SVN Stroke & Vascular Neurology

Implications of MMP-12 in the pathophysiology of ischaemic stroke

Krishna Kumar Veeravalli

To cite: Veeravalli KK. Implications of MMP-12 in the pathophysiology of ischaemic stroke. *Stroke & Vascular Neurology* 2024;**9**: e002363. doi:10.1136/svn-2023-002363

Received 3 February 2023
Accepted 5 June 2023
Published Online First 19 June 2023

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, oligodendrocytes 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 pathophysiological 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

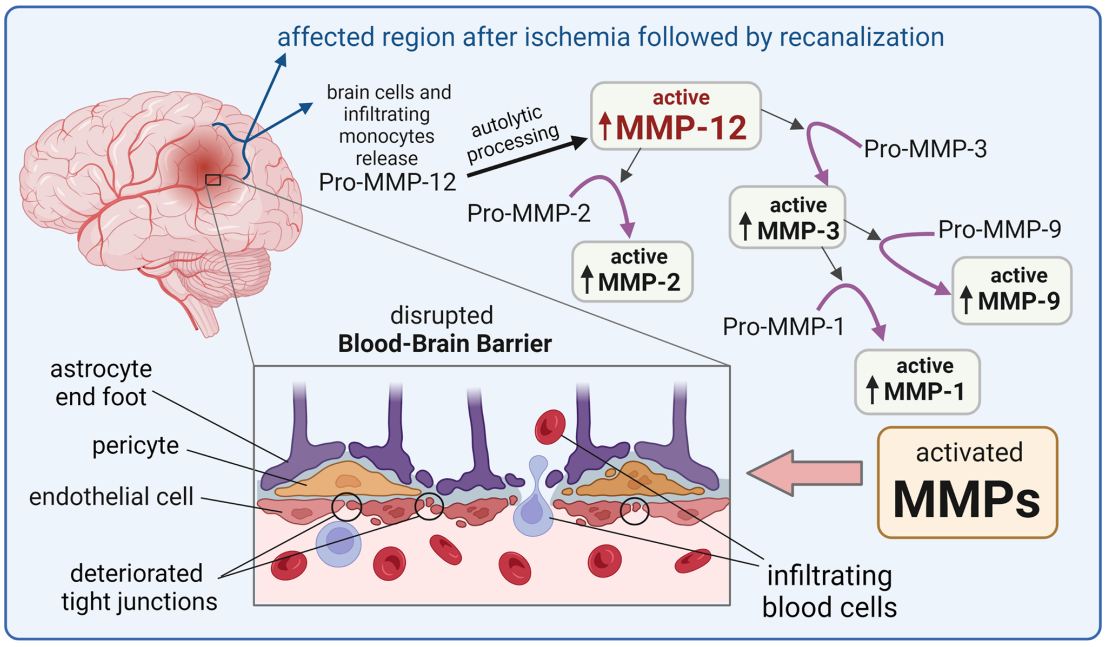

Figure 1 Activation of matrix metalloproteinases (MMPs) and blood-brain barrier (BBB) disruption after ischaemic stroke. Schematic representation of the plausible downstream activation of multiple MMPs and rupture of the BBB after transient cerebral ischaemia and reperfusion. MMP-12 is produced from 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

