Matrix Metalloproteinase-9 Pretreatment Level Predicts Intracranial Hemorrhagic Complications After Thrombolysis in Human Stroke

Joan Montaner, MD; Carlos A. Molina, MD; Jasone Monasterio, MD; Sonia Abilleira, MD; Juan F. Arenillas, MD; Marc Ribó, MD; Manolo Quintana; José Alvarez-Sabín, MD

Background—Matrix metalloproteinase (MMP) expression is related to blood brain barrier disruption after cerebral ischemia. Moreover, MMP inhibitors reduce hemorrhagic transformation (HT) after embolic ischemia in tissue plasminogen activator (t-PA)–treated animals. We aimed to correlate plasmatic MMP levels with the appearance of intracranial bleeding complications in stroke patients treated with t-PA.

Methods and Results—Serial MMP-2 and MMP-9 determinations were performed (ELISA, ng/mL) in 41 strokes involving the middle cerebral artery territory in patients who received t-PA within 3 hours of stroke onset. Blood samples were obtained at baseline (pretreatment) and at 12 and 24 hours after symptom onset. Hemorrhagic events were classified according to CT criteria (petechial hemorrhagic infarctions [HI, 1 to 2] and large parenchymal hemorrhages [PH, 1 to 2]). Brain CT scan was obtained at 48 hours or when a neurological worsening occurred. HT was present in 36.5% of the patients (24.4% HI and 12.1% PH). MMP-2 values were unrelated to any subtype of HT. The highest baseline MMP-9 level (normal range 97 ng/mL) corresponded to patients who later developed a PH (PH: 270.2/87.8, non-HT: 126.3/127.5, HI: 94.6/88.7; *P* = 0.047). A graded response was found between mean baseline MMP-9 levels and the degree of bleeding (HI-1 = 37.4; HI-2 = 111.0; PH-1 = 202.5; PH-2 = 337.8). Baseline MMP-9 was the most powerful predictor of PH appearance in the multiple logistic regression model (OR = 9.62; CI 1.31 to 70.26; *P* = 0.025).

Conclusions—Baseline MMP-9 level predicts PH appearance after t-PA treatment. Therefore, we suggest that MMP determination may increase the safety profile for thrombolysis and, in the future, anti-MMP drugs might be combined with t-PA to prevent hemorrhagic complications. (Circulation. 2003;107:598-603.)

Key Words: metalloproteinases ▪ stroke ▪ thrombolysis ▪ hemorrhage

The trial conducted by the National Institutes of Neurological Disorders (NINDS) demonstrated a beneficial effect of intravenous tissue plasminogen activator (t-PA) when given <3 hours after symptom onset of ischemic stroke. However, the beneficial effect obtained by thrombolysis-induced recanalization may be decreased by the risk of hemorrhagic transformation (HT). Therefore, it is critical to identify the underlying mechanisms of this complication to improve the safety profile of thrombolytic agents for stroke treatment.

Matrix metalloproteinases (MMPs) are a family of zinc-binding proteolytic enzymes that normally remodel the extracellular matrix (ECM). MMP-2 and MMP-9 specifically attack type IV collagen, laminin, and fibronectin, which are the major components of the basal lamina around cerebral blood vessels. In animal models of cerebral ischemia, expression of these MMPs was significantly increased and related to blood brain barrier (BBB) disruption, edema formation, and HT. Recently, MMP inhibition has been demonstrated to reduce the presence and extent of hemorrhagic complications in t-PA–treated animals with the use of an embolic ischemia model.

In contrast to the evidence implicating MMPs in thrombolysis-related hemorrhage in animals, no data are available for humans receiving t-PA after an ischemic stroke. We hypothesize that after degradation of BBB by MMPs, t-PA induced reperfusion may generate blood elements extravasation leading to severe HT.

Methods

Study Population

Patients with an acute stroke admitted to the emergency department of a University Hospital were prospectively studied. Our target group consisted of patients who had had an acute ischemic stroke and were admitted within the first 3 hours after symptom onset. A total of 146 consecutive patients with a nonlacunar stroke involving the vascular
territory of the middle cerebral artery (MCA) were evaluated. Of these, 138 (94.5%) underwent urgent carotid ultrasound and transcranial Doppler (TCD) examinations. Ninety-eight (71%) patients had a documented MCA occlusion on TCD. Of these, 45 (45.9%) patients with a cardioembolic stroke received t-PA in a standard 0.9-mg/kg dose (10% bolus, 90% continuous infusion during 1 hour) <3 hours after symptom onset. We excluded patients with a known inflammatory or malignant disease. Finally, 41 patients who had had an acute cardioembolic stroke in the MCA territory who received t-PA <3 hours after symptom onset were included in the final analysis.

