

Matrix metalloproteinase-9 in hemorrhagic transformation after acute ischemic stroke (Review)

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Abstract. Hemorrhagic transformation (HT) is a devastating complication following acute ischemic stroke with high morbidity and mortality. The pathogenesis of HT mainly involves ischemia-reperfusion-induced oxidative stress, neuroinflammation, thrombolytic therapy-associated toxicity and, most critically, blood-brain barrier (BBB) disruption. Matrix metalloproteinase-9 (MMP-9) serves as a critical mediator of HT through degrading extracellular matrix components and disrupting tight junction proteins, thereby compromising BBB integrity. Thus, elaborating the underlying molecular mechanisms of MMP-9 in destroying BBB and promoting HT is essential to improve the outcome of ischemic stroke patients. Furthermore, to provide beneficial insights for the treatment of ischemic stroke, precise understanding of the potential role of MMP-9 as a biomarker and treatment target to predict and ameliorate the risk of HT is also necessary.

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1. Introduction

Stroke is the second leading cause of death and the third leading cause of disability worldwide and a great burden to patients and society (1). Ischemic stroke, the most common type of stroke, occurs when cerebral artery occlusion leads to critical reduction in blood flow. Early reperfusion remains paramount in acute ischemic stroke (AIS) management. Intravenous thrombolysis with tissue plasminogen activator (tPA) is one of the most effective reperfusion therapeutic strategies and is able to rapidly restore cerebral perfusion and alleviate the neurological deficits of patients (2). Despite its clinical efficacy, tPA thrombolysis remains underused, with only 5-10% of ischemic stroke patients receiving treatment (3). This limited application primarily stems from two key constraints: the narrow 4.5-h therapeutic window and the risk of tPA-induced hemorrhagic transformation (HT) (4). Therefore, early identification of the risk of HT and timely prevention strategies might be helpful for physicians to make more useful decisions on the treatment for AIS.

The pathophysiological mechanisms of HT are complicated, involving blood-brain barrier (BBB) disruption, ischemia-reperfusion injury, oxidative stress, neuroinflammation, tPA administration and brain cell death (3). These processes interact with each other to promote the disruption of vascular integrity and BBB dysfunction (5). BBB is a highly selective functional structure that acts as a protective interface between the central nervous system (CNS) and the circulatory system, playing a critical role in maintaining the microenvironmental homeostasis of CNS (6). BBB disruption is a prominent pathophysiological feature of ischemic stroke, accompanied by worse functional prognosis and higher mortality rate (7). The components of erythrocytes, neurotoxic plasma components and pathogens enter the brain tissue through the compromised BBB, markedly contributing to the pathogenesis of HT (8).

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Matrix metalloproteinases (MMPs) play a key role in the pathological process of BBB damage by mediating the degradation of extracellular matrix (ECM) and tight junction proteins (TJPs) (6,9). MMPs comprise a family of calcium-dependent zinc-containing endopeptidases and play pivotal roles in tissue remodeling and ECM degradation, including gelatin, elastins, collagens, matrix glycoproteins and proteoglycans (10). These enzymes are synthesized by neurons, astrocytes, endothelial cells, microglia and peripheral immune cells (11). Structurally, MMPs exhibit a highly conserved domain architecture consisting of four principal components: The amino-terminal signal peptide domain, the propeptide region (predomain), the catalytic domain containing zinc and variable C-terminal domains that mediate substrate recognition and interaction, including the transmembrane domain, the hemopexin-like domain and the fibronectin domain (12,13). At present, at least 26 human MMPs have been discovered, which are divided into six subgroups according to their different structures: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others (10,14). Previous studies have demonstrated that MMPs high expression is associated with BBB disruption and HT following AIS (15-17). Among all MMP family members, MMP-9 has emerges as the most extensively investigated protease, with numerous animal and clinical studies establishing its crucial role in HT pathogenesis (11,18-22).

The present review aimed to elaborate the mechanistic involvement of MMP-9 in HT development following AIS and evaluate its potential as biomarker and therapeutic target.

2. Physiopathological involvement of MMP-9 in ischemic stroke

Belonging to gelatinase B family, MMP-9 comprises a signal peptide domain, a prodomain, a catalytic domain with a Zn^{2+} binding site, a fibronectin domain and a hemopexin-like domain (Fig. 1) (23). The signal peptide domain mediates MMP-9 excretion from the cell (24). The prodomain maintains MMP-9 in an inactive state through binding its conserved cysteine residue to Zn^{2+} in the enzyme's active center (25). The catalytic domain is responsible for the proteolytic activity of MMP-9 (23). The fibronectin domain acts as a modulator of substrates recognition, which is able to bind to gelatins, laminins and collagens (26). The hemopexin-like domain plays a crucial role in substrates specificity and the specific substrates mainly comprise ECM components (such as collagens, gelatin and fibronectin) and non-ECM molecules including tissue inhibitor metalloproteinases and chemokines (23,26). In the brain, MMP-9 is mainly produced by astrocytes, microglia and infiltrating leukocytes (27,28). The expression of MMP-9 is regulated by transcription factors, inflammatory factors, chemokines and growth factors (29). MMP-9 is usually secreted in an inactive form and activated by thrombin, MMP-3, tPA and reactive oxygen/nitrogen species (ROS/RNS) (29-32). Notably, zinc plays a dual role in MMP-9 regulation: As a structural cofactor required for its proteolytic function at physiological levels and as a pro-oxidant stimulus that indirectly activates MMP-9 via ROS generation when accumulated in excess (33).

Following ischemic stroke, MMP-9 undergoes a dynamic change, with its expression and activity varying markedly over time (Fig. 2). The significant upregulation of MMP-9 can be

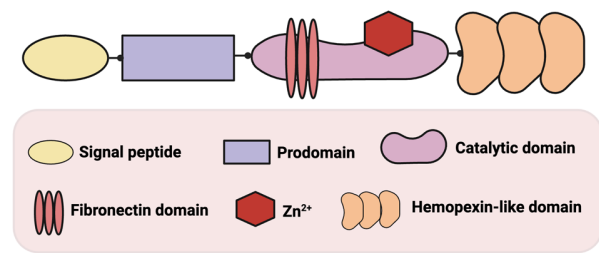


Figure 1. Domain structures of MMP-9. MMP-9 contains several domains, including a signal peptide domain, a prodomain, a catalytic domain with a Zn^{2+} binding site, a fibronectin domain and a hemopexin-like domain. The figure was generated with Biorender (<https://www.biorender.com>). MMP-9, matrix metalloproteinase-9.

found in the acute phase of ischemic stroke (within 7 days from onset). It has been demonstrated that the expression and activity of MMP-9 increases rapidly and peaks within 24 h after ischemic stroke (34,35). During this time, MMP-9 exacerbates brain injury by damaging BBB integrity, aggravating edema, enhancing neuroinflammation and brain cell death and promoting HT (36). At 7-14 days after ischemic stroke, the activity and expression of MMP-9 still remains elevated. In this delayed phase following ischemic stroke, MMP-9 facilitates the tissue repair and angiogenesis by degrading damaged ECM, thereby mitigating ischemic brain injury and prompting functional recovery (37). From weeks to months after ischemic stroke, the expression and activity of MMP-9 gradually decreases. This phase is characterized by ECM remodeling, in which impenetrable scar tissues formed by gliosis hinders the regeneration and reprojction of axons. At this time, the BBB opening mediated by MMP-9 allows cells to enter the CNS, facilitating functional recovery (38).