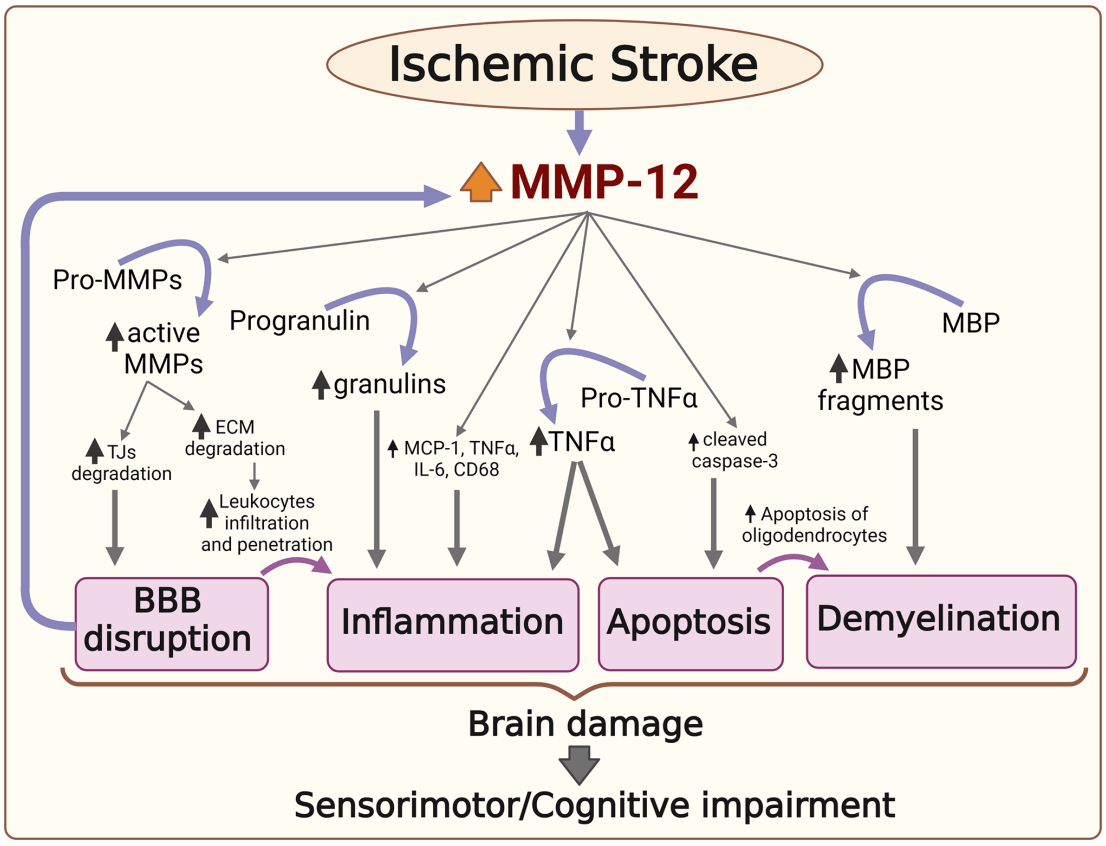

Figure 2 Role of MMP-12 in ischaemic stroke. The potential molecular changes and pathophysiological processes by which elevated MMP-12 contributes to brain injury and sensorimotor and cognitive impairments after an ischaemic stroke are depicted schematically. BBB, blood-brain barrier; ECM, extracellular 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.

Acknowledgements I thank Dr. Casimir A. Fornal for editing the manuscript, Dr. Siva Reddy Challa for assisting with the preparation of figures, and Christina Constantinidou and Erika Sung for their assistance with the formatting of the manuscript.

Contributors This review article was prepared by KKV.

Funding The National Institute of Neurological Disorders and Stroke of the National Institutes of Health awarded a research grant (Award Number: R01NS102573) to study the role of MMP-12 in ischaemic stroke.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD

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

REFERENCES

1 Furie K. Epidemiology and primary prevention of stroke. *CONTINUUM (Minneap Minn)* 2020;26:260–7.
2 World Health Organization (WHO). *Report 2019*. 2020.
3 Virani SS, Alonso A, Benjamin EJ, *et al*. Heart disease and stroke Statistics-2020 update: a report from the American Heart Association. *Circulation* 2020;141:e139–596.
4 Zivin JA. Thrombolytic stroke therapy: past, present, and future. *Neurology* 1999;53:14–9.
5 Hacke W, Kaste M, Bluhmki E, *et al*. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008;359:1317–29.
6 Adeoye O, Hornung R, Khatri P, *et al*. Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: a doubling of treatment rates over the course of 5 years. *Stroke* 2011;42:1952–5.
7 Jauch EC, Saver JL, Adams HP, *et al*. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2013;44:870–947.
8 Wardlaw JM, Warlow CP, Counsell C. Systematic review of evidence on thrombolytic therapy for acute ischaemic stroke. *Lancet* 1997;350:607–14.
9 Scheitz JF, Turc G, Kujala L, *et al*. Intracerebral hemorrhage and outcome after thrombolysis in stroke patients using selective serotonin-reuptake inhibitors. *Stroke* 2017;48:3239–44.
10 Acır İ, Erdoğan HA, Yayla V, *et al*. Incidental thrombotic thrombocytopenic purpura during acute ischemic stroke and thrombolytic treatment. *J Stroke Cerebrovasc Dis* 2018;27:1417–9.
11 Powers WJ, Rabinstein AA, Ackerson T, *et al*. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2018;49:e46–110.
12 Campbell BCV, Donnan GA, Lees KR, *et al*. Endovascular stent thrombectomy: the new standard of care for large vessel ischaemic stroke. *Lancet Neurol* 2015;14:846–54.
13 Furlan AJ. Endovascular therapy for stroke--it's about time. *N Engl J Med* 2015;372:2347–9.
14 Cohen DL, Kearney R, Griffiths M, *et al*. Around 9% of patients with ischaemic stroke are suitable for thrombectomy. *BMJ* 2015;351:h4607.
15 Chia NH, Leyden JM, Newbury J, *et al*. Determining the number of ischemic strokes potentially eligible for endovascular thrombectomy: a population-based study. *Stroke* 2016;47:1377–80.
16 Jadhav AP, Desai SM, Kenmuir CL, *et al*. Eligibility for endovascular trial enrollment in the 6- to 24-hour time window: analysis of a single comprehensive stroke center. *Stroke* 2018;49:1015–7.
17 Goyal M, Menon BK, van Zwam WH, *et al*. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet* 2016;387:1723–31.
18 Albers GW, Marks MP, Kemp S, *et al*. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med* 2018;378:708–18.
19 Josephson SA, Kamel H. The acute stroke care revolution: enhancing access to therapeutic advances. *JAMA* 2018;320:1239–40.
20 Verma RP, Hansch C. Matrix Metalloproteinases (MMPs): chemical-biological functions and (Q)Sars. *Bioorg Med Chem* 2007;15:2223–68.
21 Martel-Pelletier J, Welsch DJ, Pelletier JP. Metalloproteases and inhibitors in arthritic diseases. *Best Pract Res Clin Rheumatol* 2001;15:805–29.
22 Chen YE. MMP-12, an old enzyme plays a new role in the pathogenesis of rheumatoid arthritis *Am J Pathol* 2004;165:1069–70.
23 Werb Z, Gordon S. Elastase secretion by stimulated macrophages characterization and regulation. *J Exp Med* 1975;142:361–77.
24 Banda MJ, Werb Z. Mouse macrophage elastase. Purification and characterization as a metalloproteinase. *Biochem J* 1981;193:589–605.
25 Shapiro SD, Griffin GL, Gilbert DJ, *et al*. Molecular cloning, chromosomal localization, and bacterial expression of a murine macrophage metalloelastase. *J Biol Chem* 1992;267:4664–71.
26 Shapiro SD, Kobayashi DK, Ley TJ. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. *J Biol Chem* 1993;268:23824–9.
27 Gronski TJ Jr, Martin RL, Kobayashi DK, *et al*. Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase. *J Biol Chem* 1997;272:12189–94.
28 Ashworth JL, Murphy G, Rock MJ, *et al*. Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodelling. *Biochem J* 1999;340 (Pt 1):171–81.
29 Dwivedi A, Slater SC, George SJ. MMP-9 and -12 cause N-cadherin shedding and thereby beta-catenin signalling and vascular smooth muscle cell proliferation. *Cardiovasc Res* 2009;81:178–86.
30 Chandler S, Cossins J, Lury J, *et al*. Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. *Biochem Biophys Res Commun* 1996;228:421–9.
31 Dong Z, Kumar R, Yang X, *et al*. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* 1997;88:801–10.
32 Cornelius LA, Nehring LC, Harding E, *et al*. Matrix metalloproteinases generate angiostatin: effects on neovascularization. *J Immunol* 1998;161:6845–52.
33 Belaaouaj AA, Li A, Wun TC, *et al*. Matrix metalloproteinases cleave tissue factor pathway inhibitor. *J Biol Chem* 2000;275:27123–8.
34 Suh H-S, Choi N, Tarassishin L, *et al*. Regulation of progranulin expression in human microglia and proteolysis of progranulin by matrix Metalloproteinase-12 (MMP-12). *PLoS One* 2012;7:e35115.
35 Chelluboina B, Warhekar A, Dillard M, *et al*. Post-transcriptional inactivation of matrix metalloproteinase-12 after focal cerebral ischemia attenuates brain damage. *Sci Rep* 2015;5:9504.
36 Chelluboina B, Klopfenstein JD, Pinson DM, *et al*. Matrix metalloproteinase-12 induces blood-brain barrier damage after focal cerebral ischemia. *Stroke* 2015;46:3523–31.
37 Arruri V, Chokkalla AK, Jeong S, *et al*. MMP-12 knockdown prevents secondary brain damage after ischemic stroke in mice. *Neurochem Int* 2022;161:105432.
38 Challa S, Fornal CA, Schaibley C, *et al*. Post-stroke suppression of matrix metalloproteinase-12 attenuates the expression of M1 and M2 markers and prevents the elevation of other matrix metalloproteinases. *Stroke* 2022;53.
39 Challa SR, Nalamolu KR, Fornal CA, *et al*. Therapeutic efficacy of matrix metalloproteinase-12 suppression on neurological recovery after ischemic stroke: optimal treatment timing and duration. *Front Neurosci* 2022;16:1012812.