Clinical Protocol
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 leukocyte differential and blood biochemistry in all patients; when indicated, some patients also underwent special coagulation tests, transthoracic echocardiography, and Holter monitoring. With this information, as well as the neuroimaging data, previously defined etiologic subgroups were determined. Most of the recruited patients (85%) had a cardioembolic stroke due to an atrial fibrillation. Clinical examination was performed on admission and at 12, 24, and 48 hours from symptom onset. Stroke severity was assessed with the National Institutes of Health Stroke Scale (NIHSS). We defined neurological improvement as a decrease in the stroke score by ≥4 points and neurological deterioration as either death or an increase in score by ≥4 points at 48 hours.

Intravenous heparin was not administered during the study period. This study was approved by the Ethics Committee of the hospital, and all patients or relatives gave informed consent.

Computed Tomography
On admission, all patients underwent a CT within the first 3 hours of stroke onset, which was repeated after 48 hours (or earlier when rapid neurological deterioration occurred) to evaluate the presence of HT.

CT scans were reviewed by a neuroradiologist with extensive experience in acute stroke who was blinded to clinical details and MMP results. Presence and type of HT were defined according to previously published criteria. Hemorrhagic infarction (HI) was defined as a petechial infarction without space-occupying effect, and parenchymal hematoma (PH) was defined as hemorrhage with mass effect. For statistical analysis, both subtypes of HI and PH were considered together (HI-1, small petechiae along the margins of the infarct; HI-2, more confluent petechiae within the infarcted area; PH-1, when hematoma involved ≤30% of the infarcted area with some slight space-occupying effect; and PH-2, when hematoma involved >30% of the infarcted area with substantial mass effect, or clot remote to the infarcted area). Symptomatic intracranial hemorrhage was defined as blood at any site in the brain on the CT scan and documentation of neurological deterioration.

The presence of hyperdense MCA sign, early focal hypodensity, or swelling as a result of developing infarction on baseline CT was assessed according to ECASS criteria. No patient with a hypodensity involving >33% of the MCA territory received t-PA in this study.

MMP-2 and MMP-9 Immunoassays
Peripheral blood samples were drawn from each patient at study entry (before t-PA administration) and at 12 and 24 hours from stroke onset. EDTA tubes were used to collect the blood. Plasma was immediately separated by centrifugation at 3000 rpm for 15 minutes and stored at −80°C. MMP-2 and MMP-9 levels were determined in duplicate by commercially available enzyme-linked immunosorbent assay (ELISA, Biotrak Amersham Pharmacia) and the mean value of both determinations was used. All ELISAs were performed according to the manufacturer’s instructions. Our laboratory reference ranges for healthy controls were: 41±27.8 ng/mL for MMP-9 (n=62, 58% males, mean age 43 years, normal range <97 ng/mL) and 630.8±101.8 ng/mL for MMP-2 (n=40, 47% males, mean age 43 years, normal range 427 to 835 ng/mL). The mean intra-assay coefficients of variation were <10% for both MMPs.

Statistical Analyses
Descriptive and frequency statistical analysis were obtained and comparisons were made by use of the SPSS statistical package, version 9.0. Statistical significance for intergroup differences was assessed by the Fisher’s exact test for categorical variables and the Student’s t test and ANOVA for continuous variables. MMP values were normally distributed (Kolmogorov-Smirnov and P-P plot). Baseline MMP or mean MMP levels (mean value of the 3 time-points) were used for different analyses. When indicated, Mann-Whitney U and Pearson tests were used. To calculate the sensitivity and specificity for MMP values to predict HT, a receiver operator characteristic curve was configured. A logistic regression analysis was performed to determine factors that could be considered independent predictors of HT subtypes. A probability value <0.05 was considered statistically significant.

Results
We included in the study 41 patients (61% women) with an acute cardioembolic stroke in the MCA territory. Mean age was 70±10.6 years (range 41 to 86 years). A total of 68.4% of patients were hypertensive, 24.3% were dyslipemic, and 23.7% had a history of diabetes mellitus. NIHSS score of the series on admission was 16 (range 7 to 22). Baseline TCD detected a proximal MCA occlusion in 65.9% and a distal occlusion in 34.1% of the patients.

