Implications of MMP-12 in the pathophysiology of ischaemic stroke

Krishna Kumar Veeravalli 🅘

Cancer Biology and Pharmacology, University of Illinois College of Medicine at Peoria, Peoria, Illinois, USA

Correspondence to
Dr Krishna Kumar Veeravalli; krishnav@uic.edu

ABSTRACT

This article focuses on the emerging role of matrix metalloproteinase-12 (MMP-12) in ischaemic stroke (IS). MMP-12 expression in the brain increases dramatically in animal models of IS, and its suppression reduces brain damage and promotes neurological, sensorimotor and cognitive functional outcomes. Thus, MMP-12 could represent a potential target for the management of IS. This article provides an overview of MMP-12 upregulation in the brain following IS, its deleterious role in the post-stroke pathogenesis (blood-brain barrier disruption, inflammation, apoptosis and demyelination), possible molecular interactions and mechanistic insights, its involvement in post-ischaemic functional deficits and recovery as well as the limitations, perspectives, challenges and future directions for further research. Prior to testing any MMP-12-targeted therapy in patients with acute IS, additional research is needed to establish the effectiveness of MMP-12 suppression against IS in older animals and in animals with comorbidities. This article also examines the clinical implications of suppressing MMP-12 alone or in combination with MMP-9 for extending the currently limited tissue plasminogen activator therapy time window. Targeting of MMP-12 is expected to have a profound influence on the therapeutic management of IS in the future.

INTRODUCTION

Stroke ranks as the fifth top cause of death in the USA and as a second-leading cause of mortality worldwide.[1 2] Stroke is the primary reason for long-term neurological impairments and disability. The most common type, accounting for approximately 87% of all strokes, is an ischaemic stroke (IS), which occurs when blood arteries in the brain get blocked.[3]

Current therapies for IS and their limitations

The only Food and Drug Administration (FDA)-approved drug for treating IS is recombinant tissue plasminogen activator (tPA), which recanalizes blocked blood arteries.[4–7] Although the percentage of acute IS patients eligible for tPA therapy increased in certain stroke centers with improved door-to-needle time, a vast portion of patients still fail to receive this treatment due to the short tPA treatment window (within 4.5 hours after the onset of symptoms). Furthermore, due to the elevated mortality rate and subsequent haemorrhagic transformation, individuals with bleeding disorders are not candidates for tPA treatment.[4 5 8] The clinical efficacy of recanalization with tPA treatment is limited due to incomplete reperfusion, risk of haemorrhagic transformation and occlusion of the recanalized blood vessels.[9 10] For eligible patients (those with large vessel occlusion) who present with acute IS up to 24 hours after the onset of symptoms, endovascular thrombectomy is available for recanalization.[11] Only about 19% of patients, on average, are eligible for endovascular thrombectomy.[12–16] Even with 3 months after endovascular thrombectomy, 50% of patients still unfortunately have disabilities.[17 18] Moreover, many stroke centres lack the resources and expertise necessary to provide this therapy.[19]

The FDA approved recanalization treatments (thrombolysis therapy with recombinant tPA and endovascular thrombectomy) end ischaemia and re-establish blood flow (referred to as reperfusion). However, lingering brain damage as well as the damage caused by recanalization (known as reperfusion injury) leads to occurrence of severe and persistent secondary effects that result in functional deficits due to tissue damage. There are no medications currently available to extend the tPA treatment time window, mitigate the progressive brain damage following recanalization with either tPA treatment or endovascular thrombectomy or enhance functional recovery, despite years of intensive research. Matrix metalloproteinases (MMPs), which play a crucial role in injury and recovery after IS, have been extensively investigated.

Structure and substrates of MMPs, including MMP-12

MMPs are calcium-dependent zinc-containing endopeptidases.[20] Several features, including the ability to degrade various extracellular matrix (ECM) components, dependence on metal ions as cofactors and specific DNA sequence, distinguish MMPs from other endopeptidases. There are currently at least 23 MMPs that are known, and they are capable of degrading a range of ECM components

and processing a variety of bioactive chemicals.[21] Every MMP has a similar domain structure that includes the haemopexin-like domain at the carboxy-terminal which controls the substrate selectivity, the catalytic domain that binds zinc ion, and the amino-terminal propeptide domain that is essential for enzyme latency. MMPs are grouped into collagenases (1, 8 and 13), gelatinases (2 and 9), matrilysins (7 and 26), stromelysins (3, 10 and 11), enamelysin (20), metalloelastase (12), membrane-type MMPs (14, 16, 17, 24 and 25) and further unclassified MMPs (19, 21, 23, 27 and 28).[22]

MMP-12 is the only MMP that belongs to the class of metalloelastases. MMP-12, which breaks down elastin and is produced by macrophages, was discovered in 1975.[23 24] Macrophage elastase (ME), human ME (HME) and macrophage metalloelastase (MME) are other names for MMP-12. Similar to other MMPs, MMP-12 is made up of a propeptide domain, a catalytic domain and a hemopexin-like domain. In addition to elastin, MMP-12 can also degrade laminin, type IV collagen, vitronectin, fibrillin-1, fibronectin, chondroitin sulphate and heparan sulphate proteoglycans.[22 25–28] In addition to these ECM components, several other biomolecules (myelin basic protein (MBP), plasminogen, progranulin, N-cadherin, α1-antitrypsin, tissue factor pathway inhibitor and pro-tumour necrosis factor-α (TNFα) serve as substrates for MMP-12.[29–35] Overall, MMP-12 substrates include a wide range of biomolecules and ECM components.

