Matrix metalloproteinases and their multiple roles in neurodegenerative diseases

Gary A Rosenberg

Matrix metalloproteinases (MMPs) and proteins containing a disintegrin and metalloproteinase domain (ADAM) are important in neuroinflammation, and recent studies have linked their actions to neurodegenerative disorders. MMPs act as cell-surface sheddases and can affect cell signalling initiated by growth factors or death receptors. Four tissue inhibitors of metalloproteinases (TIMPs) regulate metalloproteinase activity. These proteases increase the permeability of the blood–brain barrier, which can cause oedema, haemorrhage, and cell death. MMPs also participate in tissue repair by promoting angiogenesis and neurogenesis. In vascular cognitive impairment, MMPs change permeability of the blood–brain barrier and might contribute to white matter damage. MMPs and ADAMs might contribute to the formation and degradation of amyloid proteins in Alzheimer’s disease and cause death of dopaminergic neurons in Parkinson’s disease. In this Review, by examining the effects of neuroinflammation, we try to understand the role that MMPs might have in neurodegenerative diseases. Therapeutic strategies that use inhibitors of MMPs could represent potential novel treatments for neurological diseases.

Introduction

Metalloproteinases are a large family of important proteases that include matrix metalloproteinases (MMPs) and proteins with a disintegrin and metalloproteinase domain (ADAM).1 A major role of MMPs is that of sheddases at the cell surface, where they control activation of growth factors, death receptors, and other signalling molecules. These enzymes are produced in a latent form but, once activated, regulate many physiological and pathological processes. Among these processes, MMPs increase the permeability of the blood–brain barrier as part of the neuroinflammatory response in hypoxia–ischaemia, multiple sclerosis, and infection.1

MMPs cause the increase in permeability of the blood–brain barrier by attacking the extracellular matrix, basal lamina, and tight junctions in endothelial cells, resulting in the final common pathway downstream of acute neuroinflammatory damage. When acute hypoxia–ischaemia initiates the cellular damage, MMPs target the matrix proteins of blood vessels and brain cells, resulting in cytotoxic and vasogenic oedema, haemorrhagic transformation, and apoptosis of neurons and oligodendrocytes. Neuroinflammation without hypoxia–ischaemia, as occurs in infection and immunological reactions, follows another pattern of MMP-induced injury: the blood vessel remains the main site of pathological changes with mainly vasogenic oedema (which increases the extracellular space) but, without hypoxia, neuronal cell death might not occur.1

Recent studies have also implicated MMPs in the chronic neurodegeneration associated with vascular cognitive impairment, Alzheimer’s disease, and Parkinson’s disease. Additionally, MMPs have key roles in tissue repair,1 for which they activate angiogenesis and neurogenesis.

Several reviews have been published on MMPs, ADAMs, and tissue inhibitors of metalloproteinases (TIMPs) in acute neuroinflammation, however, a comprehensive discussion of the emerging role of MMPs and ADAMs in neurodegeneration is not available. Therefore, their role in neurodegenerative diseases is discussed in this Review.

Metalloproteinase structure, activation, and inhibitors

MMPs

MMPs share a common structure that comprises four main domains: the propeptide, catalytic, haemopexin-like, and transmembrane domains (figure 1). The propeptide domain contains a cysteine residue that binds zinc in the active site to form the cysteine switch. The binding of cysteine in the catalytic domain blocks the active zinc site, maintaining the latent or inactive state.1 Although there is continuous production of constitutively expressed MMPs and ADAMs, the proteins remain latent until they are activated by free radicals or enzymes that free the cysteine bond or cleave the propeptide region. Most MMPs, with the exception of the membrane-type MMPs (MT-MMPs), are secreted and act in the extracellular space. An intracellular role for MMP3 (also known as stromelysin-1) in cell death of dopaminergic neurons has been recently identified.7

MMPs are divided into four main subgroups on the basis of domain structure: collagensases, gelatinases, stromelysins, and MT-MMPs. Collagensases degrade triple-helical fibrillar collagens, which are the main components of bone and cartilage. In the brain, gelatinase A (MMP2) and gelatinase B (MMP9) have been the most intensively studied because of the ease with which they can be identified by gelatin zymography and their prominent role in injury and repair. Gelatinases degrade molecules in the basal lamina around capillaries, facilitate angiogenesis and neurogenesis, and contribute to instigating cell death (table 1). Stromelysins (MMP3, MMP10, MMP11, and MMP7 [also known as matrilysins]) are small proteases that degrade components of the extracellular matrix, although not the triple-helical fibrillar collagens. MT-MMPs contain a furin cleavage site near the propeptide region and are activated intracellularly by the proconvertase furin, and the serine protease plasmin. MT-MMPs are membrane bound and act at the cell surface as sheddases with several important functions, including activation of other proteases and growth factors.
As described above, MMPs are secreted as latent enzymes and require activation. Proteolysis is tightly regulated to prevent tissue damage. Products of a series of inducible genes, including MMP3 and MMP9, are normally present at low concentrations, but rapidly increase in quantity to cause more extensive damage to the injury site. MMP2 is activated at the cell surface by membrane-bound MMP14 (MT1-MMP). Activation implicates formation of a trimolecular complex of pro-MMP2, TIMP2, and MMP14. Binding the complex to regions close to the membrane, MMP14 constrains the action of MMP2. Because MMP2 is constitutively expressed, this constraint controls the extent of damage to the extracellular matrix, whereas MMP3 and MMP9, which are secreted into the extracellular space where they can move around freely, cause more extensive damage to the injury site.