The temporal profile of MMP-9 and MMP-2 after stroke is shown in Figure 1. The highest level of both MMPs was found within 3 hours, at the pretreatment baseline determination, and both peaks (MMP-9=135.4±122.8 ng/mL and MMP-2=1021.7±326.9 ng/mL) exceeded the normality interval of our laboratory for healthy controls.
No differences in baseline MMP-2 or MMP-9 levels existed regarding age, sex, or early signs of infarction on CT. A positive correlation was found between mean MMP-9 and glucose levels at arrival ($r = 0.34$, $P = 0.03$). Among cardiovascular risk factors, only patients with diabetes had significantly higher baseline levels of MMP-9 (225.8 ± 144.3 ng/mL versus 112.4 ± 112.3 ng/mL; $P = 0.03$).

HT was present in 15 (36.5%) patients (10 [24.4%] HI and 5 [12.1%] PH). Of the 10 patients with an HI, 3 were HI-1 and 7 were HI-2. Among the 5 PH patients, 2 were PH-1 and 3 were PH-2.

Mean MMP-9 and MMP-2 levels did not differ in terms of the presence or absence of HT considered globally (118.3 ng/mL versus 104.1 ng/mL, $P = 0.58$ for mean MMP-9; 893.7 ng/mL versus 929.0 ng/mL, $P = 0.67$ for mean MMP-2). MMP-2 values were unrelated to any subtype of HT. In contrast, a significantly increased baseline MMP-9 expression was found among patients who later on develop a PH (Figure 2). The highest baseline MMP-9 level was found in patients with an ulcerative PH and the lowest baseline MMP-9 level in those with a HI (PH: 270.2 ± 87.8 ng/mL, non-HT: 126.3 ± 127.5 ng/mL and HI: 94.6 ± 88.7 ng/mL; $P = 0.047$). Similar results ($P = 0.002$) were found for mean values of MMP-9 during the study period: high values appeared in patients with PH (214.0 ± 83.4 ng/mL), intermediate levels in patients without HT (104.1 ± 69.2 ng/mL), and low levels in patients with HI (70.5 ± 57.2 ng/mL). Temporal profile of MMP-9 according to HT subtypes is shown in Figure 3.

A graded response was found between baseline MMP-9 levels and the degree of bleeding on CT (Figure 4). We observed very low mean baseline MMP-9 levels in the HI-1 group (37.4 ng/mL), higher but almost normal values in the HI-2 group (111.0 ng/mL), abnormally higher in the PH-1 group (202.5 ng/mL), and extremely high levels in the PH-2 group (337.8 ng/mL). A cut point for MMP-9 of 191.3 ng/mL had a sensitivity of 100% and a specificity of 78% to detect the presence of PH with a positive predictive value of 67% and a negative predictive value of 100%.

Clinical assessment revealed that 7 (17%) patients worsened, 25 (61%) improved, and 9 (22%) remained stable during the first 48 hours after admission. Among patients with an HT, those with a PH worsened (4 cases, 80%) in a significantly greater proportion ($P < 0.001$) than those with HI (0 cases, 0%) who had a very good outcome (90% improved). As expected, a higher baseline MMP-9 level was observed in patients with symptomatic hemorrhages (289.0 ng/mL) compared with patients with nonsymptomatic hemorrhages or without HT (119.5 ng/mL, $P = 0.020$). Again the only variable related to symptomatic hemorrhages apart from very high
levels of baseline MMP-9 was the presence of dyslipemia (Table 1).

**Discussion**

This study shows for the first time that a biological marker (plasmatic MMP-9) measured before the administration of thrombolytic therapy in acute stroke patients accurately predicts intracerebral hemorrhage, the most feared complication and safety concern of this treatment.

**MMP-9 Association With Parenchymal and Symptomatic Hemorrhages**

Different definitions of HTs have been used in the large stroke t-PA trials. Using both clinical and radiological classifications, we have demonstrated the accuracy of MMP-9 pretreatment levels to predict severe large intracranial bleedings.

It has been demonstrated that only PH-2 increases the risk of early clinical deterioration; in contrast, other types of HT, particularly HI-1 and HI-2, were not associated with clinical deterioration. Similar results are found in our series: baseline MMP-9 levels were very low in patients who later displayed an HI, most of whom improved, and very high MMP-9 baseline levels were found in PH patients, most of whom worsened or died.

