL) and significantly decreased on follow-up determinations (12 hours 668.8±254.6 ng/mL, P=0.011; 24 hours 599±195.4 ng/mL, P<0.001; and 48 hours 580.1±188 ng/mL, P<0.001). Mean MMP-2 level for all of these times was 644.2±185.8 ng/mL. All MMP-2 levels just indicated were included in the normality interval of our laboratory values for healthy control subjects (427 to 835 ng/mL) (Figure 1).

Baseline MMP-9 level was 147.1±118.6 ng/mL, and no significant changes on MMP-9 levels were found on follow-up determinations (12 hours 140.4±120.7 ng/mL, 24 hours 172.6±139 ng/mL, and 48 hours 144.5±127.6 ng/mL). Mean MMP-9 level for all of these times was 149.6±99 ng/mL. All MMP-9 levels just indicated exceeded the reference interval for healthy control subjects (<97 ng/mL) (Figure 1).
No difference was found for MMP-2 or MMP-9 regarding age, sex, or any vascular risk factor, except for higher MMP-2 mean levels in patients with history of a previous stroke (764.2 ± 208.7 ng/mL for patients with a previous stroke and 608.2 ± 165.6 ng/mL for first stroke patients, P = 0.025).

Mean plasma levels of MMP-2 and MMP-9 were both correlated (r = 0.340, P = 0.034). Moreover, a significant correlation was found between MMP-9 and other laboratory parameters such as glucose (r = 0.349, P = 0.037), leukocyte count (r = 0.395, P = 0.014), and mean temperature (r = 0.332, P = 0.039).

Relation to NIHSS
Median baseline NIHSS score of the series was 17 (range 5 to 25), and median final NIHSS score was 15 (range 0 to 30). No correlation was found between MMP-2 and NIHSS score at any time point. In contrast, MMP-9 was increasingly correlated to NIHSS scores at different time points (baseline: r = 0.302, P = 0.083; 12 hours: r = 0.364, P = 0.023; 24 hours: r = 0.408, P = 0.012; 48 hours: r = 0.439, P = 0.011).

There was a trend of association between stroke severity on admission and MMP-9 overexpression at 48 hours. Final MMP-9 was lower in patients with a baseline NIHSS score of <8 (15.9 ± 15 ng/mL) compared with those with a baseline NIHSS score between 8 to 20 (133.9 ± 124.3 ng/mL) and with those with a baseline NIHSS score of >20 (212.0 ± 121.9 ng/mL) (P = 0.053). Baseline NIHSS–final MMP-9 correlation was r = 0.416 (P = 0.016).

Interestingly, there also was a positive correlation between mean MMP-9 and final NIHSS score (r = 0.486, P = 0.002) as shown in Figure 2. Patients with a good neurological status at the end of the study (final NIHSS score <8, n = 13) were those who had lower MMP-9 levels at anytime (baseline: 97.8 ± 84.2 versus 177.6 ± 128.1 ng/mL, P = 0.05; 12 hours: 94.2 ± 84.7 versus 163.5 ± 130.5 ng/mL, P = 0.09; 24 hours: 87 ± 65.5 versus 213.7 ± 146.9 ng/mL, P = 0.001; 48 hours: 48.6 ± 62.4 versus 199.2 ± 123.5 ng/mL, P < 0.001) (Figure 3). Other parameters that influenced final NIHSS score are shown in Table 1.

MMP-9 value was the only factor associated with the final NIHSS score in the multiple logistic regression model (OR 4.54, 95% CI 1.5 to 13.75). A cut-point for MMP-9 of 142.18 ng/mL had a positive predictive value of 94.4% to assess a patient’s NIHSS score (<8 or ≥8) by the end of the study (Table 3).

Because NIHSS scores were obtained at different times, we classified patients according to whether they improved, remained stable, or worsened. During the study period, 14 patients improved, 10 remained stable, and 15 deteriorated. When the improving group was analyzed, we found lower MMP-2 and MMP-9 levels than in the nonimproving group (Figure 4). Other parameters related to neurological improvement are shown in Table 2. MMP-9 was the main factor associated with neurological recovery in a logistic regression model.
model (OR 12, 95% CI 1.64 to 88.09) in which a cut-point for MMP-9 of 142.18 ng/mL gave a positive predictive value of 83.33% for the detection of neurological improvement (Table 3).

