Neurovascular Proteases in Brain Injury, Hemorrhage and Remodeling After Stroke

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Abstract—Matrix metalloproteinases (MMPs) mediate tissue injury during acute stroke. Clinical data show that elevated MMPs in plasma of stroke patients may correlate with outcomes, suggesting its use as a biomarker. MMP-9 signal has also been detected in clinical stroke brain tissue samples. Because tissue plasminogen activator can upregulate MMPs via lipoprotein receptor signaling, these neurovascular proteolytic events may underlie some of the complications of edema and hemorrhage that plague thrombolytic therapy. However, in contrast to its deleterious actions in acute stroke, MMPs and other neurovascular proteases may play beneficial roles during stroke recovery. MMPs are increased in the subventricular zone weeks after focal stroke, and inhibition of MMPs suppress neurogenic migration from subventricular zone into damaged tissue. In peri-infarct cortex, MMPs may mediate neurovascular remodeling. Delayed inhibition of MMPs decrease markers of remodeling, and these phenomena may be related to reductions in bioavailable growth factors. Acute versus chronic protease profiles within the neurovascular unit are likely to underlie critical responses to stroke, therapy, and recovery. (Stroke. 2007;38[part 2]:748-752.)

Key Words: matrix proteins ■ neuroregeneration ■ pathology ischemia

Extracellular protease systems become dysregulated after stroke and brain trauma. Over the past 10 years, a role for matrix metalloproteinases (MMPs) and plasminogen activators has been delineated. MMPs comprise a family of zinc-endopeptidases that are collectively capable of degrading essentially all components of neurovascular matrix in the central nervous system.1 And it is now recognized that plasminogen activators are not only important in blood, but also play critical roles in brain matrix physiology.2

MMPs in Acute Brain Injury

MMPs are rapidly upregulated after cerebral ischemia in animal models and clinical stroke patients.3,4 By degrading neurovascular matrix, MMPs may mediate blood-brain barrier leakage, edema and hemorrhage.5 By disrupting cell-matrix homeostasis, MMPs may trigger cell death.6,7 Although there are many MMPs, an important role for MMP-9 in particular has been suggested. MMP-9 knockout mice are protected against brain trauma, focal cerebral ischemia, and transient global cerebral ischemia.8–10 A relatively specific inhibitor of MMP-9, SB-3CT, reduces infarction after transient focal cerebral ischemia.11

In a mouse model of intracerebral hemorrhage, MMP-9 is upregulated in neurons and reactive astrocytes surrounding the hematoma.12 MMP-9 knockout mice experience less brain edema compared with wild-type mice. Interestingly, MMP-9 from both brain and blood seem to participate in this pathogenic process. Deletion of MMP-9 from blood alone or brain alone seemed to impart equal protection as deleting MMP-9 from both compartments. Ultimately, oxidative stress may be involved because free radical scavengers reduce hemoglobin-induced MMP-9 in astrocyte cultures.

Emerging clinical data now supports the relevance of the MMP hypothesis. MMP-9 is increased in plasma of acute stroke patients and demonstrates some correlation with clinical outcomes, perhaps suggesting its potential use as a biomarker.13,14 And MMP-9 is detected in brain tissue sections from ischemic and hemorrhagic strokes as well.15

Nevertheless, the sources of MMPs remain to be fully clarified. In global cerebral ischemia, MMP-9 appears to be increased in hippocampal neurons.10 But in some models of focal ischemia, significant MMP-9 signal seems to come from microvessels and associated astrocytic endfeet.9 The contribution of inflammatory cells may also need to be considered. In some studies, neutrophil depletion did not significantly alter MMP-9-mediated edema and infarction,16 whereas in other studies, infiltrating neutrophils and reactive microglia seemed to be critical sources of MMP-9 signal.17,18 In part, some of these differences may be attributable to technical issues such as differences between antibodies, antigen exposure within damaged matrix, and inherent differences between in situ zymography and immunostaining. The former detects net enzyme activity that may not be entirely specific for MMPs, whereas the latter is more...
specific for MMP isoforms depending on the antibody used, although it may not distinguish between proform zymogens and active cleaved enzyme. Nevertheless, taking all studies into account suggests that multiple brain cells can synthesize MMPs. These interactions between parenchymal and blood-borne MMPs further underlie the importance of the neurovascular unit concept, wherein integrative responses at the blood-brain interface may mediate the pathophysiology of stroke and neurodegeneration.19–21