Until now only clinical or radiological parameters such as age, the severity of neurological deficit, or the extent of initial hypodensity on baseline CT scan have been associated with the occurrence of a PH. PH-2 occurs more frequently in patients with hypodensity >33% on early CT, but patients

| TABLE 1. Main Baseline Characteristics of Patients According to the Presence or Absence of a Parenchymal Hemorrhage or to the Presence or Absence of a Symptomatic Hemorrhage |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                   | No PH (n=36)    | PH (n=5)        | P               | No SH (n=37)    | SH (n=4)        | P               |
| Women            |                 |                 |                 |                 |                 |                 |
| Age, y           | 69.5 (66.7)     | 73.6 (80.0)     | 0.42            | 69.3 (67.6)     | 73.7 (75.0)     | 0.44            |
| Hypertension     | 22 (66.7)       | 4 (80.0)        | 0.64            | 23 (67.6)       | 3 (75.0)        | 1.0             |
| Dyslipemia       | 6 (16.7)        | 4 (80.0)        | 0.009*          | 7 (18.9)        | 3 (75.0)        | 0.039*          |
| Diabetes mellitus| 8 (24.2)        | 1 (20.0)        | 1.0             | 9 (26.5)        | 0 (0)           | 1.0             |
| Previous stroke  | 3 (8.7)         | 1 (25.0)        | 0.39            | 4 (12.5)        | 0 (0)           | 1.0             |
| NIHSS score      | 16.1 (15.8)     | 15.8 (12.5)     | 0.92            | 16.0 (16.5)     | 16.25 (10.0)    | 0.92            |
| Proximal occlusion| 23 (63.9)      | 4 (80.0)        | 0.64            | 24 (64.9)       | 3 (75.0)        | 1.0             |
| CT early signs   | 19 (54.3)       | 4 (80.0)        | 0.37            | 20 (55.6)       | 3 (75.0)        | 0.62            |
| Systolic blood pressure, mm Hg | 153.7 | 161.0 | 0.54 | 154.7 | 155.0 | 0.98 |
| Diastolic blood pressure, mm Hg | 82.6 | 84.7 | 0.76 | 82.9 | 82.6 | 0.97 |
| Glucose, mg/dL   | 156.4 | 168.6 | 0.76 | 157.7 | 151.5 | 0.89 |
| Platelets        | 247 868 | 175 500 | 0.14 | 248 322 | 136 000 | 0.052 |
| Leukocyte, x10E9/L | 9090 | 9566 | 0.81 | 9241 | 8200 | 0.68 |
| Partial thromboplastin time, s | 31.2 | 29.2 | 0.51 | 30.9 | 30.1 | 0.80 |
| Fibrinogen, g/L  | 3.5 | 3.1 | 0.33 | 3.5 | 3.2 | 0.55 |
| MMP-2, ng/mL     | 1027.1 | 983.7 | 0.80 | 1017.8 | 1059.3 | 0.83 |
| MMP-9, ng/mL     | 116.1 | 270.2 | 0.016* | 119.5 | 289.0 | 0.020* |

Values are mean or n (%), as applicable. PH indicates parenchymal hemorrhage; SH, symptomatic hemorrhage.

*Factors with a value of \( P < 0.05 \).
TABLE 2. Factors Associated With Parenchymal Hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>Univariate Analysis OR (95% CI)</th>
<th>Logistic Regression OR (95% CI)</th>
<th>logLR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline MMP-9</td>
<td>8.63 (1.43–51.71)</td>
<td>9.62 (1.31–70.26)</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Dyslipemia</td>
<td>6.94 (1.28–37.38)</td>
<td>7.85 (1.13–54.63)</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td>Initial NIHSS score</td>
<td>0.72 (0.14–3.59)</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

with such hypodensities were excluded in our study. MMP-9 findings may thus be considered very useful, especially in a subset of patients without CT protocol violations in which almost no data may predict PH appearance. In addition, MMP-9 is potentially modifiable in contrast to other HT risk factors.

MMP-9 Effects in t-PA–Induced Hemorrhages

The precise mechanisms that underlie this negative effect of t-PA remain unclear, but they seem related to the severity of the ischemic insult as well as to the timing of t-PA-induced reperfusion. Previous results of our group support these hypotheses and stress the deleterious role of MMPs in acute stroke. We have demonstrated that delayed t-PA–induced recanalization is related to PH and that a time-occlusion dependence exists for MMPs release.