MMP-12 may be a potential target for IS treatment

MMP-12's negative role on post-stroke brain damage and pathogenesis (ie, blood-brain barrier (BBB) breakdown, infarct development, inflammation, apoptosis and demyelination) as well as in neurological and functional impairments and recovery has been observed in rodent models of IS.[35–39] In these studies, suppressing MMP-12 gene expression shortly after IS decreased brain injury and promoted the recovery of sensorimotor and cognitive function. Reduced BBB breakdown and neuroinflammation in MMP-12-suppressed animals indicate that MMP-12 inhibition can be a promising strategy for preventing the haemorrhagic transformation caused by delayed tPA therapy, thereby allowing more patients to receive tPA treatment.

MMP-12 EXPRESSION INCREASES AFTER IS

Several studies in rodent models (table 1) shows that MMP-12 is markedly elevated in the brain following IS.[35–37 39–42] The time course of MMP expression showed that MMP-12 was upregulated substantially more than any other MMP. MMP-12 mRNA/protein levels increase as early as 1 hour and remained elevated for at least 14 days. Furthermore, the increase of MMP-12 was specific to the ipsilateral brain hemisphere.

Under normal conditions, the expression of MMP-12 in the adult brain is extremely low. However, following ischaemia, several brain cell types express MMP-12. For

Table 1 MMP-12 levels increase in the brain after cerebral ischaemia followed by reperfusion

Species	Stroke induction method	Results (related to MMP-12 expression)	Reference
Mouse	Permanent CAO and 50 min hypoxia	MMP-12 expressed in microglia and neurons on day 1 post-CAO. Increased MMP-12 mRNA and protein expression on day 3.	Svedin *et al*, *Developmental Neuroscience* 2009[40]
Rat	2 hour MCAO	Increased MMP-12 mRNA and protein expression on post-ischaemic days 1, 3, 5, 7 and 14. MMP-12 expressed in neurons, oligodendrocytes and microglia/macrophages on day 7.	Chelluboina *et al*, *Scientific Reports* 2015[35]
Rat	2 hour MCAO	Increased MMP-12 expression immediately after ischaemia (1 hour, 2 hours and 4 hours MCAO); increase more pronounced on day 1.	Chelluboina *et al*, *Stroke* 2015[36]
Mouse	1 hour MCAO	Increased MMP-12 mRNA expression on post-ischaemic day 1.	Nalamolu *et al*, *Stroke and Vascular Neurology* 2018[41]
Mouse	Photothrombotic cortical ischaemia	Increased MMP-12 mRNA expression on post-ischaemic day 3. MMP-12 expressed in microglia.	Hohjoh *et al*, *Neuroscience Letters* 2020[42]
Mouse	1 hour MCAO	Increased protein expression of MMP-12 on post-ischaemic day 1.	Arruri *et al*, *Neurochemistry International* 2022[37]
Rat	2 hour MCAO	Increased mRNA expression of MMP-12 on post-ischaemic days 1, 3, and 7. MMP-12 expression not elevated in contralateral hemisphere.	Challa *et al*, *Frontiers in Neuroscience* 2022[39]

CAO, carotid artery occlusion; MCAO, middle cerebral artery occlusion.

example, MMP-12 expression was found in neurons, oligo-dendrocytes and microglia following hypoxia-ischaemia in mice.[40] MMP-12 expression in these brain cells was also noticed in rat brains after cerebral ischaemia and reperfusion (IR).[35] Microglia of mice was subjected to photothrombotic stroke-expressed MMP-12.[42] Overall, it is evident that, with the exception of astrocytes, almost all brain cells express MMP-12.

Monocytes in the blood do not express MMP-12.[26 43] The biological function and protein secretion of these cells can be significantly impacted by the differentiation of monocytes into macrophages when they enter tissues. Monocyte-derived macrophages have shown substantial MMP-12 mRNA and protein.[43] MMP-12 was detected initially in conditioned media of mouse peritoneal macrophages.[24] This explains why the MME was the original name given to MMP-12. BBB disruption following cerebral ischaemia leads to the infiltration of blood monocytes, which differentiates into macrophages in the brain. It was reported that monocytes are recruited mainly into the ischaemic brain between days 3 and 7 following IS.[44] The infiltrating macrophages can survive in the ischaemic brain for weeks or months.[45] This explains the increased MMP-12 expression seen after 14 days.[35]

The extent of BBB breakdown following cerebral ischaemia increases over time with simultaneous increase of infiltrating monocytes into the ischaemic brain. This explains why MMP-12 levels raised from ~50-fold on day 1 to ~260-fold on day 7.[35 39] MMP-12 is expressed by a variety of brain cells; however, it is clear that following IS, invading monocytes/macrophages are the main source of MMP-12 in the brain.