3. MMP-9 mediated blood-brain barrier dysfunction after ischemic stroke

BBB is a unique structure formed by blood vessels in the CNS, maintaining neuronal activity and blocking pathogenic damage (39). The maintenance of BBB function primarily relies on the interaction of endothelial cells, astrocytes, pericytes and ECM around the vessels (Fig. 3). Endothelial cells express high levels of TJPs, which help to prevent blood entering brain by limiting para-cellular permeability (40). TJPs mainly include the transmembrane proteins (claudin-5, claudin-3, claudin-12 and occludin) and zonula occludens proteins (ZO-1, ZO-2 and ZO-3), in which transmembrane proteins, especially claudin-5, interact with the similar proteins in the adjacent cell to form tight junctions and zonula occludens proteins connect the aforementioned proteins to the cytoskeleton (41,42). The basement membrane (BM), surrounding the lumen surface of endothelial cells, is composed of collagen IV, fibronectin, heparin sulfate and laminins secreted by endothelial cells, astrocytes and pericytes (43,44). Pericytes and astrocytic endfeet are embedded in the BM, playing a significant role in maintaining BBB integrity (45). The brain ECM consists of neuro-ECM, BM and luminal ECM. The neuro-ECM serves as the connective framework of the brain, forming a dynamic scaffold that supports neurons and glia, while both the BM and the luminal ECM are involved in forming BBB (Fig. 3) (46).

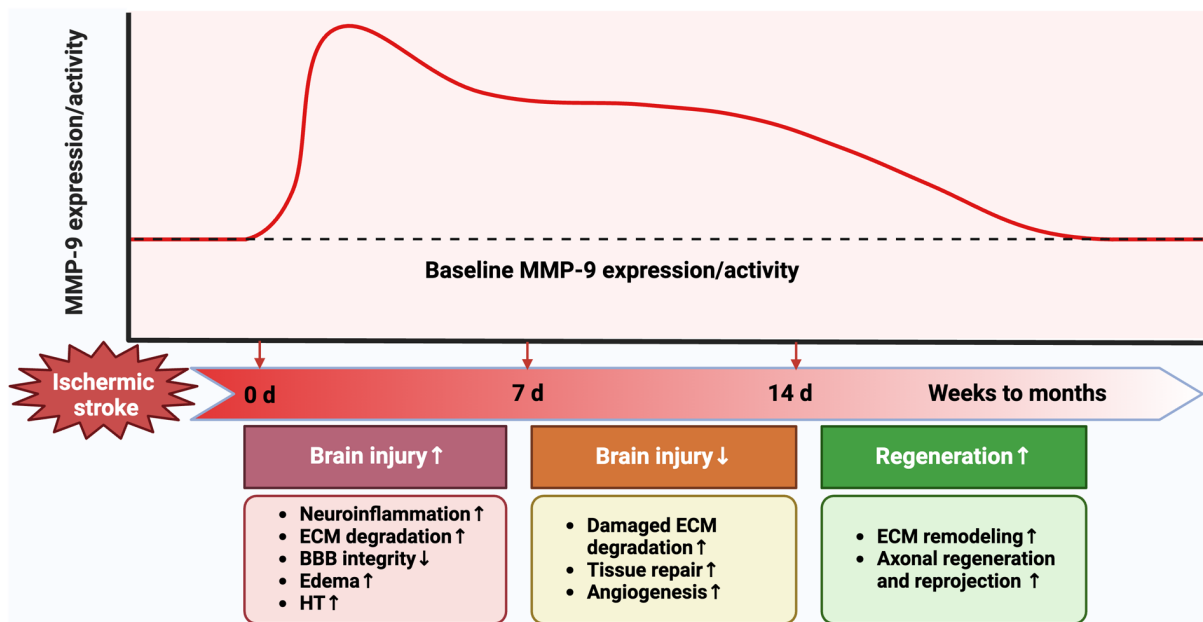


Figure 2. Schematic representation of the dynamic changes in MMP-9 following ischemic stroke. Within 7 days from onset, MMP-9 expression/activity increases rapidly and peaks within 24 h, which exacerbates BBB disruption, edema and neuroinflammation, thereby promoting HT. At 7-14 days after ischemic stroke, MMP-9 expression/activity remains at a high level, facilitating tissue repair and angiogenesis through degrading damaged ECM. From weeks to months after ischemic stroke, MMP-9 expression/activity decreases gradually, promoting ECM remodeling and functional recovery. The figure was generated with Biorender (<https://www.biorender.com>). MMP-9, matrix metalloproteinase-9; BBB, blood-brain barrier; HT, hemorrhagic transformation; ECM, extracellular matrix.