40 Svedin P, Hagberg H, Mallard C. Expression of MMP-12 after neonatal hypoxic-ischemic brain injury in mice. *Dev Neurosci* 2009;31:427–36.
41 Nalamolu KR, Chelluboina B, Magruder IB, *et al*. Post-stroke mRNA expression profile of MMPs: effect of genetic deletion of MMP-12. *Stroke Vasc Neurol* 2018;3:153–9.
42 Hohjoh H, Horikawa I, Nakagawa K, *et al*. Induced mRNA expression of matrix metalloproteinases MMP-3, MMP-12, and MMP-13 in the infarct cerebral cortex of photothrombosis model mice. *Neurosci Lett* 2020;739:135406.
43 Wu L, Fan J, Matsumoto S i, *et al*. Induction and regulation of matrix metalloproteinase-12 by cytokines and CD40 signaling in monocyte/macrophages. *Biochem Biophys Res Commun* 2000;269:808–15.
44 Beuker C, Strecker J-K, Rawal R, *et al*. Immune cell infiltration into the brain after ischemic stroke in humans compared to mice and rats: a systematic review and meta-analysis. *Transl Stroke Res* 2021;12:976–90.
45 Gonzalez-Mejia ME, Doseff AI. Regulation of monocytes and macrophages cell fate. *Front Biosci (Landmark Ed)* 2009;14:2413–31.
46 Mahdessian H, Perisic Matic L, Lengquist M, *et al*. Integrative studies implicate matrix metalloproteinase-12 as a culprit gene for large-artery Atherosclerotic stroke. *J Intern Med* 2017;282:429–44.
47 Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 2005;57:173–85.
48 Bazzoni G, Martinez-Estrada OM, Orsenigo F, *et al*. Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. *J Biol Chem* 2000;275:20520–6.
49 Lochhead JJ, Yang J, Ronaldson PT, *et al*. Structure, function, and regulation of the blood-brain barrier tight junction in central nervous system disorders. *Front Physiol* 2020;11:914.
50 Butt AM, Jones HC, Abbott NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *J Physiol* 1990;429:47–62.
51 Keaney J, Campbell M. The dynamic blood-brain barrier. *FEBS J* 2015;282:4067–79.
52 Stamatovic SM, Keep RF, Andjelkovic AV. Brain endothelial cell-cell junctions: how to "open" the blood brain barrier. *Curr Neuropharmacol* 2008;6:179–92.
53 Luissint A-C, Artus C, Glacial F, *et al*. Tight Junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS* 2012;9:23.
54 Jiao H, Wang Z, Liu Y, *et al*. Specific role of tight junction proteins claudin-5, occludin, and ZO-1 of the blood-brain barrier in a focal cerebral ischemic insult. *J Mol Neurosci* 2011;44:130–9.
55 McCarthy KM, Skare IB, Stankewich MC, *et al*. Occludin is a functional component of the tight junction. *J Cell Sci* 1996;109 (Pt 9):2287–98.
56 Furuse M, Itoh M, Hirase T, *et al*. Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. *J Cell Biol* 1994;127:1617–26.
57 Itoh M, Furuse M, Morita K, *et al*. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol* 1999;147:1351–63.
58 Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis* 2008;32:200–19.
59 Mun-Bryce S, Rosenberg GA. Matrix metalloproteinases in cerebrovascular disease. *J Cereb Blood Flow Metab* 1998;18:1163–72.
60 Gasche Y, Fujimura M, Morita-Fujimura Y, *et al*. Early appearance of activated matrix metalloproteinase-9 after focal cerebral ischemia in mice: a possible role in blood-brain barrier dysfunction. *J Cereb Blood Flow Metab* 1999;19:1020–8.
61 Heo JH, Lucero J, Abumiya T, *et al*. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. *J Cereb Blood Flow Metab* 1999;19:624–33.
62 Rosenberg GA. Matrix metalloproteinases in neuroinflammation. *Glia* 2002;39:279–91.
63 Zhao BQ, Tejima E, Lo EH. Neurovascular proteases in brain injury, hemorrhage and remodeling after stroke. *Stroke* 2007;38:748–52.
64 Yang Y, Estrada EY, Thompson JF, *et al*. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J Cereb Blood Flow Metab* 2007;27:697–709.
65 del Zoppo GJ. Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. *Neuroscience* 2009;158:972–82.
66 Montaner J, Alvarez-Sabín J, Molina C, *et al*. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. *Stroke* 2001;32:1759–66.
67 Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999;19:819–34.