ROLE OF MMP-12 IN POST-STROKE PATHOGENESIS

Clinical and experimental investigations demonstrated the involvement of MMP12 in the development of large-artery stroke by promoting elastin degradation and macrophage infiltration in atherosclerotic plaques.[46] The critical role of MMP-12 in poststroke pathogenesis has been emphasised in a number of articles published over the past decade (table 2). MMP-12's role in patho-physiological processes such as BBB disruption, inflammation, apoptosis, and demyelination after cerebral IR is described below.

BBB and its disruption by MMP-12

The BBB is predominantly composed of endothelial cells of cerebral microvessels. The absence of fenestrations, limited pinocytotic activity and presence of tight junctions (TJs) distinguish these cerebral microvessels from peripheral blood vessels.[47] The TJs play a vital role in maintaining BBB integrity and restricting the entry of blood-borne substances into the brain.[48] Thus, the BBB serves as a dynamic interface between the brain and the peripheral circulation. Additionally, TJs maintain the transendothelial electrical resistance of the BBB by regulating the paracellular transport of ions.[49]

Ion balance and energy homeostasis, which are essential for the function of neurons, are maintained by the BBB's strong transendothelial electrical resistance, low rate of transcytosis and constrained paracellular permeability.[50 51]

The TJ protein complexes largely determine the properties of the BBB. Transmembrane proteins such as occludin and claudin-5 and membrane-associated intracellular scaffolding proteins such as ZO-1 make up the TJ protein complexes.[52 53] These TJ proteins, each of which has a unique molecular structure and set of regulatory properties, are essential for maintaining of TJ structure and function. Aberrant distribution and decreased protein expression of these TJs after cerebral IR leads to BBB disruption and increased permeability.[54] TJ density is maintained by claudin-5, although TJ stability and barrier function are maintained by occludin.[55] The integrity of TJs is maintained by the interaction of ZO proteins (1, 2 and 3) with claudin-5 and occludin. ZO-1 forms heterodimers with ZO-2 and ZO-3, and functions as a scaffold for the attachment of occludin and claudin to the actin cytoskeleton.[56 57]

Besides endothelial cells, the BBB comprises pericytes, astrocytes and neurons.[58] Proteins such as fibronectin, laminin, elastin, collagen type-IV, thrombospondin and a variety of proteoglycans make up the ECM that surrounds the BBB's endothelial cells and pericytes.

Following ischaemia, BBB disruption is the primary factor contributing to extensive brain injury. The expression of MMPs upregulates in the brain rapidly after cerebral ischaemia.[59–61] Moreover, reperfusion also contributes to the release and elevated expression of additional MMPs.[35 41] During the acute phase following IS, elevated MMPs in the brain degrade TJ proteins of the BBB, increasing its permeability and causing brain oedema, leucocyte infiltration and haemorrhage.[58] Because MMPs 2 and 9 degrade microvascular basal lamina and TJ proteins, it was previously hypothesized that they are the primary regulators of BBB rupture.[62–65] However, a subsequent study reported MMP-9's role in brain swelling and secondary brain injury following IS, while MMP-2 is involved in tissue repair and nerve regeneration.[66]

Recently, the involvement of MMP-12 in the breakdown of the BBB was demonstrated in rodent models of IS.[36 37] In these studies, MMP-12 suppression by siRNA/shRNA gene silencing reduced the degradation of claudin-5, occludin and ZO-1. While TJ protein expression was noticed in endothelial cells of the cerebral blood vessels in MMP-12 shRNA-treated animals, it was weaker in untreated stroke-induced animals.[36] Interestingly, in stroke-induced animals, while TJ proteins were found in astrocytes in untreated group (indicating that the proteins were degraded), the proteins were less associated with astrocytes in MMP-12 shRNA-treated group. Furthermore, suppression of MMP-12 reduced the breakdown of the BBB, as indicated by decreased extravasation of Evan's blue dye into the ischaemic brain after

Table 2 MMP-12 suppression/inhibition following ischaemic stroke is beneficial

Species	Stroke induction method	Test item	Dose (ROA)	Time of injection	Results	Reference
Rat	2 hour MCAO	MMP-12 shRNA	1 mg/kg (IV)	1 day AR	Reduced infarct size, apoptosis and TNFα expression. Decreased MMP-9 expression and MBP degradation.	Chelluboina et al, Scientific Reports 2015[35]
Rat	2 hour MCAO	MMP-12 shRNA	1 mg/kg (IV and IA)	Immediately (within 30 min) AR	Reduced BBB disruption, tight junction proteins degradation and infarct size. Decreased expression of MMP-9 and tPA.	Chelluboina et al, Stroke 2015[36]
Rat	2 hour MCAO	MMP-12 shRNA	1 mg/kg (IV)	Immediately (within 30 min) AR	Reduced expression of microglial markers (CD68, IL-10, Arg1 and TGFβ) and MMPs (7, 9, 11 and 14).	Challa et al, Stroke 2022[38]
Mouse	1 hour MCAO	MMP-12 siRNA	35 nmol (IV)	Immediately (5 min) AR	Reduced infarct volume, tight junction protein degradation, expression of inflammatory mediators (MCP-1, TNFα and IL-6) and cleaved caspase-3. Improved motor and cognitive functions.	Arruri et al, Neurochemistry International 2022[37]
Rat	2 hour MCAO	MMP-12 shRNA	1 mg/kg (IV)	Immediately AR, 3 hour AR, or 6 hour AR Immediately AR and on day 7 and 14 AR	Improved sensory and motor functions. Immediate treatment was superior to delayed treatments. Acute and chronic MMP-12 suppression are equally effective.	Challa et al, Frontiers in Neuroscience 2022[39]