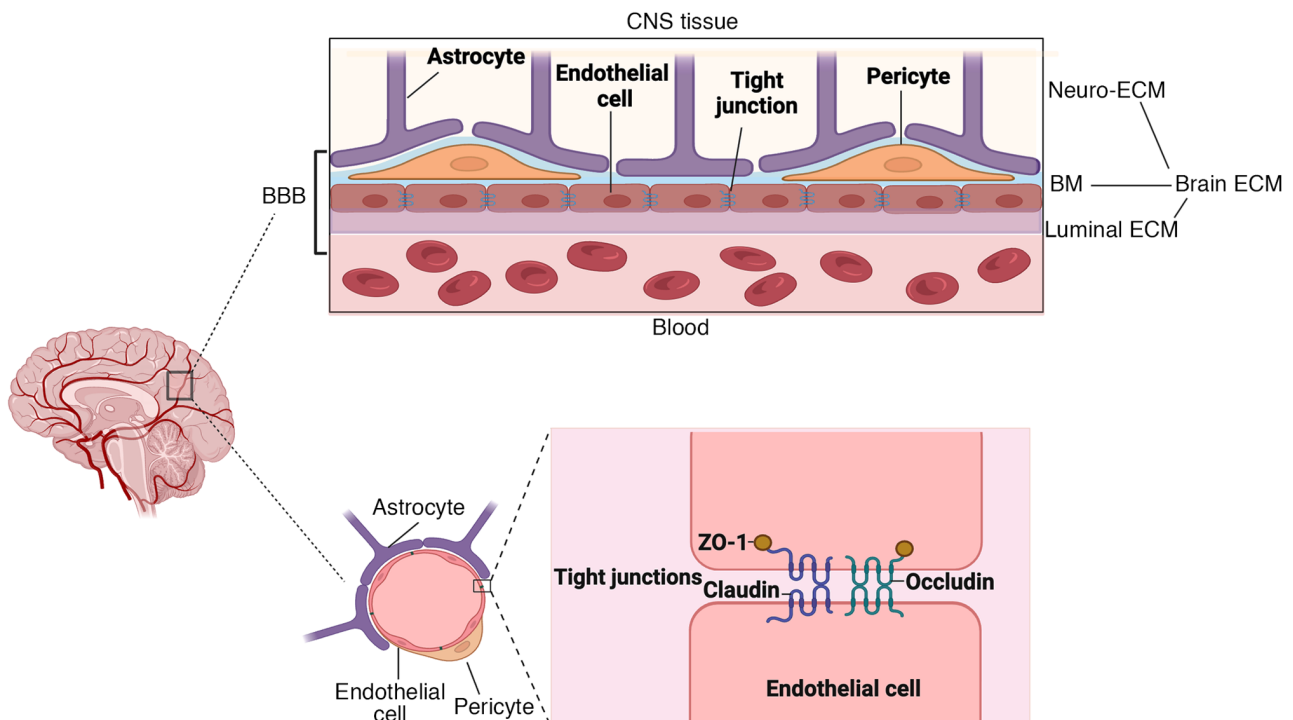


Figure 3. The biological structure of blood-brain barrier. The components of BBB mainly include endothelial cells with tight junctions, astrocytes, pericytes and ECM. Claudin, occludin and ZO-1 are common TJPs secreted by endothelial cells. ECM of the brain consists of neuro-ECM, BM and luminal ECM. Astrocytes and pericytes are embedded in the BM. All these different cells and structures are involved in maintaining the integrity and function of BBB. The figure was generated with Biorender (<https://www.biorender.com>). BBB, blood-brain barrier; BM, basement membrane; ECM, extracellular matrix; CNS, central nervous system.

These integrated cells and structures collectively promote the integrity of BBB, thereby playing a critical role in regulating the brain homeostasis and protecting the CNS.

BBB dysfunction initiates from the onset of ischemic stroke and aggravates with the sustained hyperfusion. The disruption of TJPs and ECM is the main reason underlying the

Table I. ECASS classification of hemorrhagic transformation (54).

Type	Radiographic features
HI-1	Small scattered petechial hemorrhage at the infarct margins
HI-2	Numerous confluent petechial hemorrhages within the infarction, without signs of mass effect
PH-1	Hematoma within the infarction, occupying <30%, with minor signs of mass effect
PH-2	Hematoma within the infarction, occupying $\geq$ 30%, with obvious mass effect

ECASS, the European Cooperation Acute Stroke Study; HI, hemorrhagic infarction; PH, parenchymal hematoma.

increased permeability of BBB after ischemic stroke, in which MMP-9 plays a critical role (47). MMP-9 primarily disrupts BBB through proteolytic degradation of key ECM components, including collagen IV, laminin and fibronectin (48,49). Beyond its role in ECM degradation, MMP-9 also targets TJPs, with evidence demonstrating its specific proteolytic effects on occludin, claudin-5 and ZO-1 (50). In addition, MMP-9 mediates BBB disruption by degrading dystroglycan, a critical ECM receptor on astrocyte endfeet that anchors them to the BM through the high-affinity interactions with laminin (51,52).

4. Involvement of MMP-9 in hemorrhagic transformation after ischemic stroke

HT is a common complication of ischemic stroke, with an incidence of 10-40%, leading to increased stroke mortality (53). The classification of HT following ischemic stroke, as defined by the European Cooperative Acute Stroke Study criteria, encompasses two main categories with distinct radiographic and prognostic implications: Hemorrhagic infarction (HI) and parenchymal hematoma (PH) (54). They are further subclassified into four clinically relevant subtypes based on computed tomography characteristics: HI-1, HI-2, PH-1 and PH-2 (Table I). The clinical prognosis of HT exhibits marked heterogeneity across different subtypes. Compared with ischemic stroke patients without HT, PH-2 markedly increases the risk of early neurological deterioration, 3-month mortality and disability, whereas HI-1, HI-2 and PH-1 show no statistically significant differences in these outcomes (55). HT can be also classified into symptomatic HT and asymptomatic HT on basis of the presence or absence of neurological decline (56). While the pathogenesis of HT following ischemic stroke is complicated, current evidence points to three interconnected mechanisms, including oxidative stress induced by ischemia-reperfusion, neuroinflammation and thrombolytic therapy-associated toxicity (53,57,58). MMP-9 functions as a pivotal effector molecule in these pathological processes (Fig. 4).

Oxidative stress is a crucial pathological process mediating ischemia-reperfusion injury and HT, in which excess production and activation of ROS/RNS play an important role (32,59). After stroke, ischemia and hypoxia of certain brain tissue induces the release of excitotoxic substance-glutamate, which subsequently activates the calcium channel-related receptors, especially N-methyl-D-aspartate receptor, leading to the influx of calcium (22,60). Calcium influx initiates a series of deleterious cascades, such as the mitochondrial depolarization

and nicotinamide adenine dinucleotide phosphate oxidase activation, subsequently triggering an excessive generation and activation of ROS/RNS (61,62). Mounting evidence indicates that oxidative stress mediates BBB dysfunction through both direct and indirect mechanisms, with MMP-9 serving as a key downstream mediator in this pathological cascade. Direct effects involve disrupting TJPs (occludin, ZO-1 and claudin-5) through oxidative modification and redistribution and activating MMP-9 to catalyze the ECM degradation (63,64). Indirectly, ROS/RNS modulate BBB permeability by oxidatively activating multiple signaling pathways and transcriptional regulators. Primarily, ROS/RNS-mediated activation of the high-mobility group box1 (HMGB1)/toll-like receptor 4 (TLR4) signaling axis induces nuclear factor- κ B (NF- κ B)-dependent transcriptional upregulation of MMP-9, which subsequently exacerbates BBB disruption (32). Furthermore, ROS activate phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) signaling cascades, which promote nuclear translocation of transcription factors, such as NF- κ B and activator protein-1 (AP-1), thereby enhancing MMP-9 expression through specific promoter binding (65,66).

