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Acta Med. Okayama, 2011
Vol. 65, No. 4, pp. 219–223
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Acta Medica Okayama
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Review

Therapeutic Approaches to Vascular Protection in Ischemic Stroke

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Reperfusion with recombinant tissue plasminogen activator (tPA) sometimes causes catastrophic hemorrhagic transformation (HT) in the ischemic brain. Consequently, the application of tPA has been strictly limited. Recent studies have indicated that matrix metalloproteinases (MMPs), especially MMP-9, play a critical role in blood brain barrier (BBB) disruption in the ischemic brain, leading to brain edema and HT. In the ischemic brain, free radicals and exogenous tPA itself can trigger MMP-9 activation through several signaling pathways containing LDL receptor-related protein (LRP) and proteinase-activated receptor 1 (PAR1). Therapeutic targeting of free radicals and MMP-9/t-PA related signaling pathways might be promising approaches to minimizing catastrophic HT in acute stroke patients. We provide an overview of the available scientific reports to improve our understanding of the mechanisms leading to HT, and highlight recent progress in the development of new therapeutic strategies for preventing HT in the post-stroke brain.

Key words: cerebral ischemia, hemorrhagic transformation, tissue plasminogen activator, free radical, matrix metalloproteinase-9

Strokes are a major cause of death and result in a severe reduction in the quality of life. If cerebral blood flow is restored by tissue plasminogen activator (tPA), ischemic brain damage can be ameliorated [1]. However, since delayed reperfusion with tPA can cause hemorrhagic transformation (HT) [2], the application of tPA is strictly limited in a clinical setting. An understanding of the mechanism underlying HT and a new therapeutic strategy prohibiting HT are both needed. In this paper, we focus on therapeutic approaches to vascular protection that can help prevent HT in the ischemic brain when treated with tPA.

Received March 29, 2011; accepted April 20, 2011.
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Microvascular Integrity and MMPs

Cerebral microvascular integrity mainly depends on three components: the vascular wall formed by endothelial cells, the blood brain barrier (BBB) provided by endothelial tight junctions, and the basal membrane lining the endothelial cells [3] (Fig. 1). Recent studies indicated that matrix metalloproteinases (MMPs), especially MMP-9, can play critical roles in BBB disruption in the ischemic brain [4]. MMP-9 comprises a family of zinc endopeptidases, and cerebral vascular endothelial cells and infiltrating leukocytes are regarded as the main cellular sources of MMP-9 in the ischemic brain [5–7]. MMP-9 knock-out mice showed a significant reduction in BBB

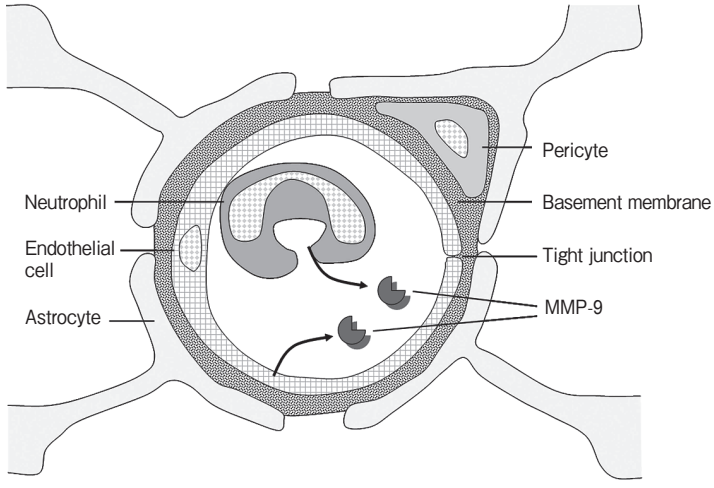

Fig. 1 Schematic diagram of vascular unit that comprises endothelial cells, astrocytes, and pericytes. MMP-9 is mainly derived from brain endothelial cells and infiltrating neutrophils in the acute phase of a stroke. MMP-9 can degrade the basement membrane, which links the endothelial cells, and maintains the integrity of the vascular unit.

disruption and brain edema, and this effect was associated with reduced degradation of the MMP-9 substrate of a tight junction protein, ZO-1 [8]. Recent scientific papers have suggested that MMP-9 can disrupt the BBB by degrading not only tight junction proteins but also basal membrane proteins (*e.g.*, fibronectin, laminin, collagen, and others), thereby leading to BBB disruption, brain edema, and HT in the post-ischemic brain in animal models [9, 10].

MMP-9 Expression in Stroke Patients

By using gelatin zymography, Clark *et al.* reported that MMP-9 activity was markedly elevated in infarcted human brain tissue 2 days after an ischemic stroke [11]. Montaner *et al.* reported that a high level of plasmatic MMP-9 expression in stroke patients could predict HT after thrombolysis [12]. A substrate of MMP-9, fibronectin, has also been reported to be a useful biomarker for predicting HT [13]. Faster analytic methods for the above 2 biomarkers are now required to develop a new strategy for predicting HT in routine clinical practice.