68 Samson Y, Lapergue B, Hosseini H. Inflammation and ischaemic stroke: current status and future perspectives. *Rev Neurol (Paris)* 2005;161:1177–82.
69 Chamorro A, Hallenbeck J. The harms and benefits of inflammatory and immune responses in vascular disease. *Stroke* 2006;37:291–3.
70 Iadecola C, Anrather J. The Immunology of stroke: from mechanisms to translation. *Nat Med* 2011;17:796–808.
71 Yilmaz G, Granger DN. Cell adhesion molecules and ischemic stroke. *Neurol Res* 2008;30:783–93.
72 Nalamolu KR, Smith NJ, Chelluboina B, *et al*. Prevention of the severity of post-ischemic inflammation and brain damage by simultaneous knockdown of toll-like receptors 2 and 4. *Neuroscience* 2018;373:82–91.
73 Nalamolu KR, Challa SR, Fornal CA, *et al*. Attenuation of the induction of TLRs 2 and 4 mitigates inflammation and promotes neurological recovery after focal cerebral ischemia. *Transl Stroke Res* 2021;12:923–36.
74 Ceulemans A-G, Zgavc T, Kooijman R, *et al*. The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. *J Neuroinflammation* 2010;7:74.
75 Jin R, Liu L, Zhang S, *et al*. Role of inflammation and its mediators in acute ischemic stroke. *J Cardiovasc Transl Res* 2013;6:834–51.
76 Peruzzotti-Jametti L, Donegá M, Giusto E, *et al*. The role of the immune system in central nervous system plasticity after acute injury. *Neuroscience* 2014;283:210–21.
77 Shichita T, Sakaguchi R, Suzuki M, *et al*. Post-ischemic inflammation in the brain. *Front Immunol* 2012;3:132.
78 Marchant DJ, Bellac CL, Moraes TJ, *et al*. A new transcriptional role for matrix metalloproteinase-12 in antiviral immunity. *Nat Med* 2014;20:493–502.
79 Yong VW, Power C, Forsyth P, *et al*. Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci* 2001;2:502–11.
80 Astrup J, Symon L, Branston N, *et al*. Thresholds of cerebral ischemia. In: Schmiedek P, Gratzl O, Spetzler RF, eds. *Microsurgery for stroke*. 1977: 16–21.
81 Ferrer I. Apoptosis: future targets for neuroprotective strategies. *Cerebrovasc Dis* 2006;21 Suppl 2:9–20.
82 Radak D, Katsiki N, Resanovic I, *et al*. Apoptosis and acute brain ischemia in ischemic stroke. *Curr Vasc Pharmacol* 2017;15:115–22.
83 Uzdensky AB. Apoptosis regulation in the penumbra after ischemic stroke: expression of pro- and antiapoptotic proteins. *Apoptosis* 2019;24:687–702.
84 Chelluboina B, Klopfenstein JD, Gujrati M, *et al*. Temporal regulation of apoptotic and anti-apoptotic molecules after middle cerebral artery occlusion followed by reperfusion. *Mol Neurobiol* 2014;49:50–65.
85 Lee S-R, Lo EH. Induction of caspase-mediated cell death by matrix metalloproteinases in cerebral endothelial cells after hypoxia-reoxygenation. *J Cereb Blood Flow Metab* 2004;24:720–7.
86 Copin J-C, Goodyear M-C, Gidday JM, *et al*. Role of matrix Metalloproteinases in apoptosis after transient focal cerebral ischemia in rats and mice. *Eur J Neurosci* 2005;22:1597–608.
87 Gu Z, Cui J, Brown S, *et al*. A highly specific inhibitor of matrix metalloproteinase-9 Rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. *J Neurosci* 2005;25:6401–8.
88 Fujimoto M, Takagi Y, Aoki T, *et al*. Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. *J Cereb Blood Flow Metab* 2008;28:1674–85.
89 Broughton BRS, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. *Stroke* 2009;40:e331–9.
90 Deoni SCL, Mercure E, Blasi A, *et al*. Mapping infant brain myelination with magnetic resonance imaging. *J Neurosci* 2011;31:784–91.
91 Pantoni L, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischemia. *Stroke* 1996;27:1641–6.
92 Bristow MS, Simon JE, Brown RA, *et al*. MR perfusion and diffusion in acute ischemic stroke: human gray and white matter have different thresholds for infarction. *J Cereb Blood Flow Metab* 2005;25:1280–7.
93 Marin MA, Carmichael ST. Mechanisms of demyelination and Remyelination in the young and aged brain following white matter stroke. *Neurobiol Dis* 2019;126:5–12.
94 Chen C, Bivard A, Lin L, *et al*. Thresholds for infarction vary between gray matter and white matter in acute ischemic stroke: a CT perfusion study. *J Cereb Blood Flow Metab* 2019;39:536–46.

