Letters to the Editor

Dysregulation of the Levels of Matrix Metalloproteinases and Tissue Inhibitors of Matrix Metalloproteinases in the Early Phase of Cerebral Ischemia

To the Editor:

Castellanos et al.1 reported increased matrix metalloproteinase (MMP)-9 plasma levels to be an independent risk factor for hemorrhagic transformation in all stroke subtypes. Plasma levels of MMP-9 in patients without hemorrhage were not significantly different from controls, suggesting that MMP-9 does not play an important role in early pathophysiological events in cerebral ischemia. Elevated plasma levels of MMP-9 may therefore play a role in hemorrhagic transformation. Interestingly, similar findings were reported in an animal model of nonhuman primates: MMP-9 was only significantly increased in subjects with hemorrhagic transformation. Plasma levels of MMP-9 are elevated in such diseases as diabetes,1 carotid artery plaques,4 and atherosclerosis.5 We recently found elevated levels of MMP-9 in the plasma of patients with Alzheimer’s disease.6

We investigated levels of MMPs and tissue inhibitors of MMPs (TIMPs) in 24 patients who were admitted with a first episode of cerebral ischemia to the New York Presbyterian Hospital at Weill-Cornell Medical Center. Controls were from the outpatient department and were admitted because of headache or seizures. The mean age was not significantly different between the groups (Table). The research ethics committee approved the study, and written informed consent was obtained from all patients (or from family members when necessary). Ten milliliters of blood were collected by venipuncture within 24 hours of the onset of symptoms. Samples were immediately centrifuged at 3000g for 10 minutes and the plasma was stored at −80°C. The gelatinolytic activity of MMP-2 and MMP-9 was evaluated by zymography and TIMP-1 and TIMP-2 by reverse zymography. Additionally, ELISA kits (Amersham Pharmacia, UK) were used to determine the levels of MMP-2, MMP-9, and MMP-8 as well as TIMP-1 and TIMP-2. Differences between groups were tested by the Mann-Whitney test and were considered significant at P<0.05.

As reported by Castellanos et al.,1 levels of MMP-9 in stroke patients were not significantly different from 15 controls. Levels of MMP-9 did not correlate with infarct size (data not shown) nor with time after onset of stroke (blood drawn between 0.5 hours and 24 hours after stroke). Only 1 patient subsequently had an MRI diagnosis of hemorrhage. Levels of MMP-9 did not correlate with infarct size (data not shown) nor with time after onset of stroke (blood drawn between 0.5 hours and 24 hours after stroke). Only 1 patient subsequently had an MRI diagnosis of hemorrhage. Levels of MMP-9 did not correlate with infarct size (data not shown) nor with time after onset of stroke (blood drawn between 0.5 hours and 24 hours after stroke). Only 1 patient subsequently had an MRI diagnosis of hemorrhage.

Levels of MMP-2, MMP-8, MMP-9, TIMP-1, and TIMP-2 in the Plasma of Patients in the Early Phase of Cerebral Ischemia as Compared With Controls

<table>
<thead>
<tr>
<th></th>
<th>Stroke Patients (n=24)</th>
<th>Controls (n=15)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>71±14</td>
<td>67±17</td>
<td>0.498</td>
</tr>
<tr>
<td>MMP-2</td>
<td>314±81</td>
<td>289±64</td>
<td>0.272</td>
</tr>
<tr>
<td>MMP-8</td>
<td>117±19</td>
<td>163±63</td>
<td>0.042</td>
</tr>
<tr>
<td>MMP-9</td>
<td>101±70</td>
<td>76±35</td>
<td>0.676</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>112±11</td>
<td>88±20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>141±30</td>
<td>140±30</td>
<td>0.829</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

Levels of TIMP-1 were significantly increased in plasma samples from stroke patients as compared with controls. Generally, TIMPs inhibit active MMPs by forming noncovalent stoichiometric complexes within the catalytic site. Several recent reports suggest a role of TIMPs in brain and peripheral nerve injury and repair. TIMPs are induced after kainate-induced excitotoxic seizures in mice.7 TIMP-1 mRNA expression is decreased 2 or 3 days after injury and remains elevated in areas where initial degeneration occurs. Furthermore, TIMP-1 is expressed by Schwann cells and macrophages after sciatic nerve injury in humans.8 These studies indicate that TIMPs, by virtue of their ability to limit the extent of injury-induced matrix proteolysis, may be associated with the remodeling of neuronal circuits after injury. Brain microvascular endothelial cells upregulate TIMP-1 in response to a variety of cytokines, with the strongest effect exerted by the combination of IL-1β and TNF-α.9 Additionally, TIMP-1 blocks degradation of IL-1β by several MMPs.10 These cytokines are implicated in neurodegeneration in the early phase of stroke and are frequently found in the plasma of stroke patients. Levels of TIMP-2 in stroke patients were not different from controls. TIMP-2, which is constitutively expressed, is not significantly influenced by cytokines and growth factors.12 This further supports the hypothesis that TIMPs are upregulated in the context of cytokine expression after stroke. In an animal model of stroke, TIMP-2 inhibited MMP-2 activity and reduced proteolytic opening of the blood-brain barrier by MMP-2.13 Therefore the absence of an increase of TIMP-2 in combination with upregulated MMP-9 could also contribute to an increased risk of hemorrhage after cerebral ischemia.