Neuroinflammation following ischemic stroke is another widely recognized contributor to BBB breakdown and HT development. After ischemic stroke, dying brain cells release damage-associated molecular pattern (DAMP) molecules, including HMGB1, heat shock protein and DNA, thereby triggering microglia and astrocytes activation and pro-inflammatory cytokines, chemokines and MMPs production (67-69). The pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α play a profound role in mediating BBB breakdown (70). IL-1 β enhances chemokines production, promoting neutrophil infiltration and subsequent MMP-9 release (71,72). IL-6 directly compromises BBB integrity by reducing the trans-endothelial electrical resistance value in cerebral endothelial cells (73). TNF- α activates MAPK and PI3K/Akt signaling pathways, stimulating MMP-9-mediated degradation of ECM and TJPs (74). Meanwhile, upregulated chemokines, such as monocyte chemokine protein-1 (MCP-1), macrophage inflammatory protein-1 α and stromal cell-derived factor 1, mediate BBB disruption by inducing the endothelial cells retraction and intercellular gap formation (75). These pro-inflammatory factors and proteases (especially MMP-9) collectively contribute to BBB dysfunction, promoting the infiltration of peripheral immune cells, mainly neutrophils, through the compromised BBB (70). The infiltrated and activated neutrophils release more pro-inflammatory cytokines

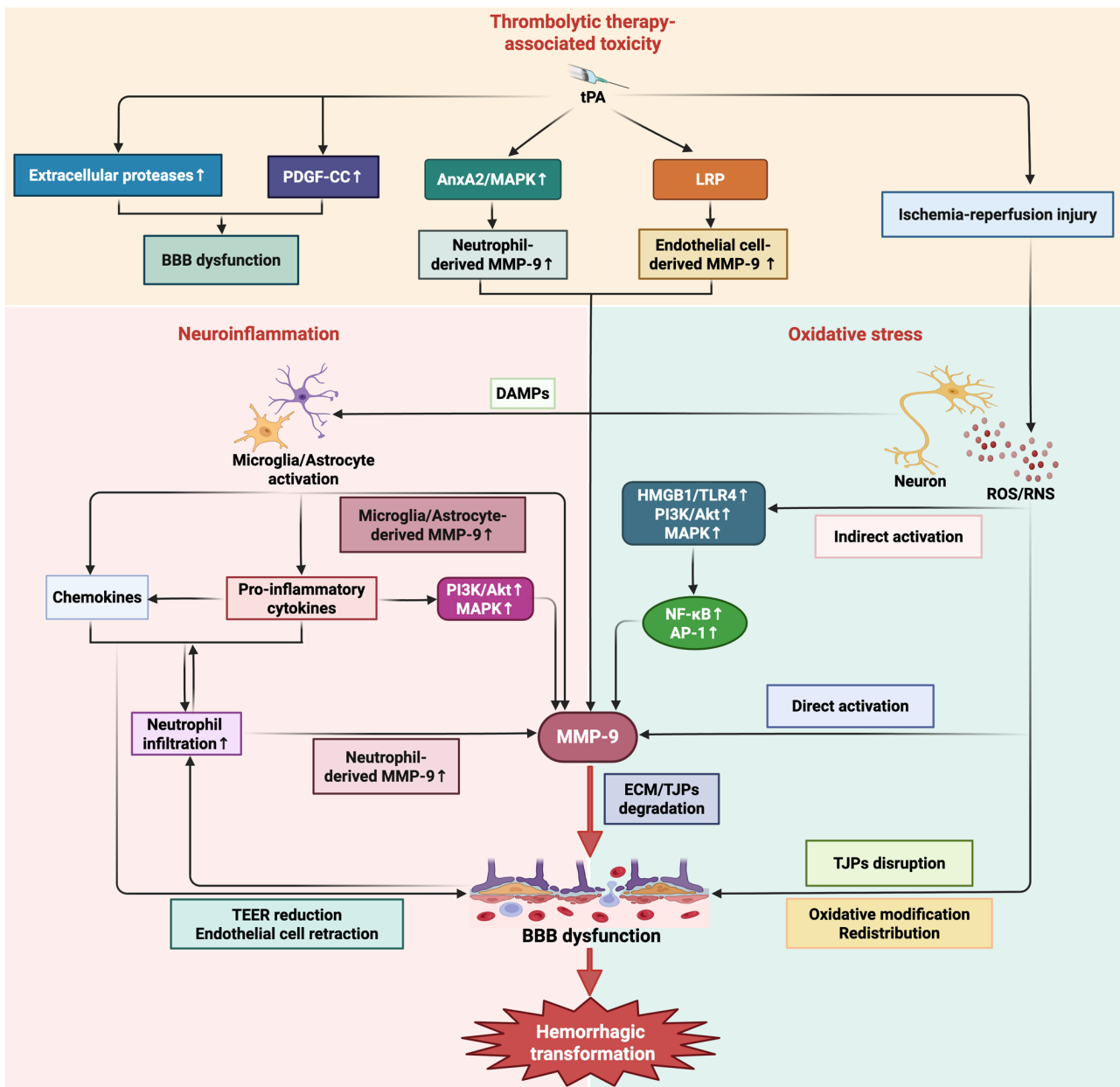


Figure 4. Involvement of MMP-9 in HT after ischemic stroke. HT following ischemic stroke is primarily driven by BBB dysfunction, which involves three interconnected pathological processes including oxidative stress, neuroinflammation and thrombolytic therapy-associated toxicity. These mechanisms collectively disrupt BBB integrity through direct structural injury and indirect MMP-9-dependent pathways. The figure was generated with Biorender (<https://www.biorender.com>). MMP-9, matrix metalloproteinase-9; HT, hemorrhagic transformation; PDGF, platelet-derived growth factor; AnxA2, annexin A2; MAPK, mitogen-activated protein kinase; LRP, low-density lipoprotein receptor-related protein; BBB, blood-brain barrier; DAMPs, damage-associated molecular patterns; ROS, reactive oxygen species; RNS, reactive nitrogen species; HMGB1, high-mobility group box1; TLR4, toll-like receptor 4; PI3K, phosphatidylinositol-3 kinase; Akt, protein kinase B; NF-κB, nuclear factor-κB; AP-1, activator protein-1; ECM, extracellular matrix; TJP, tight junction protein; TEER, trans-endothelial electrical resistance.

and chemokines to enhance the activation of microglia and astrocytes. Additionally, the upregulation of neutrophil-derived MMP-9 further aggravates BBB dysfunction. These interconnected pathological cascades synergistically exacerbate BBB disruption and promote the progression of HT.