MMP14 has an NF-κB binding site, suggesting that this gene can also be induced during inflammation. Cytokines, such as tumour necrosis factor α (TNFα) and interleukin 1β, induce transcription of MMP3 and MMP9, which is important in both acute and chronic neuroinflammation. In the case of MMP9, several activation mechanisms have been suggested, such as other proteases (eg, MMP3) and free radicals (eg, nitric oxide, which acts through -nitrosylation).9,10

ADAMs
ADAMs are transmembrane proteins that bind to integrins and are important in intracellular signalling and cell adhesion.19 Although several of these metalloproteinases have been identified, only a few are known to have roles in the brain (table 1).20 The subunits of the ADAMs comprise a catalytic domain at the end of the extracellular extension.
which is composed of three domains: a disintegrin, a cysteine-rich domain, and epidermal growth factor repeats. The cytoplasmic tail attached to the epidermal growth factor domain protrudes through the membrane and signals cell-surface events to the cytoplasm. The disintegrin domain binds to integrins whereas the cysteine-rich region interacts with proteoglycans. The catalytic region of the ADAM molecules releases bound proteins from the extracellular matrix and cell surface through a process called ectodomain shedding. Several important examples of the function of the ADAMs in the CNS include processing of amyloid precursor protein and transforming growth factor α (TGFα). ADAMs are implicated in cellular proliferation, migration, differentiation, and survival, as well as in axonal growth and myelination. For example, ADAM10 is possibly an α-secretase that cleaves amyloid precursor protein (table 1); and ADAM17 (also known as TNFα converting enzyme or TACE) activates TNF.  

**TIMPs**  
TIMPs are small proteins with molecular weights between 21 and 28 kDa; these enzymes are codified by highly conserved genes and have overlapping functions (table 2). So far, four TIMPs have been identified. TIMPs have inhibitory actions against most MMPs with some predilections: TIMP1 mainly inhibits MMP9, whereas TIMP2 inhibits MMP2 and, paradoxically, contributes to activation of pro-MMP2. TIMP3 is the only TIMP bound to the extracellular matrix. TIMP3 inhibits several membrane-bound molecules with sheddase functions, such as MMP14, MMP3, and TACE, indicating that TIMP3 plays a central part in several important reactions, including cellular growth, cellular death, and tissue repair (figure 2). TIMP3 is expressed early after ischaemia and contributes to apoptosis of neurons in the middle cerebral artery suture occlusion model. TIMP3 mRNA is overexpressed in developing brain tissue and after injury in rats. Stimulation of cultured astrocytes by lipopolysaccharide occlusion model. TIMP3 mRNA is overexpressed in developing brain tissue and after injury in rats.

**Nomenclature, molecular weights, functions, and location of TIMPs**

<table>
<thead>
<tr>
<th>TIMP</th>
<th>Molecular weight (kDa)</th>
<th>MMPs inhibited</th>
<th>Other functions</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP1</td>
<td>28</td>
<td>All MMPs</td>
<td>Strong inhibitor of MMP9</td>
<td>Secreted into the ECM</td>
</tr>
<tr>
<td>TIMP2</td>
<td>21</td>
<td>All MMPs</td>
<td>Forms trimolecular complex with pro-MMP2 and MMP14 (also known as MT1-MMP) to activate MMP2</td>
<td>Secreted into the ECM</td>
</tr>
<tr>
<td>TIMP3</td>
<td>24 (unglycosylated) or 27 (glycosylated)</td>
<td>All MMPs</td>
<td>Apoptosis, inhibits angiogenesis</td>
<td>Bound to the ECM</td>
</tr>
<tr>
<td>TIMP4</td>
<td>22</td>
<td>All</td>
<td>Inhibits angiogenesis</td>
<td>Secreted into the ECM</td>
</tr>
</tbody>
</table>

**ADAM**: a disintegrin and metalloproteinase. **ECM**: extracellular matrix. **MMP**: matrix metalloproteinase. **MT**: membrane type. **TIMP**: tissue inhibitor of metalloproteinase. **TNF**: tumour necrosis factor. **TNFR**: tumour necrosis factor receptor. **sAPP**: soluble APP. **sFas**: soluble Fas. **sFasL**: soluble Fas ligand. **sTNF**: soluble TNF. **sTNFR**: soluble TNFR. **TACE**: TNFα converting enzyme. **TGFα**: transforming growth factor α.  