Although higher MMP-9 levels were found for patients who deteriorated or remained stable (deterioration 170.6 ± 82.1 ng/mL, stability 176.2 ± 124.9 ng/mL, improvement 90.5 ± 60.3 ng/mL; \( P < 0.036 \)), when a post hoc analysis was conducted, this significance was mainly due to the difference between high MMP-9 levels in the deteriorating group and low MMP-9 levels in the improving group.

Relation to TCD

Baseline TCD showed an occluded MCA in 37 (97.4%) patients: a distal occlusion in 16 (42.1%), and a proximal occlusion in 21 (55.3%). Another patient had a normal TCD (1 missing datum existed for baseline TCD). By the end of the study (48-hour TCD), 16 patients still had occluded arteries (44.4%); 9 were distal (25%) and 7 were proximal (19.4%). Finally, a normal TCD was found in the other 20 (55.6%) patients. The remaining 3 patients died before the study period was finished.

MMP expression was related to the time the vessel remained occluded and to the extent of the occlusion (distal or proximal MCA occlusion) as observed in Table 4.

MMP-2 levels were significantly higher when the MCA remained proximally occluded during the study period compared with patients with a distal occlusion or a normal MCA at 48-hour TCD (\( P < 0.05 \) for all time points). MMP-9 levels were also higher when a proximal occlusion was recorded at 48-hour TCD (\( P < 0.05 \) for baseline and 24-hour determinations).

![Figure 3. MMP-9 levels during the study period for patients with a final NIHSS score of <8 (48 h.) and for patients with a final NIHSS score of ≥8. Values are mean±SEM. Dashed line indicates MMP-9 reference interval value for health control subjects (<97 ng/mL).](http://stroke.ahajournals.org/)

| Table 1. Main Characteristics of Patients Regarding Final NIHSS Score |
|-----------------|-----------------|---------|
| NIHSS <8 (n=13) | NIHSS ≥8 (n=26) | \( P \)  |
| Male, n (%)     | 6 (46.2)        | 14 (53.8) | 0.651  |
| Age, mean y (SD)| 76.5 (9.9)      | 72.7 (17.3) | 0.474  |
| Hypertension, n (%) | 6 (46.2) | 14 (53.8) | 0.651 |
| Hyperlipidemia, n (%) | 1 (7.7) | 5 (22.7) | 0.377  |
| Diabetes, n (%)  | 2 (15.4)        | 7 (26.9)  | 0.420  |
| Previous stroke, n (%) | 2 (15.4) | 7 (26.9) | 0.420  |
| Temperature, mean °C (SD) | 36.4 (0.3) | 36.9 (0.4) | 0.002* |
| TCD (N/DO/PO), n (%) | 0 (0)/10 (76.9/3 (23.1)| 1 (4.5)/6 (24)/18 (72) | 0.007* |
| SBP, mean mm Hg (SD) | 168.3 (20.3) | 153.0 (39.8) | 0.144 |
| DBP, mean mm Hg (SD) | 93.1 (14.3) | 81.9 (22.4) | 0.117 |
| Glucose, mean mg/dL (SD) | 128.7 (81.7) | 165.8 (94.1) | 0.253 |
| Platelets, mean (SD) | 233 538 (66 817) | 188 360 (44 900) | 0.018* |
| Leukocytes, mean X10E9/L (SD) | 8653 (1827) | 10 356 (3720) | 0.130 |
| Fibrinogen, mean g/L (SD) | 4.0 (0.8) | 3.9 (1.0) | 0.610 |
| MMP-2, mean ng/mL (SD) | 614.4 (120.3) | 659.1 (211.8) | 0.487 |
| MMP-9, mean ng/mL (SD) | 82.1 (55) | 183.43 (99.5) | 0.000* |