Thrombolytic therapy with tPA is a well-established risk factor for HT. As the only US Food and Drug Administration-approved agent for AIS, tPA can markedly improve functional outcomes by restoring cerebral blood flow when administered within 4.5 h of symptom onset (76). However, the fibrinolytic effect of tPA simultaneously

increases the risk of hemorrhagic complications. Compelling clinical evidence indicates that intravenous tPA administration in AIS patients markedly elevates HT risk, particularly when treatment is delayed beyond the 4.5-h therapeutic window, compared with non-thrombolytic treatment approaches (77). Multiple mechanisms are involved in the development of tPA-mediated HT. First, tPA promotes the formation of HT through its pharmacological effect. Beyond its fibrinolytic activity in clot dissolution, tPA also activates some extracellular proteases and mediates the dysregulation of ECM proteolysis, leading to BBB leakage (78). Second, tPA can act

on the platelet-derived growth factor receptor alpha (PDGFR α) on perivascular astrocyte end feet and promote the expression of PDGF-CC, leading to the upregulation of vascular permeability (79). Last but not least, tPA can also increase the risk of HT via multiple MMP-9-dependent pathways (80-83): i) tPA mobilizes and activates neutrophils, via annexin A2-dependent MAPK signaling pathway, promoting neutrophil-derived MMP-9 secretion; ii) tPA binds the low-density lipoprotein receptor-related protein receptor on endothelial cells to enhance endothelial cell-derived MMP-9 expression. It is worth mentioning that although the role of tPA in promoting HT has been well established, it is not the sole contributing factor to HT during ischemia-reperfusion. The excessive ROS/RNS production during ischemia-reperfusion also plays a critical role in HT through activating MMP-9 and mediating BBB dysfunction (53). Of particular clinical relevance, the delayed administration of tPA (>4.5 h post-ischemia) potentiates this pathological process, thereby amplifying BBB disruption and HT risk during cerebral ischemia-reperfusion (84). This pathophysiological cascade elucidates the clinical observation of elevated HT risks when thrombolysis is administered beyond the 4.5-h therapeutic window.

5. MMP-9 as biomarker for hemorrhagic transformation

The role of MMP-9 in mediating BBB disruption highlights its potential as a predictive biomarker for HT. Previous reviews revealed that MMP-9 concentration in peripheral blood was positively associated with larger infarct size, worse prognosis and higher HT risk after AIS (85,86). There was a clinical study supporting the aforementioned viewpoints as well. With 168 patients with ischemic stroke and 40 healthy controls included in this study, Yuan *et al* (87) determined the plasma MMP-9 concentrations of these patients through enzyme-linked immunosorbent assay, finding that higher plasma MMP-9 concentration was markedly associated with increased spontaneous HT risk and MMP-9 value >181.7 ng/ml within 24 h after stroke onset was an independent marker to predict HT risk in ischemic stroke patients. However, Montaner *et al* (16) noted that the predictive value of plasma MMP-9 level differed in different HT subtypes. The authors found that baseline plasma MMP-9 levels positively correlated to HT severity following tPA administration: Lowest MMP-9 levels in HI-1 group (lower than non-HT group), higher but almost normal levels in HI-2 group, markedly higher in PH-1 group and highest in PH-2 group, which highlights the predictive value of baseline plasma MMP-9 in severe HT. This phenomenon may be attributable to the role of MMP-9 in mediating BBB dysfunction and promoting HT following ischemic stroke. Although substantial clinical evidence has established the specific predictive role of MMP-9 for HT following ischemic stroke, most clinical trials to date rely exclusively on peripheral blood MMP-9 measurements. This methodological limitation primarily reflects the significant ethical constraints and technical difficulties involved in accurately quantifying MMP-9 concentrations in the human brain. The results from an animal study clarified that MMP-9 activity within brain was positively associated with edema and infarct volume after ischemic stroke (88). Another study performed in mice and endothelial cells cultured *in vitro* reported that tPA-induced

HT could be markedly attenuated when suppressing the expression of brain MMP-9 (89). These experimental findings from animal models and cell cultures provide indirect evidence supporting the predictive role of brain MMP-9 in HT. Additionally, some researchers suggested that an imbalance of MMP-9 and TIMP-1 contributed to the occurrence of HT (90). TIMP-1 is secreted together with MMP-9 and inhibits its proteolytic activity. The higher MMP-9/TIMP-1 ratio usually indicates the higher MMP-9 activity and HT risk. Notably, preclinical evidence from rat AIS models revealed the parallel MMP-9/TIMP-1 ratio changes in serum and brain tissue (91), indicating their comparable predictive capacity for HT.

As an important factor regulating MMP-9 expression, MMP-9 gene polymorphism represents a promising biomarker for predicting HT risk after ischemic stroke. In a case-control study involving 1,274 ischemic stroke patients and 1,258 healthy controls, researchers investigated four critical polymorphic sites of MMP-9 (rs17156, rs3787268, rs3918241 and rs3918242), finding that rs3918242 (-1562C/T) polymorphism was markedly associated with serum MMP-9 levels (92). In another clinical study including 222 ischemic stroke patients stratified by magnetic resonance imaging findings into HT and non-HT groups, researchers genotyped the -1562C/T polymorphism using PCR-restriction fragment length polymorphism analysis, revealing that 1562C/T polymorphism was markedly associated with HT risk, with the T allele potentially serving as a predictive biomarker for HT susceptibility (93). Contrary to previous findings, another study performed in the cerebrovascular disease patients found no correlation between -1562C/T polymorphism and spontaneous HT (94). Additionally, Fernández-Cadenas *et al* (95) genotyped 14 single nucleotide polymorphisms (SNPs) in MMP-9 gene, demonstrating no correlation between MMP-9 genetic variations and HT. These inconsistent results can be explained by several contributing factors. First, population-specific genetic differences play a crucial role in these observed variations. A comprehensive meta-analysis revealed the correlation between MMP-9 gene -1562C/T polymorphism and stroke risk among Asians, but not among Caucasians (96). Second, the pathophysiological mechanisms underlying HT encompass a complex cascade of molecular and cellular events, including BBB disruption, inflammatory responses, oxidative stress and MMPs activation, which collectively contribute to the development and progression of this condition. Therefore, genetic polymorphisms in MMP-9 may not markedly influence the occurrence of HT, as the development of HT is mediated through complex interactions among multiple genetic, molecular and environmental factors. Third, the interaction of different genetic variants in MMP-9 gene should not be ignored. Yi *et al* (97) demonstrated that the increased HT risk was attributable to the synergistic interaction between rs3918242 and rs3787268 genetic variants, rather than being caused by individual variant alone. Finally, variations in sample size and statistical methodologies may also contribute to the inconsistencies in research findings.

6. Reduction of hemorrhagic transformation by targeting MMP-9

Reducing HT following ischemic stroke may be beneficial to extend the therapeutic window of tPA, increase eligibility for

thrombolytic therapy and improve the prognosis of patients. As a key mediator in HT pathogenesis, MMP-9 may be a prospective pharmacological target for HT. Although the predictive role of MMP-9 in severe HT following ischemic stroke has been well established, whether therapies targeting MMP-9 have varying efficacy based on HT subtypes remains unknown. To date, no selective MMP-9 inhibitors have passed clinical trials. The present review evaluated clinically available neuroprotective agents that demonstrate indirect regulatory effects on MMP-9 activity or expression, aiming to elucidate their molecular pathways of MMP-9 regulation and provide evidence-based recommendations for optimizing therapeutic strategies to mitigate post-ischemic HT risk (Table II).