**Review**  
Inflammation without hypoxia  
Cells that have not been exposed to hypoxia might survive inflammation initiated by immunological reactions or infection. In the absence of hypoxia, inflammation mainly attacks blood vessels because of leucocyte infiltration. Injury to vessels might enable extravasation of serum proteins, leading to vasogenic cerebral oedema. For example, in an acute exacerbation of multiple sclerosis, the immunological reaction is focused on the blood vessel and might not necessarily damage the surrounding cells.
However, secondary to the damaged blood vessels, cells might die as toxins enter the brain or as intracranial pressure increases. Occasionally, inflammation leads to infarction and distinction between inflammation due to hypoxia–ischaemia and immunological factors is obscured. Additionally, chronic infections or immunological reactions can lead to neurodegeneration. Chronic inflammation activates microglia and triggers macrophages to repair the tissue. During the removal of debris and remodelling of the damaged regions, toxic proteases might be released and free radicals formed. When macrophages are recruited to regions with myelinated fibres, demyelination might result as a bystander effect. However, evidence for this effect is derived from in vitro studies of the effect of plasmin and MMPs on myelin proteins and needs confirmation in vivo.22,23

Acute neuroinflammation

Acute ischaemic injury causes excitotoxic cell death through release of glutamate, production of free radicals, and activation of endonucleases that destroy DNA in the nucleus. Although blood vessels are the target of all forms of inflammation in the brain, cell death is more prominent in ischaemic injury than in infection and immunological disorders. Duration of inflammatory response also affects response to injury. When macrophages are recruited to a chronic injury site they secrete proteases, which might cause sustained damage to surrounding tissue. This chronic response to injury might actually be interpreted as an attempt at tissue repair.

Cerebral blood vessels have tight junctions and are surrounded by basal lamina, astrocytes, and neurons, forming the neurovascular unit.24 In hypoxia–ischaemia, the acute inflammatory process includes a final common pathway formed by free radicals and MMPs that attack proteins in tight junctions and components of the basal lamina, causing oedema, haemorrhage, and cell death.25 Loss of oxygen and energy substrates releases glutamate into the extracellular space, initiating molecular events in the injured cells that might result in loss of membrane integrity and necrosis.

MMPs in hypoxia–ischaemia

The basal lamina around cerebral blood vessels contains extracellular matrix proteins, including laminin, fibronectin, heparan sulphate, and type IV collagen. Proteolysis of the blood–brain barrier by MMPs results in loss of basal lamina proteins, which increases the risk of haemorrhage.26–28 MMP2, MMP3, and MMP9 increase the permeability of the blood–brain barrier. Inhibitors of MMPs can reduce damage to the blood–brain barrier.29 mice that are deficient in superoxide dismutase have an extreme response to hypoxic–ischaemic insults and have greater damage to the blood–brain barrier than do wild-type mice. In ischaemia with reperfusion, MMPs are induced and disrupt the blood–brain barrier; for instance, in an Mmp9 knockout model, focal ischaemic lesions decreased the damage to the blood–brain barrier and the infarct size.29 Cyclo-oxygenases are linked to the production of MMP9, possibly as part of the free-radical response, and inhibitors of cyclo-oxygenases restrict MMP9 production after intracerebral injection of TNFα in rodents.30 MMP3 is an inducible enzyme and its concentration increases in hypoxia–ischaemia and immunological reactions.30,31 When Mmp3 is knocked out, the normal disruption of the blood–brain barrier that occurs after intracerebral injection of lipopolysaccharide is attenuated and Mmp3 knockout mice have fewer neutrophils recruited to the site of inflammation than do wild-type mice.32

Apoptotic cell death is dependent on cell-surface death receptors.23,33 When these receptors of the TNFα family such as Fas and the TNFα receptor 1 (p55TNF-R1; TNFR1) are bound to their ligands (FasL and TNFα, respectively), apoptosis occurs. MMP3 cleaves FasL from the cell surface (cell culture studies indicate this is most probably from an adjacent neuron, although origination of FasL might be different in vivo) and apoptosis is attenuated. However, neurons deprived of glucose and oxygen undergo TIMP3-mediated apoptosis, in which TIMP3 prevents MMP3 from cleaving FasL from the cell surface.33–35 TACE cleaves latent TNFα from the cell surface of macrophages or microglia, producing the mature, active 17-kDa form. This active TNFα then binds TNFR1, promoting apoptosis. However, TACE can also release TNFα from the neuronal cell surface, most likely preventing apoptosis. TIMP3 inhibits TACE; therefore, inhibition of released active TNFα attenuates cell death, whereas inhibition of TNFR1 shedding facilitates cell death. Because of the dual actions of TACE, the exact effect of inhibition by TIMP3 is difficult to predict. Figure 3 shows the possible roles of TIMP3 in neuronal cell death via Fas and TNFR1.

