Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia

GARY A. ROSENBERG, M.D.,1,2,3 AND YI YANG, M.D., PH.D.1

Departments of 1Neurology, 2Neurosciences, and 3Cell Biology and Physiology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

Vasogenic edema, which occurs due to breakdown of the blood–brain barrier (BBB). Cytotoxic edema results from energy failure, and vasogenic edema occurs when the blood vessels are damaged. Proteases and free radicals are the end result of a molecular injury cascade. Matrix metalloproteinases (MMPs) are a gene family of extracellular matrix-degrading enzymes that disrupt the BBB. Tight junction proteins (TJPs), occludin and claudin-5, which form the endothelial barrier, are vulnerable to attack by MMPs. Basal lamina proteins, such as fibronectin, laminin, and heparan sulfate, are also degraded by MMPs. Reperfusion injury leads to a biphasic opening of the BBB, with the early opening occurring several hours after the onset of reperfusion due to activation of the constitutive enzyme gelatinase A (MMP-2). This initial opening is transient and followed 24 to 48 hours later by more intense damage to the blood vessel, which is associated with the expression and activation of gelatinase B (MMP-9) and stromelysin-1 (MMP-3). Synthetic MMP inhibitors restore the early integrity of the BBB but are ineffective in the later opening. Because these inhibitors block MMPs involved in angiogenesis and neurogenesis, they also slow recovery. The challenge is to identify agents that will protect the BBB, blocking vasogenic edema without interfering with recovery.

Key Words • blood–brain barrier • edema • ischemia • matrix metalloproteinase • neuroinflammation • tight junction protein

Abbreviations used in this paper: BBB = blood–brain barrier; MCAO = middle cerebral artery occlusion; LPS = lipopolysaccharide; MMP = matrix metalloproteinase; MR = magnetic resonance; MT1-MMP = membrane type 1 MMP; rt-PA = recombinant tissue plasminogen activator; TIMP = tissue inhibitor of metalloproteinase; TJP = tight junction protein.
as metastasis of cancer, arthritic destruction of collagen in joints, rupture of atherosclerotic plaques in the heart and carotid artery, acute inflammation in multiple sclerosis, vasogenic edema in stroke, brain infections, and brain trauma. In stroke, the MMPs as well as cytokines and free radicals are induced as part of an inflammatory response related to hypoxia. The action of MMPs is regulated by four endogenous TIMPs. In addition to their destructive role in stroke, MMPs and TIMPs are important in the recovery phase when they facilitate angiogenesis and neurogenesis. Because of the wide range of diseases that involve pathological changes in the MMPs, there is great interest in the development of synthetic inhibitors of MMPs that could be used in therapy. These endeavors have met with mixed success because of the complex roles that MMPs play not only in injury but also in normal healing processes.

Proteolysis of TJP

Interfaces between the systemic circulation and brain tissues preserve the internal milieu of the brain. The BBB tightly regulates the brain fluid environment by controlling the transport of substances into and out of the brain. The junction proteins, which form the tight junctions between the endothelial cells, assemble in the clefts of the cerebral blood vessels to restrict transport across the BBB. Several TJPs have been isolated and cloned. Within the cerebral endothelial cells are zona occludin molecules anchored to the actin cytoskeleton. Claudins form the tight junctions between the endothelial cells. Occludin is another of the TJPs within the clefts between the endothelial cells. At the level of the endothelial cells, which are directly in contact with the systemic circulation, the TJPs form a seal that blocks the entry of large proteins and charged
molecules. The TJPs, including claudins and occludin, are proteolytically degraded by the MMPs. In a suture stroke model in spontaneously hypertensive rats, a 90-minute MCAO with 3 hours of reperfusion resulted in cleavage of TJPs by activated MMP-2. Degradation of the TJPs was blocked by a synthetic broad-spectrum MMP inhibitor.

Gelatinase A, or MMP-2, is a constitutively expressed MMP that is normally present in the brain in a latent form and can be detected in the cerebrospinal fluid. The mechanism of MMP-2 activation involves the formation at the cell surface of a trimolecular complex of pro-MMP-2, MT1-MMP, and TIMP-2. Small amounts of TIMP-2 facilitate activation of MMP-2, whereas larger amounts inhibit activation. The MT1-MMP is activated by the intracellular proprotein convertase furin. All components necessary to activate MMP-2 are either present or induced within 3 hours after the onset of reperfusion. In the rats exposed to 90 minutes of MCAO, there was transient BBB opening 3 hours after the initiation of reperfusion. Using confocal microscopy, we have observed fragmentation of the TJPs within 3 hours, which differed from the arrayed linear structures that seemed to be “ziplocked” together (Fig. 1).