95 Yang Y, Jalal FY, Thompson JF, *et al*. Tissue inhibitor of metalloproteinases-3 mediates the death of immature oligodendrocytes via TNF-alpha/TACE in focal cerebral ischemia in mice. *J Neuroinflammation* 2011;8.
96 Rost NS, Cougo P, Lorenzano S, *et al*. Diffuse microvascular dysfunction and loss of white matter integrity predict poor outcomes in patients with acute ischemic stroke. *J Cereb Blood Flow Metab* 2018;38:75–86.
97 Hansmann F, Herder V, Kalkuhl A, *et al*. Matrix metalloproteinase-12 deficiency ameliorates the clinical course and demyelination in Theiler's murine encephalomyelitis. *Acta Neuropathol* 2012;124:127–42.
98 Dwivedi A, George S. MMP-12 is important for VSMC proliferation and migration: role of B-Catenin signalling. *Vascular Pharmacology* 2006;45:e129.
99 Matsumoto S, Kobayashi T, Katoh M, *et al*. Expression and localization of matrix metalloproteinase-12 in the aorta of cholesterol-fed rabbits: relationship to lesion development. *Am J Pathol* 1998;153:109–19.
100 Chaturvedi M, Kaczmarek L. Mmp-9 inhibition: a therapeutic strategy in ischemic stroke. *Mol Neurobiol* 2014;49:563–73.
101 Lanone S, Zheng T, Zhu Z, *et al*. Overlapping and enzyme-specific contributions of matrix Metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest* 2002;110:463–74.
102 Challa SR, Nalamolu KR, Fornal CA, *et al*. The interplay between MMP-12 and T-PA in the brain after ischemic stroke. *Neurochemistry International* 2022;161:105436.
103 Mazzieri R, Masiero L, Zanetta L, *et al*. Control of type IV collagenase activity by components of the Urokinase-Plasmin system: a regulatory mechanism with cell-bound reactants. *EMBO J* 1997;16:2319–32.
104 Carmeliet P, Moons L, Lijnen R, *et al*. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet* 1997;17:439–44.
105 Bhandari V, Palfree RG, Bateman A. Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals Tandem cysteine-rich granulin domains. *Proc Natl Acad Sci U S A* 1992;89:1715–9.
106 Daniel R, He Z, Carmichael KP, *et al*. Cellular localization of gene expression for progranulin. *J Histochem Cytochem* 2000;48:999–1009.
107 Daniel R, Daniels E, He Z, *et al*. Progranulin (Acrogranin/PC cell-derived growth factor/Granulin-Epithelin precursor) is expressed in the placenta, epidermis, microvasculature, and brain during murine development. *Dev Dyn* 2003;227:593–9.
108 Mackenzie IRA, Baker M, Pickering-Brown S, *et al*. The neuropathology of frontotemporal Lobar degeneration caused by mutations in the Progranulin Gene. *Brain* 2006;129:3081–90.
109 Matsubara T, Mita A, Minami K, *et al*. PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in Adipose tissue. *Cell Metabolism* 2012;15:38–50.
110 Van Damme P, Van Hoecke A, Lambrechts D, *et al*. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J Cell Biol* 2008;181:37–41.
111 Martens LH, Zhang J, Barmada SJ, *et al*. Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. *J Clin Invest* 2012;122:3955–9.
112 Egashira Y, Suzuki Y, Azuma Y, *et al*. The growth factor progranulin attenuates neuronal injury induced by cerebral ischemia-reperfusion through the suppression of neutrophil recruitment. *J Neuroinflammation* 2013;10:105.
113 Tang W, Lu Y, Tian Q-Y, *et al*. The growth factor Progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science* 2011;332:478–84.