For TCD, N indicates normal; DO, distal occlusion; and PO, proximal occlusion. SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Baseline data are given except for temperature and MMP (mean values of the 4 time points). *Factors with a value of \( P < 0.1 \) were included in the regression model.
In the 37 patients with baseline MCA occlusion, we studied MMP levels according to whether a spontaneous recanalization was (n = 25) or was not (n = 12) present during the study period. Final MMP-2 levels were significantly lower when recanalization occurred (528 ± 144.3 versus 681.4 ± 239.2 ng/mL, \( P = 0.031 \)), and final MMP-9 levels were also lower when spontaneous recanalization occurred (110.2 ± 100.9 versus 244.8 ± 130 ng/mL, \( P = 0.004 \)).

**Relation to Infarct Volume**

Mean infarct volume was 89.77 ± 95.66 cm³. Patients with neurological improvement had smaller infarctions than did those who remained stable or worsened (15.1 ± 23.5 versus 128.7 ± 96.1 cm³, \( P < 0.001 \)), as well as those with a final NIHSS score of ≤8 (12 ± 15.7 versus 135.6 ± 96.6 cm³, \( P < 0.001 \)).

A positive correlation was found between mean MMP-9 and infarct volume (\( r = 0.385, P = 0.022 \)), as shown in Figure 2. Although there was a trend (\( r = 0.322, P = 0.07 \)) for baseline MMP-9 to correlate with the infarct volume measured in the 48-hour CT scan, the best correlation was obtained for the 48-hour MMP-9 determination (\( r = 0.500, P = 0.005 \)). No correlation existed for MMP-2 and infarct volume at any time point.

![Figure 4. MMP-9 levels according to neurological improvement. Values are mean ± SEM. Dashed line indicates MMP-9 reference interval value for health control subjects (<97 ng/mL).](image-url)

**TABLE 2. Main Characteristics of Patients With and Without Neurological Improvement**

<table>
<thead>
<tr>
<th></th>
<th>Neurological Improvement (n = 14)</th>
<th>Stability or Deterioration (n = 25)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>7 (50)</td>
<td>13 (52)</td>
<td>0.905</td>
</tr>
<tr>
<td>Age, mean y (SD)</td>
<td>73.6 (17.6)</td>
<td>74.2 (14.1)</td>
<td>0.909</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>5 (35.7)</td>
<td>15 (60)</td>
<td>0.146</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>1 (7.1)</td>
<td>5 (20)</td>
<td>0.391</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>1 (7.1)</td>
<td>8 (32)</td>
<td>0.077*</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>2 (14.3)</td>
<td>7 (28)</td>
<td>0.330</td>
</tr>
<tr>
<td>Temperature, mean °C (SD)</td>
<td>36.4 (0.3)</td>
<td>36.8 (0.4)</td>
<td>0.013*</td>
</tr>
<tr>
<td>NIHSS (&lt;8/8–20/&gt;20), n (%)</td>
<td>1 (7.7)/10 (76.9)/2 (15.4)</td>
<td>2 (8.3)/14 (58.3)/8 (33.3)</td>
<td>0.482</td>
</tr>
<tr>
<td>TCD (N/DO/PO), n (%)</td>
<td>0 (0)/9 (64.3)/5 (35.7)</td>
<td>1 (4.2)/7 (29.2)/16 (66.7)</td>
<td>0.095*</td>
</tr>
<tr>
<td>SBP, mean mm Hg (SD)</td>
<td>158.4 (27)</td>
<td>156.4 (37.6)</td>
<td>0.866</td>
</tr>
<tr>
<td>DBP, mean mm Hg (SD)</td>
<td>86.5 (12.8)</td>
<td>84.5 (23.3)</td>
<td>0.734</td>
</tr>
<tr>
<td>Glucose, mean mg/dL (SD)</td>
<td>130.9 (76.7)</td>
<td>167.8 (97.6)</td>
<td>0.239</td>
</tr>
<tr>
<td>Platelets, mean (SD)</td>
<td>231 428 (64 484)</td>
<td>187 708 (45 901)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Leukocytes, mean X10E9/L (SD)</td>
<td>8814 (1754)</td>
<td>10 533 (3826)</td>
<td>0.104</td>
</tr>
<tr>
<td>Fibrinogen, mean g/L (SD)</td>
<td>3.9 (1)</td>
<td>3.9 (0.9)</td>
<td>0.896</td>
</tr>
<tr>
<td>MMP-2, mean ng/mL (SD)</td>
<td>570.1 (129.8)</td>
<td>685.7 (201.4)</td>
<td>0.062*</td>
</tr>
<tr>
<td>MMP-9, mean ng/mL (SD)</td>
<td>108.1 (87.8)</td>
<td>172.8 (98.9)</td>
<td>0.049*</td>
</tr>
</tbody>
</table>