Western blotting, which was done to confirm the disruption seen on confocal microscopy, showed cleavage of the two major TJPs: claudin-5 and occludin. This breakdown could be prevented by treatment with a broad-spectrum MMP inhibitor, which adds further support to a role for MMP-2 in TJP degradation (Fig. 2). After its transient early opening, the BBB remained relatively intact until 24 to 48 hours later, when a more disruptive opening occurred. We observed that by 24 hours the TJPs had moved out of the vasculature into the adjacent astrocytes. We were unable to determine whether the TJPs were formed by the astrocytes or taken up by them after being degraded.

The restored integrity of the BBB after its early opening suggests that TJPs are initially maintained within the blood vessel clefts and would remain intact if the later, more damaging stage of injury could be blocked. At later times the inflammatory response leads to the induction of MMP-3 and MMP-9. Matrix metalloproteinases are secreted as latent enzymes that require activation. In particular, MMP-9 is activated in the extracellular space by other proteases and free radicals. Results of in vitro studies have suggested that MMP-3 activates MMP-9, but the mechanisms in vivo are less well understood.

**Role of MMP-3 in Neuroinflammation**

We have studied the role of MMP-3 in a model of neuroinflammation involving the intracerebral injection of
The region around the injection site becomes inflamed and a small region of BBB damage is induced. We have shown that opening the BBB in this model involves the induction and activation of the proinflammatory enzymes MMP-3 and MMP-9. Using immunohistochemistry, we found that MMP-3 is colocalized to the pericytes and microglia (Fig. 3). Neutrophils contain formed MMP-9 that is released in packets during inflammation.

We observed the neutrophils around the site of injection. Given that both MMP-9 and MMP-3 were in the vicinity of the blood vessels, there may have been an interaction between them.

We further explored the role of MMP-3 by using mice in which the MMP-3 gene had been knocked out. Neutrophils around the site of injection were counted with unbiased stereological methods. The MMP-3 knockout mice had fewer neutrophils than the wild type, suggesting that the MMP-3 contributed to opening of the BBB either by directly attacking the basal lamina around the blood vessels or by activating MMP-9, which in turn attacked the extracellular matrix proteins (Fig. 4). We found that MMP-3 affected the TJPs; the MMP-3 knockout mice had smaller losses of claudin-5 and occludin than did the wild type (Fig. 5). Furthermore, we showed that MMP-3 attacked proteins in the basal lamina, such as laminin, given that the MMP-3 knockout mice had larger amounts of