Tissue Plasminogen Activator and MMP Interactions

Although tissue plasminogen activator (tPA) is well known for its role in blood homeostasis, tPA can also participate in neural matrix physiology. tPA knockout mice are protected against stroke.22 And tPA inhibitors such as neuroserpin reduce brain injury after cerebral ischemia.23 A clinically relevant connection between stroke outcome and MMPs rely on the observation that tPA may upregulate MMPs in cerebral endothelial cell cultures, in part by signaling through the lipoprotein receptor–related protein.24 In vivo, exogenous tPA upregulates brain MMP-9 after transient focal cerebral ischemia, and ischemic MMP-9 levels are reduced in tPA knockouts.25 MMP inhibitors reduce tPA-induced hemorrhagic transformation in hypertensive rats.26 Clinically, tPA-treated patients show higher plasma levels of MMP-9.14 Therefore, it is possible that some of the complications of hemorrhage and edema that plague thrombolytic therapy may be explained in part by this tPA-MMP hypothesis.5,21,27 These data would suggest that combining MMP inhibitors with tPA might increase the therapeutic time window for safe and effective reperfusion.

MMPs and Brain Remodeling After Stroke

The first 2 sections of this minireview summarized data supporting the idea that dysregulation in neurovascular proteases such as MMPs may underlie tissue damage during acute stroke and in part explain some of the complications of thrombolytic therapy. In this section, we explore the idea that in contrast to acute pathology, neurovascular proteases might contribute to beneficial remodeling during stroke recovery (Figure).

In the brain, MMPs are expressed during development and contribute to morphogenesis of the central nervous system.28 MMPs affect cell-cell and cell-matrix interactions by cleavage of extracellular matrix proteins and regulation of the intercellular microenvironment. MMPs may also modulate bioavailable levels of various growth factors by processing proform precursors or by liberating active molecules from matrix-hidden compartments.

The subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the hippocampus are critical structures for neurogenesis in adult brain. Increased neurogenesis can be triggered by the central nervous system insults such as stroke, trauma and seizure.29–31 Stroke leads to the expansion of SVZ and produces BrdU-labeled immature cells and doublecortin-positive neural precursors in the SVZ.32,33 Although what initiates and promotes endogenous neurogenesis has not been fully understood, upregulation of stem cells and growth factors are likely to affect stroke-induced neurogenesis.34,35 Implantation of stem cells can improve functional recovery in experimental stroke.36 Newly generated neural precursors migrate toward infarcted areas, where they differentiate and express markers of neostriatal spiny neurons near damaged areas.37 Several lines of evidence appear to support the idea that MMPs may play a role in neuroblast migration. During postnatal development, MMP-9 is associated with granule cell migration in the cerebellum,37 and migration of oligodendrocyte progenitors requires MMPs.1 By cleaving the inhibitory substrates Nogo A and myelin associated glycoprotein, MMPs can facilitate axonal regeneration.1,2 By removing insulin-like growth factor–binding protein 6 and controlling the bioavailability of insulin-like growth factor 1, MMPs participate in regulating myelogenesis during brain development.38 Mice deficient in MMP-9 show continued demyelination after injury, perhaps because of failure in clearing injury-induced deposits of NG2 proteoglycan.39 At 2 weeks after stroke in mice, MMP-9 was enhanced in the SVZ.

A schematic depicting the dual-edged nature of MMPs and other neurovascular proteases after stroke. In the acute phase, MMPs may mediate tissue injury by disrupting the blood-brain barrier (BBB) and causing cell death. There may also be a direct clinical link between MMPs and tPA-associated complications of edema and cerebral hemorrhage. In the delayed phase, neurovascular proteases may aid in the recovery process. MMPs can degrade and remove inhibitory matrix substrates, as well as process and release beneficial growth factors and stem cell signals. In rodent systems, the transition between negative to positive effects may occur within 1 to 2 days. Whether the same is true in clinical stroke remains to be determined.
and was colocalized with BrdU and doublecortin-positive neuroblast cells.33 Furthermore, inhibition of MMPs reduced the extension of neuroblast signals that extended from the SVZ into the damaged striatum. These data indicate that MMPs may contribute to endogenous repair mechanisms by helping the migration of neuroblasts after stroke.