Statins. Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are used for the primary and secondary prevention of AIS for its role of plaque stabilization, anti-inflammatory and neuroprotective effects (98). In a previous study using an embolized rabbit model, tPA was administered 1 h following embolization. The results suggested that statins treatment markedly reduced MMP-9 levels, preserved cerebrovascular integrity and lowered the hemorrhagic incidence associated with intravenous thrombolysis (99). Another study revealed that lipophilic statins (such as simvastatin and atorvastatin) markedly reduced MMP-9/TIMP-1 ratio and suppressed MMP-9 activity in human endothelial cells (90). Furthermore, both *in vivo* and *in vitro* studies demonstrated that early administration of atorvastatin or simvastatin attenuated the risk of HT by inhibiting MMP-9 expression and preserving BBB integrity (100,101).

Simvastatin is an inhibitor of the Ras homolog family member A (RhoA)/Rho-associated coiled-coil containing kinases (ROCK) signaling pathway. In a rat model of AIS where t-PA was administered 3 h after occlusion, simvastatin pretreatment markedly alleviated tPA-induced HT (91). A randomized controlled trial has demonstrated that the protective effects of simvastatin are mediated through the suppression of MMP-9 activation and the reduction of the MMP-9/TIMP-1 ratio (102). The potentially mechanisms may be associated with the inhibition of RhoA/ROCK signaling pathway. Simvastatin has been found to inhibit geranylgeranylation of RhoA, prevent its translocation to plasma membrane and suppress the subsequent activation of ROCK (103). RhoA/ROCK pathway mediates myosin light chain phosphorylation and activation, which is necessary for vesicular transport and Cyclophilin A secretion. Cyclophilin A might promote the secretion and activation of MMP-9 via enhancing free radical generation and upregulating extracellular matrix metalloproteinase inducer (EMMPRIN) (104). Concerning atorvastatin, in the middle cerebral artery occlusion (MCAO) rat model, Liu *et al* (105) found that atorvastatin markedly suppressed tPA-induced MMP-9 mRNA upregulation. This finding is further supported by clinical evidence from a randomized trial in non-ST-elevation acute coronary syndrome patients, where atorvastatin markedly suppressed serum MMP-9 activity (106). A preclinical study conducted in embolic stroke rat model indicated that combined therapy of atorvastatin administered at 4 h and delayed tPA at 6 h after stroke decreased tPA-induced MMP-9 upregulation and HT, indicating that atorvastatin could help extend the therapeutic window of tPA for ischemic

stroke (107). In addition, Bellosta *et al* (108) investigated the effects of fluvastatin on MMP-9 activity in mouse and human macrophages and found that fluvastatin markedly suppressed MMP-9 activity in a dose-dependent manner.

Despite these promising preclinical findings, clinical evidence supporting the efficacy of statins in reducing HT risk following ischemic stroke remains limited. Although statins have demonstrated an established safety in clinical practice, its potential adverse effects should not be ignored, particularly myopathy, new-onset diabetes mellitus and cognitive dysfunction (109). These adverse events may occur under specific clinical circumstances, but their absolute risks remain substantially lower than the demonstrated therapeutic benefits.

Free radical scavengers. Edaravone, a potent free radical scavenger, has been widely used in AIS patients due to its anti-inflammatory and neuroprotective properties. Clinical data have highlighted that edaravone markedly reduces neurological deficits and improve the life quality in ischemic stroke patients, with an excellent safety profile (110). A minimal proportion of edaravone-treated patients experienced mild adverse events, such as headache and dizziness, both of which were self-limiting and did not require treatment withdrawal (111). Growing evidence suggests that edaravone may play a crucial role in maintaining BBB integrity (112,113). A number of clinical studies have demonstrated its potential in reducing HT risk. A randomized controlled trial involving 65 AIS patients with diabetes showed markedly lower HT incidence in the edaravone-treated group Compared with the controls (114). Further evidence revealed that edaravone administration not only decreased the incidence of HT, but also markedly attenuated its severity in ischemic stroke patients (115). These clinical findings are supported by robust preclinical evidence. Okamura *et al* (116) reported that edaravone markedly reduced the hematoma volumes in a rat ischemic stroke model. Further animal study revealed that edaravone downregulated the expression and activity of MMP-9, thereby decreasing tPA-induced HT risk (117). Similar protective effects were observed in a cerebral hypoperfusion mouse model, where edaravone preserved BBB integrity through MMP-9 inhibition (118). The underlying mechanism appears to involve the suppression of the NF- κ B pathway, leading to reduced MMP-9 expression and enhanced BBB stabilization (119,120). NF- κ B is a crucial transcriptional regulator that becomes rapidly activated during the acute phase of ischemic stroke. This activation occurs primarily through the canonical pathway, where inflammatory stimuli, such as TNF- α , IL-1 β and ROS, trigger the phosphorylation and subsequent degradation of inhibitor κ B (I κ B) by the I κ B kinase (IKK) complex (121). The liberated NF- κ B dimers (predominantly p50/p65) then translocate to the nucleus, where they bind to specific κ B sites in promoter regions to upregulate the expression of numerous pro-inflammatory mediators, including cytokines (TNF- α and IL-6), chemokines (MCP-1) and MMP-9 (122). Edaravone exerts its inhibitory effect on NF- κ B activation through multiple mechanisms (119,123): i) Edaravone scavenges ROS that serve as upstream activators of the NF- κ B pathway; ii) Edaravone suppresses the phosphorylation and subsequent proteasomal degradation of I κ B α , thereby preventing the nuclear translocation of liberated

Table II. List of agents targeting MMP-9 and reducing HT after stroke.