---

**Fig. 3.** Western gel blots and photomicrographs revealing the effects of MMP-3 on inflammation. Lipopolysaccharide was injected into the caudate nucleus, and tissue around the injection site was removed 24 hours later for analysis of protein expression. The LPS induced an MMP-mediated disruption of the BBB. A: Western blotting for MMP-3 showed the proform at 57 kD and the active form at 45 kD along with two lower, unidentified bands. The MMP-3 knockout mouse did not show the 57- or 45-kD bands on staining with MMP-3. The MMP-3 colocalized with Iba-1–immunoreactive microglia/macrophages. B: Note the MMP-3 staining in sham brain. C: An immunostain for microglia/macrophages in mice, Iba-1, was positive. D: Merged image of panels B and C. In pericytes stained for desmin, MMP-3 was seen in LPS-injected wild-type mice. E: Staining with MMP-3. F: Desmin staining. G: Merged composite of panels E and F. KO = knockout; WT = wild type. Bars = 10 μm (from Gurney et al., 2006).
Animals with MCAO for variousThus, the beneficial effect of rt-PA requires
Data in clini-
The challenge is to identify the optimal
Both of these
The rt-PA in blood is prevented from enter-
Inhibitors have been developed to block the
Vasogenic edema due to tight junction disruption
this phenomenon has been unraveled over the past sever-
Neurosurg. Focus / Volume 22 / May, 2007
morbidity and death, in treated patients.
Although the incidence of hemorrhagic transformation is
mice were done using an inflammation model rather than
surfaces. Although the studies in the MMP-3 knockout
creased significantly. The effect of reperfusion on the
BBB was studied with \(^{13}C\)-sucrose. After 6 hours of occlu-
8. Treatment of stroke with rt-PA improves outcome.\(^2\)
transformation that is estimated to be as high as 60% in
embolic stroke.\(^13\) Reperfusion in itself has been recog-
nized for more than 50 years as a major cause of bleeding
in the brain.\(^9\) The rt-PA in blood is prevented from enter-
ing the brain by the BBB. In the early stages of infarction,
there is a transient opening of the BBB. When rt-PA is
given by 3 hours after the onset of stroke, as is recom-
manded, the BBB is compromised and rt-PA extravasates
into the brain. Once in contact with brain cells, the rt-PA
activates the MMPs, causing an attack on the BBB with
hemorrhage.\(^19\) Thus, the beneficial effect of rt-PA requires
that it remain within the vessels.
Synthetic inhibitors of MMPs block the opening of the
BBB.\(^29\) Inhibitors have been developed to block the
MMPs involved in the metastasis of cancer, and most of
the clinical trials have been focused on cancer treat-
ment.\(^22\) The most effective MMP inhibitors currently
used are hydroxamate-based compounds.\(^13\) Data in clini-
cal trials have shown some benefit for their use in the
treatment of advanced cancer, but side effects related to
joint pain due to the loss of extracellular matrix remodel-
ing have forced the closure of these trials. Given the role
of the MMPs in the early opening of the BBB and in hem-
orrhage, it was reasoned that these agents would be useful
in rt-PA treatment. An MMP inhibitor was tested in the
multiple emboli model of stroke and was shown to reduce the
number of hemorrhages.\(^17\)
Personnel at our laboratory have studied the effect of a
broad-spectrum MMP inhibitor, BB-94, administered in-
traperitoneally in rats.\(^25\) Animals with MCAO for various
times underwent reperfusion. After 1.5 hours of occlusion
and 16.5 hours of reperfusion, only 1 of 12 animals died,
even when given 10 mg/kg of rt-PA, which is the dose
used in rats (Fig. 7A). After 6 hours of occlusion and 12
hours of reperfusion, however, death occurred in a signifi-
cantly increased number of animals, that is, one of six
animals without treatment and five of six with rt-PA treat-
ment. When BB-94 was given shortly before treatment
with rt-PA in animals that had undergone 6 hours of occlu-
sion and 12 hours of reperfusion, the death rate was
decreased significantly. The effect of reperfusion on the
BBB was studied with \(^{13}C\)-sucrose. After 6 hours of occlu-
sion and 1 hour of reperfusion, the sucrose space (the ratio
of sucrose in the brain to that in the blood) was greater
than 7%. Treatment with BB-94 significantly reduced the
opening (Fig. 7B). This result indicates that the protection
provided by the MMP inhibitor was most likely attribut-
able to the prevention of opening the BBB and the inabil-
ity of the rt-PA to enter the brain.

Matrix Metalloproteinase Inhibitors for
Treatment of Vasogenic Brain Edema
The toxicity of rt-PA occurs when there is extravasation
of rt-PA from the blood into the brain, where it activates
glutamate receptors in neurons and increases the activity
of the MMPs.\(^23,35\) The challenge is to identify the optimal
MMP inhibitor and the appropriate time window for its
use. Authors of several studies have identified MMP in-
hibitors as possible treatments for vasogenic edema. In an
earlier study, we found that another broad-spectrum MMP
inhibitor, BB-1101, was also effective.\(^29\) Both of these

Role of MMPs in rt-PA–Induced
Intracerebral Bleeding
Treatment of stroke with rt-PA improves outcome.\(^2\)
Although the incidence of hemorrhagic transformation is
similar in treated and untreated patients, there is a greater
risk of symptomatic intracerebral hemorrhage, resulting in
morbidity and death, in treated patients.\(^4\) The reason for
this phenomenon has been unraveled over the past sever-
al years. Normally there is an incidence of hemorrhagic
laminin than did the wild type (Fig. 6). Most likely, the
MMP-3 directly attacked the laminin considering that it is
a known substrate of the enzyme, although it is possible
that other MMPs, such as MMP-9, contributed to the at-
tack on the TJPs. Because they are also formed as latent
enzymes, a mechanism for activation is needed.
When the inflammatory MMPs are present, they are
free to move within the extracellular space, where they
could create greater damage than those attached to the cell
surfaces. Although the studies in the MMP-3 knockout
mice were done using an inflammation model rather than
an ischemic one, the mechanisms of damage to the BBB
are probably similar between the two types of injuries and
could explain the marked loss of TJPs from the cerebral
endothelial cells seen at the time of the second opening of
the BBB. The early opening was dominated by the MMP-
2 bound to the cell surface by the MT1-MMP, which
restricted damage to the vicinity of the cell surface in the
immediate region. When the more generalized inflamma-
tory response took place at 24 to 48 hours, there was a
release of the unbound MMP-9 and MMP-3, and the dam-
age caused by the unconstrained proteases in the extracel-
ular space was more extensive.