MCAO, middle cerebral artery occlusion; ROA, route of administration; IV, intravenous; IA, intra-arterial; AR, after reperfusion; tPA, tissue-type plasminogen activator; MBP, myelin basic protein; BBB, blood-brain barrier; MCP-1, monocyte chemoattractant protein-1; TNF, tumour necrosis factor; IL, interleukin; Arg, arginase; TGF, transforming growth factor.

intravenous administration.[36] These findings show how MMP-12 contributes to BBB breakdown after IS.

MMP-12 exacerbates inflammation after IS

During IS, the brain undergoes an intense inflammatory response, which plays a significant role in pathogenesis.[67–69] It is hypothesized that activated inflammatory processes (innate and adaptive immunity mechanisms) are the primary contributors to the pathogenesis of cerebral ischaemia.[70] Studies in animal models of stroke demonstrate that reducing the severity of inflammation during the acute phase reduces brain damage and improves neurological and functional outcomes.[71–73] The failure of anti-inflammatory drugs to improve post-stroke outcomes in patients suggests that inflammation following IS is not entirely harmful.[74] While severe inflammation during the acute phase worsens brain damage, it

aids tissue repair (neurogenesis, oligodendrogenesis, dendritogenesis, axon sprouting, and matrix remodelling) during the late phase.[75 76] Due to its dual nature, it is difficult to target post-ischaemic inflammation.

The contribution of MMP-12 to BBB disruption following ischaemia was discussed in the previous section. Through a leaky BBB, leucocytes infiltrate the ischaemic brain. Invading leucocytes (and injured brain cells) produce various inflammatory mediators, causing inflammation.[77] MMP-12 aids in the release of IFN-α, which stimulates the generation of TNFα, interleukins (1, 2 and 6) and IFN-γ.[78] In addition, MMP-12 has been implicated in the release of the pro-inflammatory mediator, tumour necrosis factor-α (TNFα) and the activation of pro-TNFα.[30 79] After cerebral IR in rats, MMP-12 suppression reduced TNFα expression and its colocalisation

with MMP-12 in the ischaemic brain.[35] In addition, MMP-12 suppression reduces the expression of M1 and M2 markers.[38] Furthermore, in a mouse model of cerebral IR, MMP-12 inhibition decreased the expression of monocyte chemoattractant protein-1 (MCP-1), TNFα and interleukin-6.[37] The role of IFN-α in the MMP-12-mediated elevation of pro-inflammatory mediators in the ischaemic brain is unknown. Reduced BBB permeability and leucocyte infiltration may account for the attenuation of post-ischaemic neuroinflammation mediated by MMP-12 suppression.

MMP-12 promotes apoptosis following IS

Following IS, the affected region of the brain can be divided into two regions: the core and the penumbra. The penumbra is the region of brain tissue surrounding the irreversibly damaged ischaemic core that receives sufficient blood to maintain cell viability but insufficient blood to function.[80] The ischaemic penumbra refers to brain tissue that is susceptible to infarction if blood flow is not restored within a specific time frame.

A large body of research suggests that necrosis and apoptosis significantly contribute to brain cell death following cerebral ischaemia. In the ischaemic core, necrosis is the primary mode of cell death, whereas in the penumbra, apoptosis predominates.[81–83] A previous study revealed the extent of apoptosis and the temporal expression profile of apoptotic signalling pathway molecules at various time intervals after cerebral IR.[84]

ShRNA-mediated gene silencing of MMP-12 decreased apoptosis and reduced the expression of TNFα and caspase-3.[35] Inhibition of MMP-12 also reduced the colocalisation of MMP-12 and TNFα. The role of MMP-12 in the release of TNFα and the conversion of pro-TNFα to active TNFα has previously been reported.[30 79] Suppression of MMP-12 also reduces the expression and activity of MMP-9, which has been shown to induce apoptosis following cerebral ischaemia.[35 36 38 85–88] The activation of caspase-3 following IS has been reported to exacerbate neuronal apoptosis.[89] Recently, in mice subjected to cerebral IR, MMP-12 suppressed animals exhibited a significant decrease in cleaved caspase-3 expression.[37] In addition, animals treated with MMP-12 siRNA showed a significant reduction in the number of apoptotic neurons in the peri-infarct region. These studies clearly demonstrate the function of MMP-12 in apoptosis following IS.