Authors, year	Class	Agents	Model	Subject	Dosage/path	Main findings	Target pathway	(Refs.)
Yin <i>et al</i> , 2019	HMG-CoA reductase Inhibitors	Simvastatin	MCAO	Rat	60 mg/kg/day (p.o.)	Reduction of MMP-9 levels and MMP-9/TIMP-1 ratio in brain and plasma; Mitigation of tPA-induced HT	RhoA/ ROCK	(91)
Kurzepa <i>et al</i> , 2006			AIS	Human	40 mg/day (p.o.)	Reduction of serum MMP-9/TIMP-1 ratio	/	(102)
Liu <i>et al</i> , 2006		Atorvastatin	MCAO	Rat	20 mg/kg, twice (s.c.)	Reduction of brain MMP-9 mRNA levels		(105)
Gómez- Hernández <i>et al</i> , 2008			NSTEACS	Human	80 mg/day (p.o.)	Reduction of plasma MMP-9 activity and incidence of cardiovascular events	/	(106)
Zhang <i>et al</i> , 2009			MCAO	Rat	20 mg/kg, twice (s.c.)	Inhibition of brain MMP-9 upregulation and lowering of HT incidence induced by tPA; Extension of the therapeutic window of tPA for stroke to 6 h	/	(107)
Bellosta <i>et al</i> , 1998		Fluvastatin	MPMs/ HMs	Cell	5-100 μ mol/l	Reduction of MMP-9 expression and activity in macrophages from mice and humans	/	(108)
Zheng <i>et al</i> , 2016	Free radical scavenger	Edaravone	AIS	Human	60 mg/day (i.v.)	Reduction of HT incidence and mitigation of neurological deficits	/	(114)
Toyoda <i>et al</i> , 2004			AIS	Human	60 mg/day (i.v.)	Alleviation of HT and decrease of mortality during the acute phase of stroke	/	(115)
Okamura <i>et al</i> , 2014			MCAO	Rat	3 mg/kg, twice (i.v.)	Reduction of hemorrhage volumes after stroke	/	(116)
Yagi <i>et al</i> , 2009			MCAO	Rat/Cell (HBECs)	Rat (3 mg/kg/ day, i.v.); HBECs (20 μ g/ml)	Reduction of endothelial-derived MMP-9 expression and activity; Attenuation of tPA- induced HT	NF- κ B	(117)
Miyamoto <i>et al</i> , 2014			BCCAO	Mouse	3 mg/kg, twice (i.v.)	Reduction of brain MMP-9 expression; Preservation of BBB integrity	/	(118)

Table II. Continued.

Authors, year	Class	Agents	Model	Subject	Dosage/path	Main findings	Target pathway (Refs.)
Harada <i>et al</i> , 2012			OSI	Cell (bEnd.3)	100 μ M	Attenuation of MMP-9 upregulation induced by the combination of ROS and tPA	NF- κ B (119)
Chen <i>et al</i> , 2013	Tetracycline antibiotic	Minocycline	OGD	Cell (PC12)	200 nM	Reduction of MMP-9 expression and activity	Akt (131)
Murata <i>et al</i> , 2008			MCAO	Rat	3 mg/kg/day (i.v.)	Reduction of plasma MMP-9 levels; Lowering of tPA- induced HT risk; Extension of the therapeutic window of tPA to 6 h	/ (133)
Liu <i>et al</i> , 2021			ICH	Mouse	90 mg/kg/day (i.p.)	Reduction of brain EMMPRIN and MMP-9 expression; Alleviation of BBB disruption	/ (134)
Song <i>et al</i> , 2016			BCP	Rat	160 mg/kg/day (i.p.)	Inhibition of the NF- κ B signaling pathway in spinal astrocytes	NF- κ B (136)
Zhang <i>et al</i> , 2013	Thiazolidin e-diones	Rosiglitazone	MCAO	Rat	2 mg/kg/day (i.p.)	Reduction of HT incidence; Improvement of neurobehavioral deficits	/ (144)
Li <i>et al</i> , 2019			MCAO	Mouse	6 mg/kg, twice (i.p.)	Preservation of BBB integrity; Mitigation of tPA-induced HT	/ (145)
Marx <i>et al</i> , 2003			CAD	Human	8 mg/day (p.o.)	Reduction of plasma MMP-9 levels	/ (146)
Wang <i>et al</i> , 2009			MCAO	Rat	2 mg/kg/day (i.g.)	Prevention of collagen type IV reduction through inhibiting MMP-9 activation	/ (148)
Lee <i>et al</i> , 2009			VSMCs	Cell	15 and 25 μ M	Inhibition of MMP-9 activation	GSK-3 β (151)
Nonaka <i>et al</i> , 2009	Phosphodie sterase III inhibitor	Cilostazol	MCAO	Mouse	10 mg/kg, (once i.p.)	Protection against ischemic brain injury and HT	/ (155)
Kasahara <i>et al</i> , 2012			MCAO	Mouse	0.3% in the diet	Reduction of brain MMP-9 activity and cerebral hemorrhage risk induced by tPA	/ (157)
Chuang <i>et al</i> , 2011			THP-1	cell	100 μ M	Reduction of MMP-9 promoter activity	NF- κ B (158)

MMP-9, matrix metalloproteinase-9; HT, hemorrhagic transformation; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MCAO, middle cerebral artery occlusion; TIMP-1, tissue inhibitor of metalloproteinase 1; RhoA, Ras homolog family member A; ROCK, Rho-associated coiled-coil containing kinases; AIS, acute ischemic stroke; NSTEMI, non-ST elevation acute coronary syndrome; MPMs, mouse peritoneal macrophages; HMs, human monocyte-derived macrophages; HBECs, human microvascular endothelial cells; NF- κ B, nuclear factor- κ B; BCCAO, bilateral common carotid artery occlusion; BBB, blood-brain barrier; OSI, oxidative stress *in vitro* model; bEnd, brain-derived endothelial cells; ROS, reactive oxygen species; OGD, oxygen and glucose deprivation; Akt, protein kinase B; ICH, intracerebral hemorrhage; EMMPRIN, extracellular matrix metalloproteinase inducer; BCP, bone cancer pain; CAD, coronary artery disease; VSMCs, vascular smooth muscle cells; GSK-3 β , glycogen synthase kinase-3 β ; THP-1, human monocytic leukemia cell line.

NF- κ B dimers (particularly p50/p65), ultimately inhibiting their DNA-binding activity and transcriptional regulation of downstream target genes. Contrary to previous findings, a large case-control study (n=613) found increased HT incidence in ischemic stroke patients with edaravone treatment (124). These discrepancies highlight the need for further large-scale studies to clarify the precise pharmacological effects of edaravone on HT following ischemic stroke.

Minocycline. Minocycline, a semi-synthetic second-generation tetracycline antibiotic, demonstrates significant neuroprotective properties and exhibits therapeutic potential for multiple neurological disorders, such as ischemic stroke, intracerebral hemorrhage (ICH), epilepsy, multiple sclerosis, Parkinson's disease, Alzheimer's disease and spinal cord injury (125,126). Multiple randomized controlled trials have consistently established the favorable safety profile of minocycline in acute stroke management, while its therapeutic efficacy requires further validation (127-129). In a clinical trial evaluating the efficacy and safety of minocycline as an adjunct to tPA therapy in ischemic stroke patients within 6 h of onset, no severe hemorrhages were observed in minocycline-treated cohort (130). Further clinical investigation demonstrated that combining minocycline with tPA appeared to mitigate thrombolytic therapy-associated complications, primarily through the inhibition of MMP-9 activity. Meanwhile, comprehensive preclinical studies have established that the neuroprotective properties of minocycline are largely attributable to its inhibitory effects on MMP-9 activity or expression. A previous study has revealed that minocycline, at concentrations ranging from 20 nM-20 μ M, effectively alleviated oxygen-glucose deprivation-induced cell cytotoxicity by suppressing both MMP-9 expression and enzymatic activity (131). Minocycline also exerted a protective role in reducing ischemic lesion volume and decreasing HT risk by inhibiting MMP-9 (132). Combined administration of minocycline with tPA could lower the tPA-induced HT risk and extend the narrow treatment time windows of tPA in experimental stroke models, which was mediated by plasma MMP-9 downregulation (133). Our previous research demonstrated that the administration of minocycline could inhibit the expression of EMMPRIN, a key inflammatory mediator promoting MMPs production, thereby downregulating MMP-9 expression and alleviating BBB injury (134,135). Minocycline likely regulates EMMPRIN expression through attenuating IKK/I κ B α phosphorylation, impeding NF- κ B nuclear translocation and subsequently suppressing EMMPRIN transcriptional activation (136,137). While this proposed mechanism is plausible, rigorous experimental validation through approaches such as chromatin immunoprecipitation assays to demonstrate the reduced binding of NF- κ B to the EMMPRIN promoter following minocycline treatment is currently lacking in the literature. In addition, minocycline also protected the integrity of BBB and reduced the risk of HT by targeting neuroinflammation via suppressing the migration and infiltration of neutrophils into the brain and inhibiting the activation of microglia (57,138).