![Fig. 4. Bar graph demonstrating results of stereological meth-
ods for neutrophil counts 24 hours after LPS or saline (SAL)
injection into the caudate nucleus. Counts of myeloperoxidase-
immune reactive neutrophils were greater in the LPS-injected caudate
nucleus in both wild type (WT) and MMP-3 knockout (KO) mice. In the MMP-3 knockout mice, however, there were signifi-
cantly fewer neutrophils in the caudate nucleus compared with
those in the wild type mice, in both saline- and LPS-injected
hemispheres. Four mice for each WT and KO group. *p < 0.01,
**p < 0.001. (From Gurney et al., 2006.)](image-url)
agents are very poorly soluble, making it unlikely that they will be used in clinical trials.

Confounding the use of the MMP inhibitors is the fact that MMPs play an important role in the recovery phase and possibly in the normal turnover of extracellular matrix. In a recent study, the authors showed that MMPs are critical for recovery because they are involved in both angiogenesis and neurogenesis; MMP inhibitors slowed recovery after cerebral infarction. An antibody to MMP-9 has been shown to reduce the size of an infarct in a model of stroke in rats. Genetically modified animals lacking the MMP-9 gene have less damage to the BBB and smaller strokes than wild-type animals. These studies have been focused on the acute phase of stroke, and the long-term effects of these agents are unclear. We have used MR imaging to document early injury to the BBB noninvasively and to measure the size of the infarct at 48 hours. After the initial MR imaging studies, animals were tested behaviorally for 4 weeks. We found that treatment for 3 days with the broad-spectrum inhibitor BB-1101 dramatically reduced the initial BBB opening, had no effect on infarct size, and caused a slowing in recovery (Sood et al., unpublished data). The use of these agents for the early prevention of BBB opening must include long-term follow-up to identify a safe therapeutic window for their use. In addition, as more is learned about the MMPs involved in the recovery phase, selective MMP inhibitors can be developed and tested.

Future Studies

Many laboratories are now engaged in studies on the role of MMPs in damage to the BBB that results in vasogenic edema. Rational use of MMP inhibitors to protect from MMP-mediated proteolysis of TJPs in stroke treatment will require a better understanding of the mechanisms of the proteolysis of TJPs by MMPs. Synthetic inhibitors have shown promise in controlling damage to both the extracellular matrix proteins that comprise the basal lamina and the tight junctions. Which inhibitors will be useful clinically remains an open question. Newer ones are being developed that act selectively against one or more of the MMPs, sparing others; such inhibitors are important because the beneficial effects may be due to one
22. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM: Ma-


---

**Fig. 7.** Bar graphs showing effects of the broad-spectrum MMP inhibitor BB-94 on the BBB in rats with cerebral ischemia induced using the suture model. Treatment with rt-PA resulted in increased deaths, which were blocked by BB-94. A: Effect of various intervals of ischemia and reperfusion with and without rt-PA treatment on different outcome parameters. In delayed reperfusion, that is, after 6 hours of ischemia, the number of deaths increased markedly in rt-PA–treated animals. Animals that had undergone permanent occlusion had much lower rates of death, which was only minimally affected by rt-PA. Groups were defined according to different ischemic intervals of 0 (sham), 1.5, 3, and 6 hours. A group of animals with permanent occlusion was included. Bars represent the means ± standard errors of the means. Significant differences between treated and untreated animals are indicated by an asterisk. The influence of different ischemic intervals on outcome parameters was analyzed using analysis of variance. B: Opening of the BBB as measured by sucrose space in rats that had undergone 6 hours of ischemia and 1 hour of reperfusion (I6R1). Compared with sham-operated animals, BBB permeability was markedly increased in untreated rats (**p < 0.01). Note that BB-94 administered 2 and 5 hours after MCAO markedly decreased BBB opening in the sham (**p < 0.01) and in the treated rats (*p < 0.05). Gray bars represent saline-treated animals; black bars, rt-PA–treated animals. N = number of rats. (Figure modified from Pfefferkorn et al., 2003.)
Vasogenic edema due to tight junction disruption


Accepted March 23, 2007.

Address reprint requests to: Gary A. Rosenberg, M.D., Department of Neurology, MSC10 5620, 1 University of New Mexico, Albuquerque, New Mexico 87131-0001. email: Grosenberg@salud.unm.edu.