MMPs in autoimmune disorders and infection

In injury initiated by a hypoxic–ischaemic insult, death of neurons and astrocytes parallels that which occurs after blood vessel damage. By contrast, when the initiating event is immunological or an infectious pathogen, the main site of injury is blood vessels alone. Whenever the blood–brain barrier is affected, MMP9 is a key factor in the injury process. Exacerbation of acute multiple sclerosis causes an increase in MMP9 concentrations in cerebrospinal fluid.36 Treatment with high-dose prednisolone, which restores blood–brain barrier integrity during a multiple sclerosis episode, lowers MMP9 concentrations in cerebrospinal fluid.37 Indexing MMP9 concentrations in the cerebrospinal fluid and blood to albumin showed that the enzyme was produced endogenously.38 In experimental allergic encephalomyelitis, an animal model of multiple sclerosis, demyelinated regions are associated with inflammation around blood vessels in the brain and the spinal cord. Treatment of animals with the MMP inhibitor GM-6001 suppresses development of clinical experimental allergic encephalomyelitis in mice.39 Experimental allergic neuritis in rats, a model for Guillain-Barré syndrome, causes an
immunologically mediated attack on peripheral nerves that is associated with inflammation around blood vessels. Inflammatory cells recruited to the blood vessels in the nerves release MMPs that disrupt the blood–nerve barrier. In rats with experimental allergic neuritis, a broad-spectrum MMP inhibitor, BB-1101, which also inhibits TACE, reduces damage to the nerves.21

**Metalloproteinases in neurodegeneration**

Evidence is emerging of long-term effects of MMPs in neurodegenerative diseases, including damage to white matter in patients with vascular cognitive impairment, degradation of amyloid peptides in Alzheimer’s disease, and apoptosis of dopaminergic neurons in Parkinson’s disease.

**MMPs in vascular cognitive impairment**

In vascular cognitive impairment, MMPs are induced by hypoxic hypoperfusion in the white matter; patients with vascular cognitive impairment express hypoxia-inducing factor 1α (HIF1α) in brain tissues with white matter hyperintensities on MRI. During hypoxia, HIF1α increases, leading to expression of many genes implicated in injury and repair.42 Furin contributes to activation of MMPs implicated in injury. HIF1α also stimulates expression of the same substances that mediate repair, including vascular endothelial growth factor (VEGF) and TGFβ.43 Because hypoxia seems to play a crucial part in vascular cognitive impairment, an understanding of the role of HIF1α is important. For example, in a rat model of vascular cognitive impairment, hypoxic hypoperfusion induces MMPs and thus increases the permeability of the blood–brain barrier in the white matter.46 Microglia involved in remodelling of blood vessels damaged by hypertension and diabetes secrete proteases, including MMPs and plasmin or plasminogen activator. These proteases secondarily break down myelin basic protein in animal brain tissue in vitro, which might also be a mechanism for demyelination associated with vascular disease in human beings.32,34,45

Patients with vascular cognitive impairment can be classified into two groups, with some overlap: patients with large-vessel pathology or multi-infarct dementia and patients with small-vessel pathology orBinswanger’s disease.48 Patients withBinswanger’s disease have extensive white matter hyperintensities on MRI, which are thought to be caused by hypoxia–ischaemia (figure 4).47 These white matter changes are also seen in patients with Alzheimer’s disease and in some healthy elderly people.46 Myelin is lost, but U-fibres and cortical cells are spared. In the small-vessel form with extensive white matter disease, the astrocytes are reactive in the white matter and oligodendrocytes are lost (figure 4). The blood vessels in the deep white matter are generally fibrotic or have fibrohyaline changes consistent with thickened basallamina and damaged endothelial cells, which would contribute to the abnormalities in the blood–brain barrier (figure 4).