We did not observe differences in MMP-2 expression between stroke patients and controls. Increased levels of MMP-2 have been found after cerebral ischemia in human brain tissue at 2 days after stroke, correlating with the poststroke time course of capillary remodeling.

Our findings suggest that there is a delicate change of the levels of matrix degrading proteins (MMPs) and their endogenous counter-regulators (TIMPs) in the early phase after cerebral ischemia. Whether these changes contribute to tissue injury or are the result of ischemia needs to be investigated in prospective studies.

Stefan Lorenzl, MD
Giovanni De Pasquale, MD
Alan Z. Segal, MD
M. Flint Beal, MD
Department of Neurology and Neuroscience
Weill Medical College of Cornell University
New York, New York


Response

We very much appreciate the letter by Lorenzl et al regarding to the levels of matrix metalloproteinases (MMPs) in patients with ischemic stroke, as their data further support our own previously published results.1

In a sample of 24 patients with a first episode of ischemic stroke, Lorenzl et al analyze the levels of MMP-2, MMP-9, and MMP-8 as well as the levels of MMP-inhibitors (TIMP-1 and TIMP-2). In accordance with our results, MMP-9 levels on admission are reported to be much higher in the only patient who subsequently developed hemorrhagic transformation of the ischemic tissue, whereas no differences are found between the levels in patients with ischemic stroke compared with control levels, a fact that may be indicating a specific role of MMP-9 in the development of hemorrhagic complications after cerebral ischemia. Levels of MMP-2 and TIMP-2 are also reported to be similar in patients and controls, whereas TIMP-1 levels are reported to be significantly higher in stroke patients. This is in agreement with previous experimental published data that demonstrate an early increase of TIMP-1, which appears at the time of maximal increase of MMP-9 in an attempt to avoid the blood-brain barrier opening, and a later increase of MMP-2 and TIMP-2 in the time course of molecules expression following cerebral ischemia at the time when the repair process begins.2

To our knowledge, no previous data have been previously published about the levels of MMP-8 in cerebral ischemia, which are reported to be significantly lower in stroke patients compared with controls in the report of Lorenzl et al.

MMP release is regulated at the transcriptional level by cytokines and growth factors, which stimulate the synthesis and secretion of pro-MMPs and also their endogenous inhibitors,3,4 so MMP levels may be reflecting the degree of the inflammatory response secondary to different processes. Due to this fact, the control subjects in the Lorenzl et al letter might have not been appropriate, since they studied patients with headache and seizures, in whom an inflammatory reaction cannot be ruled out. A rapid increase of cytokines such as interleukin (IL)-1β and TNF-α has been reported in response to limbic seizures,3 and higher levels of these cytokines have also been demonstrated in patients with some types of headache compared with healthy subjects.4 Moreover, MMP-9 levels have also been reported to be increased after seizures.5

Despite this fact, it is becoming increasingly clear that proteolytic enzymes participate in the pathophysiology of cerebral ischemia, although further and larger studies are still necessary to completely elucidate the role of MMPs and their inhibitors in acute human stroke.

Mar Castellanos, MD
Department of Neurology
Hospital Universitari Doctor Josep Trueta
Girona, Spain

José Castillo, MD, PhD
Department of Neurology
Hospital Clínico Universitario of Santiago de Compostela
Santiago de Compostela, Spain

Antoni Dávalos, MD, PhD
Department of Neurology
Hospital Universitari Doctor Josep Trueta
Girona, Spain

References


Dysregulation of the Levels of Matrix Metalloproteinases and Tissue Inhibitors of Matrix Metalloproteinases in the Early Phase of Cerebral Ischemia

Stefan Lorenzl, Giovanni De Pasquale, Alan Z. Segal and M. Flint Beal

*Stroke.* 2003;34:e37-e38; originally published online May 15, 2003;
doi: 10.1161/01.STR.0000075563.45920.24

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 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/34/6/e37

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/