The high baseline peak of MMP-9 in PH patients may be responsible for the disruption of some components of the BBB. After experimental MCA occlusion, a gradual loss of laminin-1, laminin-5, fibronectin, and collagen IV antigens within the basal lamina occurs. A significant correlation between the development of HT and the regional loss of basal lamina after MCA occlusion has been demonstrated. MMPs released from vascular endothelium and leukocytes, during the inflammatory phase of stroke, employ collagen IV and laminin as substrates. Leukocyte plugging in microvessels of brain tissue was more frequent in hemorrhagic infarcts and in t-PA–treated animals that develop HT. It is known that neutrophils utilize MMPs for their migration.

After intracerebral injection of MMP-2, brain regions displayed necrosis, hemorrhage, and migration of blood cells to the site of the injury. However, in our study, MMP-2–treated patients, was unrelated to HT and other studies did not find constitutive expressed MMP-2 to increase the risk of HT.

In non–t-PA–treated patients, the presence of a peak of MMP-9 before the appearance of a symptomatic HT has been previously reported, which may indicate that MMP-9 itself may be responsible for some bleedings. t-PA may contribute to increase the overall rate and extension of this complication by several mechanisms. Firstly, t-PA facilitates the activation of MMPs and secondly, t-PA–induced sudden clot lysis with abrupt reperfusion may promote bleedings in the areas in which MMP-9 has already disrupted the BBB. As plasmin is involved in the cascade that processes pro–MMP-9 to the active form, the administration of t-PA may activate and promote the destructive potential of this enzyme. This hypothesis explains the reduction of t-PA–induced hemorrhages after administering an MMP inhibitor (BB-94) in a rabbit cardioembolic stroke model. A recent report demonstrates that BB-94 also reduces the extension of these bleedings. In the present study, a positive graded response exists between MMP-9 production and the degree and extension of brain bleedings.

MMP-9 Relationship to Other Factors Related With t-PA–Induced HT

Several conditions, such as hypertension, diabetes, and dyslipemia, target the microvasculature and may contribute to loss of microvascular integrity, facilitating t-PA MMP-induced rupture. An increased risk of HT among t-PA–treated patients with a history of diabetes mellitus has been recently reported and supports previous findings in which high glucose levels heralded HT after thrombolysis. In our patients, a positive glucose-MMP-9 correlation exists, and patients with diabetes have higher MMP-9 levels. Interestingly, connections between glucose metabolism and metalloproteinases have been recently reported. Hyperglycemia enhances MMP-9 expression and activity in endothelial cells and increases MMP-9 production in astrocytes. Because MMP-9 promoter region contains nuclear factor-κB and activator protein-1, which are redox sensitive, a potential mechanism by which glucose-induced oxidative stress may regulate MMP-9 transcription and activity has been suggested.

Study Limitations

We have used an immunoassay that detects mainly proforms of metalloproteinases. With the same approach, an increased latent MMP-9 in baboons with HT was found. However, in a future study in humans, MMP activity methods should be conducted to demonstrate if t-PA is responsible for the activation of these proteases. Finally, in this report we have a small number of patients with PH, thereafter the results of this study should be interpreted with caution although our data clearly parallel findings obtained in several animal studies.

In conclusion, our results demonstrate that MMP-9 level is a strong predictor of occurrence of parenchymal and symptomatic hemorrhages after t-PA and therefore we suggest that the determination of MMP-9 may help the physician to estimate the risk of thrombolysis and decide whether an individual can be safely treated. Presently, the therapeutic time window is narrow but in the future, the administration of t-PA in combination with an MMP inhibitor to stroke patients beyond 3 hours might reduce the likelihood of HT and could provide further therapeutic benefit. Measurement of the plasma level of MMP-9 in order to identify patients at higher risk of symptomatic bleedings seems promising, and its potential usefulness could be tested in a prospective way if a rapid test for MMP-9 was developed.

Acknowledgments

Dr Montaner is the recipient of a grant from the Instituto de Salud Carlos III for medical research training. This study was supported by a grant of the Spanish government (FIS 02/0773). We thank Dorita Quiroga and Pilar Bermudez for their technical assistance. We also thank neuroradiologists, residents, and nurses of the emergency department for their invaluable help in performing this study.

References


Matrix Metalloproteinase-9 Pretreatment Level Predicts Intracranial Hemorrhagic Complications After Thrombolysis in Human Stroke

Joan Montaner, Carlos A. Molina, Jasone Monasterio, Sonia Abilleira, Juan F. Arenillas, Marc Ribó, Manolo Quintana and José Alvarez-Sabín

Circulation. 2003;107:598-603; originally published online January 27, 2003;
doi: 10.1161/01.CIR.0000046451.38849.90
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/107/4/598

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 Circulation is online at:
http://circ.ahajournals.org//subscriptions/