When the white matter disease causes progressive symptoms such as gait instability, executive dysfunction, and incontinence, Binswanger’s disease is suspected.46 The abnormal MRI signal in white matter is associated with cognitive decline in large population studies.49 Most of the patients with white matter changes have conditions that affect the small blood vessels, such as hypertension and diabetes. The deep white matter, which forms a watershed circulation (produced by the confluence of several end arteries that originate on the surface and that provide poor circulation to the deeper regions), is vulnerable to hypoxic events.46 In hypoxic tissue, MMP expression increases in different cell types. Patients with vascular cognitive impairment have high cerebrospinal fluid concentrations of MMP9, but patients with Alzheimer’s disease do not.46 In human tissue obtained at autopsy in patients with vascular cognitive impairment, immunohistochemical staining with antibodies to MMPs showed increased expression in white matter, particularly around blood vessels in regions with loss of myelin.46
Poor perfusion of the watershed regions in deep white matter might be the mechanism of damage.51 Hypertensive vascular disease and diabetes mellitus further reduce the perfusion of these vulnerable regions. Although development of animal models has been hampered by the heterogeneity of vascular cognitive impairment, the animal model most commonly used to study the hypoxic injury to the deep white matter is that of bilateral carotid artery occlusion in rats. This procedure results in damage to white matter that includes hypoxic hypoperfusion secondary to the permanent occlusion of both carotid arteries.52 When rats with bilateral carotid artery occlusion survive longer than 3 days, hypoxic hypoperfusion leads to astrocytosis, microglia activation, loss of oligodendrocytes, and demyelination. Rats with bilateral carotid artery occlusion have high expression of MMP2 in endothelial cells and microglia in white matter.53 In a recent study, treatment with a selective MMP2 inhibitor, AG-3340, reduced blood–brain barrier injury to the white matter and resulted in less myelin damage, suggesting that disruption of the blood–brain barrier by MMP2 was upstream of the myelin damage.44 Consistent results were found with Mmp2 knockout mice.44

In brain tissue from patients with vascular cognitive impairment, inflammatory cells accumulate around hypertensive, fibrotic blood vessels.47,54 Gliotic regions have reactive astrocytes that overexpress MMP2 (figure 4), and macrophages around damaged blood vessels are immunopositive for MMP3 (figure 4).47 Therefore, MMPs might damage blood vessels, disrupting the blood–brain barrier, thus activating microglia and recruiting macrophages that will contribute to the tissue injury as they try to repair the extracellular matrix. Demyelination of ischaemic white matter might occur in both animals and human beings through an MMP-mediated mechanism, including MMP2, MMP3, and possibly MMP9.44,45

Other factors might be involved in the progressive inflammation of the white matter in vascular cognitive impairment. MMP2 is an activator of endothelin 1, which is a strong vasoconstrictor that would further compromise blood flow to the deep white matter.55,56 Pathological studies in patients with vascular cognitive impairment detect increased endothelin 1 in the white matter47 and increases in HIF1α that correlated with white matter damage.43 Figure 5 shows a theoretical mechanism linking vascular changes to hypoxia through the central action of HIF1α. The dual function of HIF1α in injury and repair suggests that, after the initial injury phase with induction of inflammatory molecules, recovery begins with the activation of molecules that will stimulate regrowth of cells and blood vessels.

Chronic intermittent hypoxia results in recurrent apnoeas with periodic decreases in arterial blood oxygen, which predispose patients to cardiorespiratory morbidities. Patients with sleep apnoea can have white matter damage, like that seen on MRI in vascular cognitive impairment.58 In rodents and cell cultures, intermittent hypoxia activates various transcription factors, including HIF1α, c-fos, nuclear factor of activated T cells, and NF-κB. Intermittent hypoxia is more potent than chronic hypoxia in activating HIF1α and c-fos and results in prolonged accumulation of their mRNAs.59

MMPs and ADAMs in Alzheimer’s disease
Neuropathological features of Alzheimer’s disease include neuronal tangles and amyloid plaques.60 Deposition of improperly processed amyloid is thought to be a main factor in the pathophysiology of Alzheimer’s disease.
Amyloid precursor protein comprises a transmembrane and an extracellular component. Secretases degrade amyloid precursor protein and the sites of cleavage determine the fate of these protein fragments. The physiological pathway results in the cleavage of the amyloid precursor by α-secretase, which produces a soluble component that can be broken down for clearance. ADAM10 and ADAM17 are possible candidates for α-secretase. Two other secretases (β-secretase and γ-secretase) act together to produce Aβ peptides, Aβ1-40 and Aβ1-42; cleavage of amyloid precursor protein by β-secretase produces a fragment of amyloid precursor protein that can be further processed to Aβ1-42 by γ-secretase. Either produces a fragment of amyloid precursor protein that is transgenic mice overexpressing the Swedish variant of APP. Treatment with the MMP inhibitor GM-6001 increased Aβ concentrations in the cerebrospinal fluid in Alzheimer's disease. MMP9 degraded amyloid peptides expressed in hippocampal neurons in patients with Alzheimer's disease. MMP9 is also expressed in hippocampal neurons in patients with Alzheimer's disease. MMP9 degraded amyloid peptides and a latent form of MMP9 was found in the brain tissue of the patients. The study authors speculated that the absence of an activated form of MMP9 might have disrupted degradation of amyloid and contributed to the accumulation of insoluble Aβ peptides in plaques. In another study, immunohistochemistry detected MMP3 expression in hippocampal neurons, around amyloid plaques in the cortex, and in the interstitium of white matter. Plasma concentrations of MMP9 are increased in Alzheimer's disease; however, there is no increase in MMP9 concentrations in the cerebrospinal fluid in Alzheimer's disease. This discrepancy remains to be resolved.