MMP-12 is involved in demyelination and white matter damage after IS

In the central nervous system (that consists of brain and spinal cord), grey matter refers to the cell bodies of neurons and their unmyelinated axons and functions as an information processor. White matter refers to bundles of myelinated axons, myelin-producing oligodendrocytes and other glial cells (astrocytes and microglia) and is essential for signal transmission and inter regional communication. Myelinated axons facilitate rapid information transfer, which is essential for optimal behavioural and cognitive functions.[90] White matter and myelin-producing oligodendrocytes are more sensitive to ischaemic injury than grey matter.[91–94]

IS is often accompanied by myelin structure loss, oligodendrocyte death and white matter damage.[92 94–96] One of the primary components of white matter damage is demyelination, which is characterised by the loss of the myelin sheath and the demise of oligodendrocytes. Because the adult brain has a limited capacity for the regeneration of oligodendrocytes and remyelination of the demyelinated axons, adult demyelination of axons impairs impulse propagation and contributes significantly to the lasting cognitive and sensorimotor impairments.

MBP is the most abundant protein component of myelin and one of the substrates for the enzyme MMP-12. Activation of MMP-12 induces MBP degradation.[30] In a rat model of IS, suppressing MMP-12 prevented the degradation of MBP, preserved myelinated axons and reduced structural abnormalities such as rarefaction and myelin fragmentation in the ischaemic brain.[35] The role of MMP-12 in demyelination was also previously reported in a mouse encephalitis model.[97] Recently, in mice subjected to cerebral IR, siRNA-mediated gene silencing of MMP-12 decreased white matter damage.[37] As mentioned earlier, MMP-12 suppression following cerebral IR reduced apoptosis of brain cells, which may include oligodendrocytes, the myelin-producing cells.[35] These studies show that demyelination and white matter damage have been linked to increased MMP-12 in the ischaemic brain.

UNIQUE MOLECULAR INTERACTIONS OF MMP-12 AND MECHANISTIC INSIGHTS

As discussed earlier, MMP-12 processes and degrades several ECM components (elastin, laminin, fibronectin, type IV collagen, fibrillin-1, chondroitin sulphate, vitronectin and heparan sulphate proteoglycans) and biomolecules (MBP, plasminogen, N-cadherin, α1-antitrypsin, progranulin, tissue factor pathway inhibitor and pro-TNFα).[22–28 30–35 98] The enzymatic action of elevated MMP-12 on these diverse substrates (and a number of unidentified molecules) induces a variety of microenvironmental alterations in the ischaemic brain.

Possible interactions between MMP-12 and other MMPs

MMPs are synthesised as inactive zymogens that contain a pro-peptide domain and are secreted as proenzymes from cells. When the pro-peptide domain of MMPs is removed, the enzymes become active. Pro-MMP-12, a 54 kDa proenzyme released from cells, undergoes autolytic processing to generate active forms of 45 kDa and 22 kDa.[25 26] MMP-12 activates pro-MMP-2 (which then activates pro-MMP-1) and pro-MMP-3 (which then activates pro-MMP-9).[99] Due to the fact that MMPs can activate one another, the overall level of MMP activity increases as MMP-12 levels increase. This suggests that the elevation of MMP-12 in the brain may result in the processing or degradation of numerous
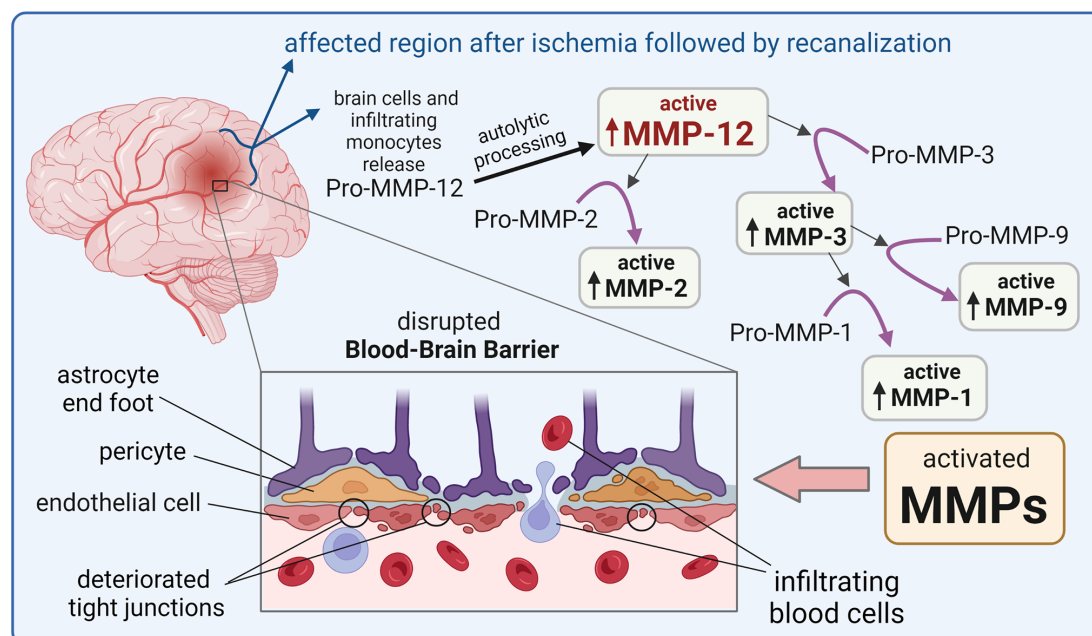
Figure 1 Activation of matrix metalloproteinases (MMPs) and blood-brain barrier (BBB) disruption after ischaemic stroke. Schematic representation of the plausible downstream activation of multiple MMPs and rupture of the BBB after transient cerebral ischaemia and reperfusion. MMP-12 is produced from cells as a proenzyme that is inactive (pro-MMP-12), which then goes through autolytic processing to become active. Other proenzymes, such as pro-MMP-2 and pro-MMP-3, are activated by activated MMP-12. Several additional proenzymes, including pro-MMP-1 and pro-MMP-9, are activated by activated MMP-3. Together, these active MMPs degrade multiple extracellular matrix (ECM) and tight junction (TJ) proteins, disrupt BBB and allow blood cells to infiltrate and enter the brain. This figure was created with biorender.com under a paid subscription.