For TCD, N indicates normal; DO, distal occlusion; and PO, proximal occlusion. SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

Baseline data are given except for temperature and MMP (mean values of the 4 time points).

*Factors with a value of \( P < 0.1 \) were included in the regression model.
In the present study, peak levels of MMP-9 were found at the 24-hour determination. This observation is in line with that of a rat model of permanent MCA occlusion, in which a large increase in the MMP-9 occurred 12 to 24 hours after the injury.18 Romanic et al detected MMP-9 in the ischemic tissue within 24 hours after MCA occlusion and was maximally observed up to the 5-day time point. MMP-2 expression was also detected in ischemic tissue but predominantly at the 5-day time point.

Although we identified a baseline peak of MMP-2 levels, we cannot rule out a delayed (>48 hours) or very early (before the baseline determination) elevation of MMP-2. In fact, in a nonhuman primate stroke model, latent MMP-2 was significantly increased in the basal ganglia 1 hour after MCA occlusion compared with control subjects.6

The temporal profile of animal MMP expression varies, depending on the author and the ischemia model. Activated MMP-9 appeared 3 hours after 60 minutes of transient ischemia,19 and pro–MMP-9 induced as soon as 2 hours after the onset of ischemia in a permanent occlusion model.4 Discrepancies in temporal profile of MMPs and in the presence of active or pro-MMP forms in these animal models of cerebral ischemia may be influenced by different zymographic techniques and MMP purification methods that have allowed the threshold of MMP-9 detection to increase. ELISA methods to measure MMPs are available, and promising results are appearing in other cardiovascular fields.20 The ELISA method used in our experiment detect the proform of the MMPs as well as the MMP/TIMP complex (pro–MMP-9 and pro–MMP-9/TIMP-1; pro–MMP-2 and pro–MMP-2/TIMP-2). Several studies suggest that enzymatic activation of MMP-9 proenzyme occurs by binding at the cell surface, and activated enzyme then is rapidly degraded to prevent excess activity.21 Therefore, only very low levels of active MMP-9 may be present in tissue at any given time.8

MMPs have been studied through immunohistochemistry in humans with strokes,22,23 showing overproduction of MMP-9 during the early and proinflammatory phases of cerebral ischemia.

**Discussion**

We found an association between MMP upregulation and neurological impairment. We evaluated the role of MMPs during the acute phase of stroke by means of several approaches, including stroke severity, infarction size, and hemodynamic status. Stroke severity as measured with the NIHSS was correlated to MMP-9 levels at different time points after symptom onset. Lower MMP-9 levels were strongly associated with neurological improvement during the first 48 hours. In contrast, higher MMP-9 levels were associated with neurological deterioration in the same time frame. MMP expression was related to the time and location of MCA occlusion. In addition, a positive correlation was found between mean MMP-9 and total infarct volume.

The temporal profile of MMPs in our series indicated MMP-9 overproduction in acute stroke patients compared with control subjects. MMP-9 levels exceeded the reference values in each time point measurement. In contrast, our patients exhibited MMP-2 levels within the normal range during the study period. Although differences in MMP levels between patients and control subjects may be influenced by the younger age of the healthy control subjects, no differences were found in MMP levels regarding age among our stroke population (21 to 95 years).