Deposition of amyloid in brain or in blood vessels in sporadic Alzheimer's disease could be due to overproduction of amyloid, as occurs in familial forms of Alzheimer's disease, or a disruption of clearance. MMP9 can catabolise Aβ. Transgenic mice carrying genes from familial forms of Alzheimer's disease, namely the APP and presenilin 1 (PSEN1) genes have increased MMP2 and MMP9 concentrations in astrocytes around amyloid plaques compared with areas without amyloid deposition. Microdialysis indicated that Mmp2 and Mmp9 knockout mice had higher levels of Aβ than wild-type mice and treatment with the MMP inhibitor GM-6001 increased Aβ in transgenic mice overexpressing the Swedish variant of APP (mutations at positions 670 and 671). MMP9 is also able to degrade the fibrillar form of Aβ. These findings suggest that MMP9 can contribute to the clearance of soluble and fibrillar Aβ. In familial forms of Alzheimer's disease, the excessive production of amyloid might be sufficient to create the amyloid deposits; however, in the more common sporadic forms, the ability to remove the amyloid that is normally produced might lead to high concentrations of amyloid in the brain (figure 6). In both of these processes, MMPs and ADAMs could have key roles.

The therapeutic strategies for Alzheimer's disease include inhibition of the enzymes implicated in amyloid peptide processing. Inhibitors of secretases offer potential to reduce the production of Aβ peptides. Deposition of amyloid peptides in tissues results in an inflammatory response that could be lessened with anti-inflammatory drugs; however, non-steroidal anti-inflammatory drugs and free-radical scavengers have not proved useful. Because ADAM10 and other secretases contribute to

![Figure 5: Possible mechanism for white matter injury in vascular cognitive impairment](image-url)

Hypertension, diabetes mellitus, and other diseases that damage blood vessels can initiate white matter injury by increasing risk of thrombosis and small strokes. If the blood flow to the watershed areas of the white matter is compromised by hypotension or by narrowing of the arteries, then hypoxia-ischaemia results, which induces inflammation. Hypoxia secondary to hypoperfusion increases HIF1α concentration, which turns on cassettes of genes associated with injury such as FURIN, and increases expression of VEGF and TGFβ, which are important in repair. Furin leads to activation of MMP2 through activation of MT-MMP. MMP2 can disrupt the tight junction proteins and open the BBB, leading to oedema. Oedema might also cause demyelination. Additionally, MMP2 might attack myelin and can activate endothelin 1, which causes vasoconstriction through calcium metabolism in the small muscle. Vasoconstriction aggravates the hypoxic state. Conversely, on the repair side, VEGF and TGFβ activate angiopoietin 2, which acts through the secretion of MMPs to initiate angiogenesis and neurogenesis. MMP=matrix metalloproteinase. MT=membrane type. TGFβ=transforming growth factor β. VEGF=vascular endothelial growth factor.

www.thelancet.com/neurology Vol 8 February 2009
production of amyloid peptides, and because MMP9 facilitates clearance of Aβ, drugs that inhibit both MMPs and ADAMs might have unexpected effects that need to be identified to determine the therapeutic potential of MMP inhibitors.

**MMP3 polymorphisms and dementia**

Genetic studies have linked polymorphisms of MMP3 to rheumatoid arthritis and cardiovascular disease, suggesting a role of these MMPs in chronic neuroinflammation.64 Homozygosity for the MMP3 6A allele was associated with worse outcome in a large cohort of patients with rheumatoid arthritis.65 In a study of lipid-lowering drugs in coronary artery disease, a beneficial effect on disease progression was associated with the 5A allele, whereas patients with coronary artery disease who were homozygous for the 6A allele (25–30% of the population) were at risk of rapid disease progression and might require more intense lipid-lowering therapy than those without that genotype to prevent disease progression.66 Homozygosity for the 6A allele has been associated with carotid artery stenosis; however, these results were obtained from only 91 patients and will need confirmation.67

In one study, patients who were *APOE* ε4 non-carriers and 6A/6A homozygous were at an increased risk of dementia.68 However, studies of MMP3 polymorphisms in the large Rotterdam cohort did not support a role for variations in MMP3 as a causal factor in dementia.69 These studies focused on dementia as a homogeneous disorder rather than separating patients into Alzheimer’s disease and vascular cognitive impairment. The identification of genetic risk factors, such as MMP3 alleles, in vascular cognitive impairment is an important goal that is currently limited by the heterogeneity of this disorder.