In normal adult brain, the existence of the neurovascular niche suggests that a close association exists between neurogenesis and angiogenesis.40 After stroke, newly born neuroblasts migrate from the SVZ to peri-infarct cortex,41 and increased vascular remodeling is also found in this area.42 Vascular endothelial cells secrete growth factors and chemokines, which may support the survival of newly formed neurons. Administration of human cord blood-derived CD34+ cells after stroke induces neovascularization in the peri-infarct cortex and increases neuroblast migration to the damaged cortex.43 Because of the close relationship between angiogenesis and neurogenesis, it is possible that neurovascular proteases may mediate some of these responses in terms of matrix remodeling. In adult brain, MMP-9 is involved in hippocampal synaptic plasticity and memory.44 The MMP inhibitor GM 6001 interferes with long-term potentiation, and MMP-9 knockout mice also show impairments in long-term potentiation and learning, whereas additional treatment with exogenous recombinant active MMP-9 to the null-mutant slices completely restored the deficient long-term potentiation. MMPs also play a role in deafferentation-induced axonal sprouting, because MMP inhibitors impair functional recovery.45 Additionally, MMPs are also implicated in the regulation of angiogenesis. In mouse stress models, MMP-9 activation enhances endothelial and hematopoietic stem cells recruitment by release of the stem cell–active cytokine Kit-ligand,46 indicating that MMP-9 is required for hematopoietic stem cell mobilization. In contrast, in MMP-9 knockout mice, release of Kit-ligand and differentiation of hematopoietic stem cells are impaired, resulting in failure of neovascularization. Stromal-derived factor-1, which modulates angiogenesis by recruiting endothelial progenitor cells, is also reduced in MMP-9 knockout mice.47 Interactions between the MMP system and blood precursor pools may also be present; basal MMP-9 expression is reduced in the bone marrow compartment of endothelial nitric oxide synthase–deficient mice, which are impaired in hematopoietic recovery.48 The functional significance of these correlates may be supported by the observation that MMP inhibition reduces vascular growth and impairs long-term cardiac recovery after myocardial infarction.49

In the context of stroke, functional MRI shows that rats subjected to focal ischemia showed increased activation signals in peri-infarct cortex at 2 weeks.50 Might these areas represent foci of neurovascular remodeling, and would MMPs be involved as well? At 7 and 14 days, increased MMP-9 signals were found in peri-infarct cortex, that colocalized with NeuN-positive and GFAP-positive cells.51 Of interest, MMP-9 expression in astrocytes is correlated with markers of endothelial cells. Consistent with these finding, inhibition of MMP at 7 days after stroke results in reduction in neuronal plasticity and vascular remodeling and additional tissue damage in peri-infarct cortex.51 Similar results were recently reported whereby inhibition of MMP early in inherited kidney disease in mice improved symptoms, but delayed MMP inhibition at later stages accelerates interstitial fibrosis and death.52

MMPs can cleave many signaling molecules such as vascular endothelial growth factor (VEGF), and promote their release from matrix-bound compartments or cell surface.53 Previous studies show that VEGF has neurotrophic and neuroprotective as well as angiogenic properties.54,55 In a limb ischemia model, low-dose irradiation induces VEGF-mediated vascular regeneration in an MMP-9–dependent manner.56 After stroke, VEGF signals emerged in peri-infarct cortex, and MMP inhibition suppressed VEGF signals and in fact worsened stroke recovery.51 The specificity of these findings were supported by the fact that administering exogenous VEGF blocks the worsening of injury induced by MMP-9 inhibition. Besides VEGF, regulation of many other growth factors by MMPs may also contribute to the dynamics of tissue recovery and remodeling after stroke. Ultimately, however, the emphasis should be that matrix proteases in general (not just MMP-9) contribute to matrix and trophic remodeling during the recovery phase after stroke and brain injury. The specificity of any particular protease may not be assured. It is likely that multiple proteases function in a complex network-like fashion as the brain seeks to heal itself.

**Conclusions**

MMPs contribute to the pathology of neurovascular dysfunction during acute stages of brain trauma, stroke, and neurodegeneration. MMP inhibitors may be useful as adjunct therapies for combination with tPA thrombolysis in acute ischemic stroke. However, in the delayed phases after injury, MMPs and other neurovascular proteases may play beneficial roles by modulating extracellular matrix and trophic factors at the neurovascular interface.

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**Disclosures**

None.

**References**

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