114 Bateman A, Belcourt D, Bennett H, *et al*. Granulins, a novel class of peptide from leukocytes. *Biochem Biophys Res Commun* 1990;173:1161–8.
115 Zhu J, Nathan C, Jin W, *et al*. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell* 2002;111:867–78.
116 Horinokita I, Hayashi H, Oteki R, *et al*. Involvement of progranulin and granulin expression in inflammatory responses after cerebral ischemia. *Int J Mol Sci* 2019;20:5210.
117 Tsao CW, Aday AW, Almarzooq ZI, *et al*. Heart disease and stroke statistics-2022 update: a report from the American Heart Association. *Circulation* 2022;145:e153–639.
118 Megyesi JF, Vollrath B, Cook DA, *et al*. In vivo animal models of cerebral vasospasm: a review. *Neurosurgery* 2000;46:448–60;
119 Ashwal S, Pearce WJ. Animal models of neonatal stroke. *Curr Opin Pediatr* 2001;13:506–16.
120 Alonso de Leciñana M, Díez-Tejedor E, Carceller F, *et al*. Cerebral ischemia: from animal studies to clinical practice. should the methods be reviewed *Cerebrovasc Dis* 2001;11 Suppl 1:20–30.
121 Traystman RJ. Animal models of focal and global cerebral ischemia. *ILAR J* 2003;44:85–95.
122 Durukan A, Tatlisumak T. Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. *Pharmacol Biochem Behav* 2007;87:179–97.
123 Bouët V, Freret T, Toutain J, *et al*. Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse. *Exp Neurol* 2007;203:555–67.
124 Noble LJ, Donovan F, Igarashi T, *et al*. Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. *J Neurosci* 2002;22:7526–35.
125 Trivedi A, Hsu J, Lin Y, *et al*. The effects of acute and extended inhibition of matrix metalloproteinases on demyelination and functional recovery after spinal cord injury. *Int J Neuroprotect Neuroregen* 2005;2:30–8.
126 Goussev S, Hsu J-Y, Lin Y, *et al*. Differential temporal expression of matrix metalloproteinases after spinal cord injury: relationship to revascularization and wound healing. *J Neurosurg* 2003;99:188–97.
127 Lee S-R, Kim H-Y, Rogowska J, *et al*. Involvement of matrix metalloproteinase in neuroblast cell migration from the subventricular zone after stroke. *J Neurosci* 2006;26:3491–5.
128 Zhao B-Q, Wang S, Kim H-Y, *et al*. Role of matrix metalloproteinases in delayed cortical responses after stroke. *Nat Med* 2006;12:441–5.
129 Koellhoffer EC, McCullough LD. The effects of estrogen in ischemic stroke. *Transl Stroke Res* 2013;4:390–401.
130 Rojas JI, Zurrú MC, Romano M, *et al*. Acute ischemic stroke and transient ischemic attack in the very old--risk factor profile and stroke subtype between patients older than 80 years and patients aged less than 80 years. *Eur J Neurol* 2007;14:895–9.
131 Philip M, Benatar M, Fisher M, *et al*. Methodological quality of animal studies of neuroprotective agents currently in phase II/III acute ischemic stroke trials. *Stroke* 2009;40:577–81.
132 Bacigaluppi M, Comi G, Hermann DM. Animal models of ischemic stroke. part two: modeling cerebral ischemia. *TONEUJ* 2010;4:34–8.
133 Sudlow CL, Warlow CP. Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. *Stroke* 1997;28:491–9.
134 Lyden P, Buchan A, Boltze J, *et al*. Top priorities for Cerebroprotective studies-a paradigm shift: report from STAIR XI. *Stroke* 2021;52:3063–71.