**MMPs in Parkinson’s disease**

Parkinson’s disease results from neurodegeneration of dopaminergic neurons in the substantia nigra, which is associated with activation of microglia. Recent studies have implicated MMPs in the death of dopaminergic neurons in this disease. In vitro, apoptotic dopaminergic neurons release MMP3, which acts as a microglia-activating molecule, suggesting that, in addition to degradation of extracellular macromolecules, MMP3 is a signalling molecule, mediating the interaction between apoptotic neurons and microglia.70 TNFα released from microglia leads to neuronal death; primary mouse mesencephalic cells in culture die when treated with BH4 (tetrahydrobiopterin), a selective dopaminergic neuronal toxin; however, treatment with the MMP3 inhibitor NNGH (N-isobutyl-N-[4-methoxyphenylsulfonyl]-glycyl-hydroxamic acid) prolongs cell survival by decreasing TNFα release from activated microglia. TNFα directly induces neuronal death, suggesting that MMP3-activated microglia might cause neuronal degeneration by releasing proinflammatory cytokines. In addition to the extracellularly triggered mechanisms of apoptosis, MMP3 acts intracellularly in the apoptotic signalling in dopaminergic cells in culture; this action of active MMP3 is linked to caspase 3.71 The mechanisms of this intracellular action of MMP3 are unclear.

**MMPs in CNS repair**

Shortly after ischaemic insult, a cascade of events is initiated that begins to repair damage. The factors involved in the repair process might be similar to those found in the healing of an epithelial wound. As in acute injury, HIF1α plays an important part by inducing genes that begin the process of growing new vessels and stimulating neurogenesis. HIF1α activates genes that are implicated in metabolism of glucose, production of red blood cells, and recruitment of cells involved in repair.42 HIF1α induces *FURIN*, which has a hypoxia-responsive element in the promoter region, as do many genes important in acute injury and repair.43 Furin is an
intracellular convertase that activates several enzymes, including MMP14.

While growing, blood vessels are dependent on the plasminogen-activator system and MMPs. Urokinase plasminogen activator, MMP2, MMP3, and MMP9 have roles in angiogenesis.77 Mechanisms of blood-vessel growth after injury have similarities to abnormal angiogenesis in brain tumours. Gliomas grow rapidly, creating a hypoxic environment with increased HIF1α concentrations; angiogenesis compensates for the absence of oxygen. Malignant glioma cell lines have increased MMP2 concentrations and low TIMP2 concentrations. The targeting of HIF1α with small interfering RNA in glioma concentrations and low TIMP2 concentrations. Gloma cells have a high level of the angiogenic regulator angiopoietin 2, which is associated with high MMP2 concentrations, suggesting that angiopoietin 2 might promote tumour cell infiltration through activation of MMP2.79

Angiogenesis is important in arteriovenous malformations. Biochemical measurements of MMPs in human brain tissue removed from arteriovenous malformations at surgery showed increased MMP9.80 Concomitant intracerebral injection of adenoviral vectors expressing VEGF and angiopoietin 2 in rats increased angiogenesis; the combination of these molecules increased angiogenesis and production of MMP9 more than VEGF alone.81 When viral vectors that deliver VEGF stimulate the growth of blood vessels, treatment with the tetracycline derivative doxycycline, an inhibitor of MMP, reduces the growth of blood vessels.82

Furthermore, angiogenesis plays an important part in recovery from stroke.83,84 In cancer, inhibition of angiogenesis is a therapeutic goal to reduce tumour growth, whereas angiogenesis is thought to be necessary after stroke. This presents a dilemma in the use of MMP inhibitors in the treatment of vascular disease. Blocking the adverse actions of the MMPs early in the course of the neuroinflammatory response might be beneficial. However, once angiogenesis begins, which seems to be within days of the initial insult in stroke, MMP inhibitors can hinder recovery.85 The migration of neural progenitor cells and the growth of vessels after stroke is dependent on multiple factors, including the stimulation of blood-vessel growth by HIF1α in the presence of decreased oxygen tension in the tissues. Endothelial cells, activated by HIF1α-induced erythropoietin, secrete MMP2 and MMP9, leading to the movement of neural progenitor cells to the injury site.86 Cells in the subventricular zone are the source of neuroblasts that migrate to the site of injury after a stroke; these cells secrete MMP9 to facilitate movement, which would be blocked with MMP inhibitors.87