Thiazolidinediones. Diabetes mellitus is characterized by hyperglycemia and microvascular damage of multiple

organs. Clinical evidence indicates that hyperglycemia is closely associated with an increased risk of symptomatic ICH, particularly in patients with blood glucose levels >200 mg/dl at stroke onset, who might face a substantially greater risk (139). The pathophysiological basis for this clinical observation may involve hyperglycemia-induced BBB disruption. It has been demonstrated that hyperglycemia disrupts BBB homeostasis by impairing the cerebral endothelial cell function through dysregulating redox signaling, inflammatory mediators and vasoactive factors (140). Furthermore, both *in vitro* and *in vivo* studies have revealed that hyperglycemia impaired BBB integrity via suppressing TJPs expression in the neurovascular unit (141,142). Additionally, clinical data from a study enrolling 287 patients demonstrated higher HT incidence among those with stress hyperglycemia, suggesting its potential role in post-stroke HT development (143).

Considering the contribution of hyperglycemia to BBB damage and HT, antidiabetic drugs might represent promising therapeutic candidates to prevent the HT following ischemic stroke. Thiazolidinediones, as peroxisome proliferator-activated receptor (PPAR) agonists, have been extensively studied, among which rosiglitazone shows promise in lowering HT risk after ischemic stroke (144). In a rat MCAO model, rosiglitazone treatment effectively protected against BBB disruption and mitigated tPA-induced HT after stroke (145). While the precise mechanisms of the BBB protective effects of rosiglitazone remain incompletely understood, accumulating evidence suggests that MMP-9 downregulation may play a pivotal role. Clinical data from type 2 diabetic patients demonstrated a markedly reduction of MMP-9 serum levels in those treated with rosiglitazone (146). In rabbit ICH models treated with minimally invasive evacuation, rosiglitazone was shown to decrease MMP-9 expression and protect BBB integrity (147). Another animal study using rat embolic stroke models indicated that rosiglitazone treatment prevented the reduction of collagen type IV and stabilized BBB function by inhibiting MMP-9 activation (148). Furthermore, experimental studies have elucidated the molecular mechanisms by which rosiglitazone modulates MMP-9 expression. Rosiglitazone activates PPAR γ -mediated transcription, which enhances adiponectin production and initiates downstream signaling pathways, leading to glycogen synthase kinase-3 β (GSK-3 β) activation (149). The activated GSK-3 β exerts its regulatory effects by stabilizing I κ B α , thereby maintaining NF- κ B in an inactive cytoplasmic state (150). This GSK-3 β -mediated suppression of NF- κ B nuclear translocation and DNA binding capacity results in significant downregulation of MMP-9 expression through the inhibition of promoter activity and subsequent attenuation of gene transcription (151). Notably, clinical evidence indicates that rosiglitazone therapy is associated with an increased risk of heart failure in patients with type 2 diabetes mellitus (152). Therefore, comprehensive cardiovascular evaluation, including assessment of left ventricular function and fluid status, should be routinely performed prior to treatment initiation and during follow-up monitoring.

Cilostazol. Cilostazol, a selective phosphodiesterase III inhibitor, is primarily used as an antiplatelet agent for patients with intermittent claudication or ischemic stroke. A multicenter

randomized controlled trial involving patients with lacunar stroke demonstrated that cilostazol markedly reduced the incidence of recurrent ischemic stroke while maintaining a favorable safety profile, with no increase in severe or life-threatening bleeding events (153). Additionally, clinical data demonstrates that cilostazol is associated with a significant lower incidence of HT, compared with other antiplatelet drugs such as aspirin (154). Meanwhile, extensive experimental evidence from animal studies has consistently corroborated this finding. Intraperitoneally administration of cilostazol (10 mg/kg) could effectively protect against HT in a transient MCAO mouse model (155). Cilostazol might protect against tPA-induced brain edema and HT by downregulating microglia-derived MMP-9 in mice with focal cerebral ischemia (156). Consistent with the previous reports, administration of cilostazol for 7 days before ischemia markedly decreased the risk of HT following injection of tPA, which was associated with the inhibition of MMP-9 activity (157). Mechanistically, it has been revealed that cilostazol inhibited the translocation of NF- κ B to nuclear and decreased the activity of MMP-9 promoter, indicating that cilostazol suppressed MMP-9 expression at transcriptional level (158). Although the detailed mechanisms underlying the inhibitory effects of cilostazol on NF- κ B pathway have not been fully elucidated, AMP-activated protein kinase (AMPK) appears to play a critical role. As reported in a previous study, cilostazol promoted AMPK phosphorylation, which subsequently blocked NF- κ B activation (159). Conversely, another study demonstrated that cilostazol attenuated warfarin-induced HT through upregulation of TJPs (claudin-5 and ZO-1) and VE-cadherin expression, while showing no significant effect on MMP-9 levels (160). These divergent results indicate that the protective role of cilostazol in HT involves multiple pathways, warranting systematic exploration of its underlying mechanisms.

7. Conclusion

MMP-9 is involved in a number of pathophysiological processes associated with BBB breakdown and HT after ischemic stroke, making it a promising predictive biomarker for HT risk. Accumulating evidence from preclinical and clinical studies indicates that pharmacological agents, such as statins, edaravone, minocycline, rosiglitazone and cilostazol, can attenuate MMP-9 expression or activity, thereby preserving BBB integrity and reducing HT incidence following ischemic stroke. The combined administration of these therapeutic agents may effectively reduce tPA-induced HT while potentially extending the therapeutic window for thrombolytic therapy in ischemic stroke.

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Authors' contributions

PG conceived and drafted the manuscript. HL and XZ performed the literature search. YL and SX revised the manuscript and created the figures. MX and VY reviewed and revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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