**TABLE 4. MMP-2 and MMP-9 Levels at Different Time Points Depending on Final MCA Status (TCD Recording at 48 Hours From Stroke Onset)**

<table>
<thead>
<tr>
<th>TCD (48 hours)</th>
<th>Normal MCA (n=20)</th>
<th>Distal Occlusion (n=9)</th>
<th>Proximal Occlusion (n=7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 Baseline</td>
<td>612.9 (128.4)</td>
<td>853.5 (242.6)</td>
<td>903.7 (130.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>12 Hours</td>
<td>543.5 (131.7)</td>
<td>804.5 (352.1)</td>
<td>821.8 (272.7)</td>
<td>0.023</td>
</tr>
<tr>
<td>24 Hours</td>
<td>514.8 (125.6)</td>
<td>653.4 (295.0)</td>
<td>722.1 (121.8)</td>
<td>0.012</td>
</tr>
<tr>
<td>48 Hours</td>
<td>508.0 (141.6)</td>
<td>596.9 (187.4)</td>
<td>751.9 (225.8)</td>
<td>0.017</td>
</tr>
<tr>
<td>MMP-9 Baseline</td>
<td>99.4 (102.4)</td>
<td>188.1 (149.3)</td>
<td>236.4 (81.1)</td>
<td>0.032</td>
</tr>
<tr>
<td>12 Hours</td>
<td>124.6 (122.2)</td>
<td>154.2 (133.0)</td>
<td>185.7 (126.7)</td>
<td>0.528</td>
</tr>
<tr>
<td>24 Hours</td>
<td>126.2 (115.8)</td>
<td>211.2 (143.4)</td>
<td>282.4 (147.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>48 Hours</td>
<td>118.1 (108.9)</td>
<td>182.3 (173.7)</td>
<td>216.1 (72.4)</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Data from patients who died before final TCD was performed (n=3) are not included. MMP values are expressed as mean (SD) ng/mL.
stroke, whereas MMP-2 was found in the later reparative phases when the blood vessels begin to regrow. These autopsy findings identified MMP-9 in neutrophils for 1 week after the infarction. Macrophages that expressed matrilysin and MMP-2 were observed after 1 week. Clark et al. found that human brain tissue after a stroke shows MMP-2 after several months, whereas MMP-9 is elevated within days after the infarct. The later rise in MMP-2 activity would correlate better with the poststroke time course of capillary remodeling than with that of cerebral edema. In accordance with these observations, we found that MMP-2 levels were higher in patients with a previous cerebrovascular event.

In animal models and postmortem studies, a causal link between MMP overexpression and brain damage has been suggested. However, whether MMP-9 overexpression contributes to the development of infarct or simply represents a marker of the extent of brain ischemia remains unclear. Our study does not elucidate this controversy. We observed a trend of correlation between baseline MMP-9 and infarct volume measured on CT scans at 48 hours, although the best correlation was obtained for the 48-hour MMP-9 determination. The study is a correlative survey of several phenomena, and one cannot choose one explanation over the other because the cause-effect relationship between the neurological impairment and the biochemical marker was not established. Because MMP expression was related to the time of persistent vascular risk factor, MMP determination could be a useful surrogate marker in the acute phase of stroke.

In conclusion, the present study demonstrates an association between MMP-9 overexpression and stroke severity, infarct size, and the time and location of MCA occlusion. These findings suggest a deleterious role for MMP-9 in the development of brain damage after human ischemic stroke.

Acknowledgment

This study was supported in part by a grant from the Catalan Society of Neurology for cerebrovascular diseases (sponsored by Uriach).

References


Matrix Metalloproteinase Expression After Human Cardioembolic Stroke: Temporal Profile and Relation to Neurological Impairment
Joan Montaner, José Alvarez-Sabín, Carlos Molina, Ana Anglés, Sonia Abilleira, Juan Arenillas, Miguel Angel González and Jasone Monasterio

Stroke. 2001;32:1759-1766
doi: 10.1161/01.STR.32.8.1759

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/32/8/1759

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/