ECM components and biomolecules that are substrates of MMP-12 and other MMPs (1, 2, 3, and 9).

The MMP most studied in relation to IS is MMP-9. It is well known for its role in post-stroke excitotoxicity, neuronal damage, apoptosis and BBB breakdown, which results in cerebral oedema and haemorrhagic transformation.[100] After IS, MMP-12 knockdown reduced the expression and activity of other MMPs, such as MMP-9, in the ischaemic brain.[35 36 38] This result was consistent with a previous study showing that MMP-12 induces other MMPs.[101] Therefore, it can be postulated that increased MMP-12 contributes to BBB disruption by increasing not only its activity but also the activity of other MMPs, thereby causing the degradation of TJ and ECM proteins (figure 1). In addition, the disruption of the BBB by MMP-12 may result in an increase in other MMPs in the brain owing to the release of these enzymes by monocytes and neutrophils. MMP-12 knockdown reduces the expression of MMP-7, MMP-9, MMP-11 and MMP-14 in the ischaemic brain.[38] Due to the fact that MMP-12 is upstream of several other MMPs, including MMP-9, targeting MMP-12 rather than MMP-9 would incorporate the benefits of targeting MMP-9 in addition to those of MMP-12 and could offer significant therapeutic benefits. Targeting MMP-9, on the other hand, may not result in clinically meaningful changes due to MMP-12's unabated negative effects. As MMP-9 upregulation may precede MMP-12 following IS, the simultaneous suppression of MMP-12 and MMP-9 may provide even greater protection than MMP-12 alone, due to MMP-9's direct suppression (as opposed to its indirect suppression).

The interplay between MMP-12 and tPA

Possible interactions between MMP-12 and tPA after IS have been reported.[36 102] In the ischaemic brain, MMP-12 and tPA interact on multiple levels, either directly or indirectly. The computational modelling of in silico protein–protein interactions revealed numerous molecular sites, where the proteases MMP-12 and t-PA may interact directly and potentially affect each other's activity. MMP-12 suppression reduces tPA expression, whereas tPA knockdown reduces MMP-12 expression. Plasmin (produced from inactive plasminogen by plasminogen activators such as uPA or tPA) activates many pro-MMPs (2, 3, 9, 12 and 13).[103 104] Since both MMP-12 and tPA contribute to BBB breakdown after cerebral IR, the decrease in MMP-12 expression or activity after tPA knockdown may be attributed to a decrease in BBB disruption and monocyte infiltration. Targeting tPA may, therefore, be an effective strategy for reducing MMP-12 expression and activity while mitigating ischaemic brain damage, as high levels of MMP-12 in the brain are primarily due to BBB disruption caused by elevated endogenous brain tPA.

Possible interaction between MMP-12 and progranulin

Progranulin (PGRN) is a substrate of the proteolytic enzyme MMP-12.[105–109] PGRN is a neurotrophic factor that increases cell survival and neurite outgrowth.[110] PGRN

deficiency causes inflammation and neuronal loss.[111] Recombinant PGRN administration reduced cerebral swelling, infarct volume and mortality rate and improved neurological scores.[112] PGRN binds to TNF receptors with high affinity, preventing TNFα from binding to its receptors and inhibiting TNFα's downstream signalling.[113] Furthermore, the conversion of PGRN to granulins by proteolytic enzymes, such as elastases, exacerbates postischaemic inflammation.[114–116] Reduced PGRN degradation following MMP-12 suppression may play a significant role in the reduction of post-ischaemic inflammation mediated by MMP-12 suppression, though this has not yet been studied.

MMP-12 INVOLVEMENT IN POSTSTROKE FUNCTIONAL DEFICITS AND RECOVERY

Strokes, including ischaemic and haemorrhagic, are the primary cause of long-term disability.[117] Strokes can cause one or more physical or cognitive impairments. Some of the disabling effects and complications of a stroke include paralysis, muscle weakness, difficulty standing or walking, slowed movements, lack of coordination, impairment of fine motor skills, sensory disturbances, speech, language, vision, memory loss and emotional and behavioural disorders. Stroke survivors experience one or more of these debilitating neurological impairments. The post-ischaemic complications and outcomes of a stroke patient are dependent on the affected brain region(s). More than 50% of stroke survivors aged 65 and older have reduced mobility.[117] Therefore, even with intensive therapy, a patient may not fully recover from a stroke and may have a persistent impairment.