**Therapeutic strategies**

MMP inhibitors have had beneficial effects in animal studies of multiple sclerosis, Guillain-Barré syndrome, meningitis, vascular dementia, and stroke. Acute cerebral ischaemia has been most intensively studied. Antibodies to MMPs and broad-spectrum MMP inhibitors, such as BB-94, BB-1101, and GM-6001, reduce blood–brain barrier damage, infarct size, and cell death.88–90 MMP inhibitors protect the brain from haemorrhagic complications of alteplase by reducing the permeability of the blood–brain barrier and preventing alteplase from entering the brain and activating MMPs.91,92 Treatment with MMP inhibitors in stroke needs to be given early in the first few days after the injury to prevent blocking the recovery phase. MRI has shown in rats that, although the broad-spectrum inhibitor BB-1101 blocked the early opening of the blood–brain barrier at 3 h after a 90-min middle cerebral artery occlusion with reperfusion, the inhibitor had no effect on lesion size at 48 h, and recovery over 3 weeks was slowed.85

Autoimmune and infectious diseases have been successfully treated with MMP inhibitors in animals. Damage to the blood–brain barrier seen in experimentally induced meningitis and experimental allergic encephalomyelitis can be reduced with MMP inhibitors. MMPs are important in the pathogenesis of bacterial meningitis and are thought to contribute to the damage seen in children with meningitis despite use of appropriate antibiotics. A water-soluble inhibitor of MMPs and TACE, TNF-484, is effective in animals.93 Doxycycline blocks secondary damage in experimental bacterial meningitis in rodents.94 Tetracycline derivate, such as doxycycline and minocycline, inhibit inflammation and reduce MMP concentrations. Minocycline given to patients with multiple sclerosis reduces the number of gadolinium-enhancing lesions on MRI.95 Doxycycline has been used in treatment of MMP-mediated vascular diseases and reduced vascular damage in an animal model of Marfan’s syndrome with improvement in aortic aneurysms after 1-year treatment.96 Tetracyclines can be given in low doses for several years without adverse effects and, when given in combination with interferon beta, were effective in controlling inflammation in mice with experimental allergic encephalomyelitis.97 The function of MMPs in the progressive damage to the white matter in vascular cognitive impairment might offer opportunities for treatment because of the possible contribution of MMPs to the damage. A caveat is that MMP9 might be important in remyelination, as oligodendrocyte regrowth is facilitated by MMP9.98

Although there are MMP inhibitors that are selective for MMP2 and MMP9,99 most available MMP inhibitors are broad-spectrum drugs.100 For the short-term treatment strategies envisioned for acute neurological diseases, such as early opening of the blood–brain barrier in ischaemia and infection, a broad-spectrum inhibitor might be useful. The problem with broad-spectrum inhibitors is that they might block important functions, such as remodelling of the extracellular matrix. In trials of MMP inhibitors in cancer, joint stiffness was the side-effect that limited treatment.101 Another challenge in the development of MMP inhibitors as acute treatments is their poor solubility;
studies are needed to facilitate improvement of delivery systems. For long-term use, selective MMP inhibitors might be needed to avoid prolonged broad-spectrum effects. Metabolism and efficacy of MMP inhibitors vary in different species. For example, drugs that effectively close the blood–brain barrier in rats after lipopolysaccharide challenge are ineffective in mice.  

In vascular cognitive impairment, decreasing white matter damage caused by ischaemic injury and hypoxic hypoperfusion is a likely therapeutic aim. Many patients with diabetes mellitus have white matter lesions, which are thought to be associated with subtle disruption of the blood–brain barrier.  

If so, treatment with a drug that blocks MMPs, such as minocycline, might be useful. Similarly, damage to the white matter caused by hypoperfusion is a likely therapeutic aim. Many patients with diabetes mellitus have white matter lesions, which are thought to be associated with subtle disruption of the blood–brain barrier.  

Even more speculative is the possible use of MMP inhibitors in the treatment of Alzheimer’s disease. The role of MMPs and ADAMs in Alzheimer’s disease is complex because of their dual function in the breakdown of amyloid to form Aβ and in the clearance of Aβ from the brain. Theoretically, treatment with MMP inhibitors could impede amyloid clearance because MMP9 seems to facilitate the removal of the amyloid peptides. Use of MMP inhibitors in Parkinson’s disease might show great promise as the death of dopaminergic neurons seems to be associated with release of MMPs by the activated cells around them. Much more information is needed before MMP inhibitors can be tested for use as treatment in these chronic disorders.

Conclusions
Much information has accumulated on the role of MMPs in acute disorders such as stroke, multiple sclerosis, and meningitis. These enzymes participate in the final common pathway after pathological changes to blood vessels and extracellular matrix around cells, which leads to cell death and increased permeability of the blood–brain barrier. Chronic neurological diseases of the elderly, namely, vascular cognitive impairment, Alzheimer’s disease, and Parkinson’s disease, are also affected by MMPs and ADAMs. However, in addition to the function of MMPs and ADAMs in disease states, these enzymes have many roles in physiological processes, such as angiogenesis and neurogenesis. This dual action of metalloproteinases complicates efforts at treatment with broad-spectrum MMP inhibitors. More research is needed to understand the diverse roles of these proteases to design specific drugs and devise therapeutic strategies.