Several animal stroke models have been developed to investigate the mechanisms underlying post-stroke injury and to develop effective therapies to reduce brain damage and improve functional recovery.[118–121] Since blockade of the middle cerebral artery (MCA) is the most common cause of IS in humans, middle cerebral artery occlusion (MCAO) was used to induce experimental focal cerebral ischaemia in rodent models.[122] Because it allows the study of reperfusion, it is less invasive than craniotomy models, and reliably produces cerebral infarcts, the MCAO procedure in rodents is often used in stroke research. Most importantly, transient MCAO followed by reperfusion results in a number of short-term and long-term cognitive, somatosensory and motor deficits in rodents.[123]

The recovery of somatosensory and motor functions in stroke-induced rats was enhanced by shRNA-mediated gene silencing of MMP-12.[39] The recovery of motor function was also significantly enhanced in mice with a stroke following MMP-12 suppression.[39] The cognitive abilities of stroke-induced mice treated with MMP-12 siRNA are significantly enhanced. The sooner MMP-12 after IS is suppressed, the greater the recovery of sensory and motor function.[39]

Different MMPs have distinct and opposing roles. Some increases in MMPs may be beneficial, while others may be detrimental. Chronic inhibition of MMPs with non-specific MMP inhibitors reduces the positive effects of acute inhibition, indicating that expression of certain MMPs during the recovery process is beneficial.[124 125] In contrast, sustained MMP-12 suppression neither increased nor decreased the useful effects of acute MMP-12 suppression, indicating that prolonged MMP-12 suppression is likely not harmful to recovery because it does not interfere with the restorative action of other MMPs in the brain.[39] Unlike other MMPs, the advantages of MMP-12 inhibition may not be time limited, as MMP-12 may only play a detrimental role during the acute and recovery phases following IS.[66 126–128] Despite the fact that MMP-12 suppression was efficacious in both sexes, the benefits are more apparent in men than in women. This is probably because higher oestrogen levels in women are associated with lower neurological deficits and improved recovery.[39 129] Reduced white matter damage, demyelination and brain injury (attenuated BBB disruption, apoptosis and inflammation) may account for the MMP-12 suppression-mediated improvement in the recovery of sensorimotor and cognitive abilities.[35–39] The potential mechanisms and pathophysiological processes implicated in MMP-12-mediated brain injury and functional impairments after IS are depicted in figure 2. In addition, inhibition of plasminogen conversion to angiostatin by MMP-12 suppression may have promoted angiogenesis in the injured brain, thereby aiding in the recovery process.[31 32]

PERSPECTIVES, LIMITATIONS, CHALLENGES AND FUTURE DIRECTIONS

Although many pharmacological agents reduced tissue damage in animals following IS, none have been translated into clinically efficacious. Due to the involvement of multiple mechanisms, pathways and molecules in the pathophysiology after cerebral IR, targeting a single molecule/mechanism/pathway may not be sufficient to achieve a significant therapeutic effect. One of the primary reasons for this translational failure is that the majority of these animal experiments were conducted on young animals, despite the fact that stroke primarily affects elderly people.[130–132]

In addition, human strokes are sexually dimorphic and have a range of aetiologies, onset ages and functional outcomes.[133] Therefore, the Stroke Treatment Academic and Industry Roundtable (STAIR) criteria for a new stroke therapeutic include the demonstration of efficacy in both sexes, aged animals and animals with comorbidities.[134]

Because MMP-12 is implicated in multiple pathologies, including BBB disruption, inflammation, apoptosis and demyelination, targeting MMP-12 may offer clinically meaningful outcomes in IS patients, in contrast to previously tested treatments. Based on the positive results of the MMP-12-targeting studies described in this article, MMP-12 seems to be a potential therapeutic target for IS treatment. However, to date, the increase in MMP-12 in the ischaemic brain and the efficacy of its suppression
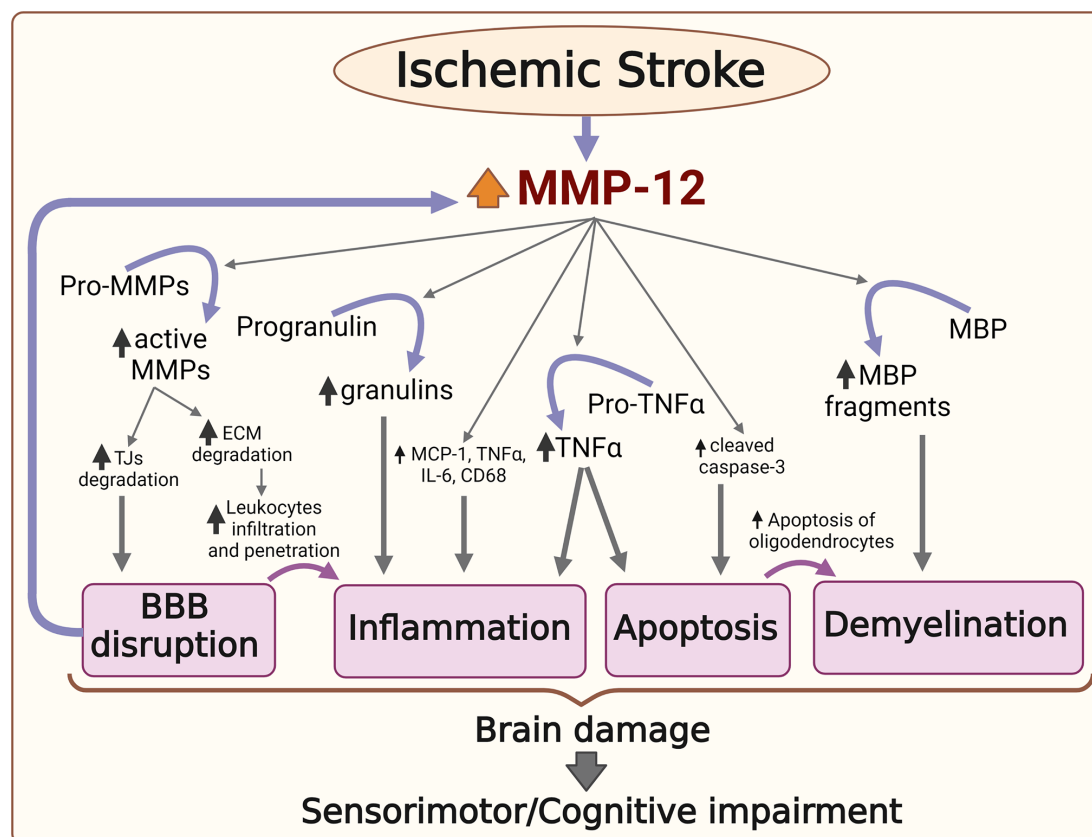
Figure 2 Role of MMP-12 in ischaemic stroke. The potential molecular changes and pathophysiological processes by which elevated MMP-12 contributes to brain injury and sensorimotor and cognitive impairments after an ischaemic stroke are depicted schematically. BBB, blood-brain barrier; ECM, extracellular matrix; IL-6, interleukin-6; MBP, myelin basic protein; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; TJs, tight junction proteins; TNFα, tumour necrosis factor α. This figure was created with biorender.com under a paid subscription.

in rodent models of IS have only been investigated and demonstrated in young animals. The potential advantages of inhibiting MMP-12 after IS in older animals and animals with comorbidities (diabetes, hypertension, etc) remain unknown. Furthermore, the efficacy of MMP-12 suppression in stroke-induced animals in which tPA is administered to induce thrombolysis and reperfusion has not been determined.

Several of the studies discussed in this review meet some of the STAIR criteria by demonstrating the therapeutic efficacy of MMP-12 in both sexes and two rodent species. Additional research is required to test the effectiveness of MMP-12 suppression on post-stroke outcomes in older animals and animals with comorbidities to determine whether targeting MMP-12 in stroke patients with these comorbidities is beneficial. Future investigations using small molecule inhibitors or therapeutic antibodies targeting MMP-12 in animal stroke models may further reveal MMP-12 inhibition as a promising strategy for stroke therapy. It appears that MMP-12 targeting therapy for stroke patients may have clinically significant advantages due to the direct and indirect interactions between MMP-12 and tPA in the ischaemic brain (as described in one of the earlier sections). Future research is needed to investigate whether combining medications that block

either MMP-12 alone or both MMP-12 and MMP-9 with recombinant tPA therapy beyond the recommended time window reduces the BBB disruption and haemorrhagic transformation associated with delayed tPA treatment.

The level of MMP-12 expression in the brain may serve as a measure of BBB disruption, as MMP-12 levels dramatically increase after monocyte infiltration through a disrupted BBB. However, it is virtually impossible to measure MMP-12 levels in the ischaemic brains of patients with acute IS. Through the compromised BBB and blood vessels, increased MMP-12 protein in the brain can reach the systemic circulation. If the level of MMP-12 expression in the brain correlates with the level of MMP-12 in the blood, plasma or serum MMP-12 can serve as a biomarker for BBB disruption and help determine whether delayed delivery of recombinant tPA is safe in patients with acute IS. Future research on stroke-induced animals and humans with IS should measure blood levels of MMP-12 to determine whether blood MMP-12 is a biomarker for BBB disruption after IS.

CONCLUSIONS
A dramatic increase in MMP-12 after cerebral IR damages the brain by rupturing the BBB and causing inflammation,

apoptosis and demyelination. Ischaemic brain injury and neurological impairments are reduced and functional recovery is enhanced by suppressing MMP-12. Given the association between increased MMP-12 in the ischaemic brain and a number of pathological processes such as BBB disruption, inflammation, apoptosis, demyelination and impaired sensorimotor/cognitive functions, MMP-12 can prove to be a potential therapeutic target for IS treatment.

ORCID iD
Krishna Kumar Veeravalli http://orcid.org/0000-0002-2243-004X

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