Free Radicals Activating MMP-9

Free radicals, the fundamental mediators of reperfusion injury, are generated soon after vessel occlusion, with explosive propagation after reperfusion in the ischemic brain [14]. Superoxide dismutase (SOD2) is the principal defense against the toxicity of free radicals, and SOD2 knock-out mice exhibit a significant increase in MMP-9 and a higher rate of brain hemorrhaging after middle cerebral artery occlusion (MCAO) [15], indicating that the excess radicals can activate MMP-9, inducing HT in the post-ischemic brain.

Exogenous tPA Activating MMP-9

Systemic administration of exogenous tPA amplified MMP-9 levels in the ischemic rat brain. Perfusion of tPA resulted in the disruption of BBB and the degradation of a basal membrane protein, laminin, in rat blood vessels [16]. In addition, MMP-9 expression, infarct size, and brain edema in tPA knock-out mouse were significantly lower than in wild-type mice [17], indicating that exogenous tPA can strongly increase the activity of MMP-9 in the brain. MMP-9 can be activated by tPA via several molecular signaling pathways including the tPA-LRP and tPA-PAR1 pathways (Fig. 2). The LDL receptor-related protein (LRP) is a member of the LDL receptor gene family that binds several ligands, such as tPA [18]. LRP is expressed in neurons and perivascular astrocytes [19], and tPA can cross the BBB by LRP-mediated transcytosis [20]. tPA treatment stimulates MMP-9 expression in cultured human

brain endothelial cells. This effect is dramatically reduced in endothelial cells treated with RNAi against LRP, but was absent in LRP-deficient MEF cells [21]. Moreover, intraventricular injection of tPA into the mouse brain increased BBB permeability, although this effect was blocked by LRP antagonists. These findings indicate that LRP signaling plays an important role in tPA-induced MMP-9 activation. Cheng *et al.* reported that activated protein C (APC) could inhibit tPA-induced MMP-9 activation in the endothelium of an ischemic brain. This inhibition was absent in protease-activated receptor 1 (PAR-1) knock-out mouse, indicating that PAR1 is required for APC-mediated down-regulation of tPA-induced MMP-9 [22].

Therapeutic Strategy for Vascular Protection Inhibiting HT

The mechanism of the vascular unit disruption after ischemia and reperfusion with tPA has been extensively studied. These results indicate that free radicals and MMP-9 are regarded as key regulators leading to the disruption of a vascular unit (Fig. 2). Therefore, many research groups have tested various kinds of drugs or reagents against free radicals and MMP-9, and some of them have been reported to be able to protect the vascular unit and inhibit HT (Table 1).

We used a spontaneously hypertensive rat model of MCAO and tested the efficacy of a free radical scav-

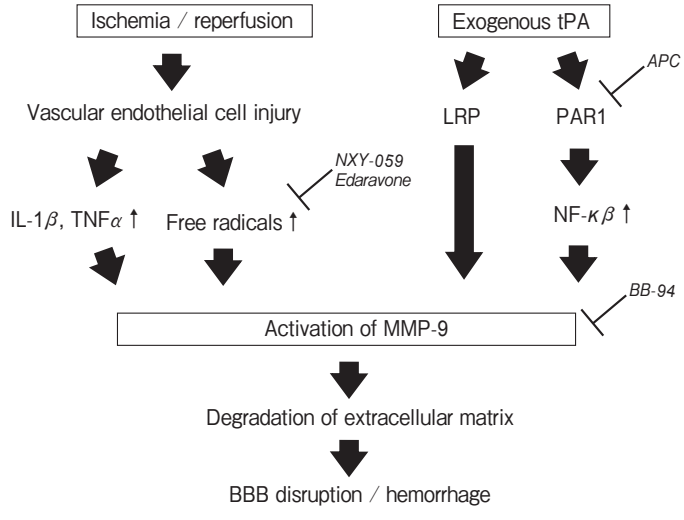

Fig. 2 Possible mechanism of vascular unit disruption after ischemia and reperfusion with tPA. MMP-9 can disrupt the BBB by degrading the basal membrane/extracellular matrix proteins, thereby leading to BBB leakage and hemorrhaging. In the acute phase of a stroke, MMP-9 can be activated by pro-inflammatory factors (*e.g.*, IL-1β, and TNF-α) and free radicals. In addition, the tPA that is administered strongly activates MMP-9 through multiple pathways. MMP-9 activation is inhibited by several reagents: NXY-059, edaravone, APC and BB-94 (Table 1).

Table 1 Scientific papers reporting reagents that can attenuate hemorrhagic cerebral infarction in animal models

Drug name	Supposed mechanisms	Animal model	References
Imatinib (PDGFR-α antagonist)	Suppressing PDGFR-α activation	Mouse hemorrhagic cerebral infarction model	Su *et al.* [28]
Activated protein C	Suppressing the tPA-PAR1-MMP9 pathway	Mouse hemorrhagic cerebral infarction model	Cheng *et al.* [22]
Melatonin	Suppressing MMP9 activity	Rat hemorrhagic cerebral infarction model	Hung *et al.* [29]
BB-94 (MMP9 inhibitor)	Suppressing MMP9 activity	Rat hemorrhagic cerebral infarction model	Sumii *et al.* [30]
Minocycline	Suppressing MMP9 activity	Rat hemorrhagic cerebral infarction model	Murata *et al.* [31]
NXY-059	Scavenging free radicals	Rabbit hemorrhagic cerebral infarction model	Lapchck *et al.* [32]
Edaravone	Scavenging free radicals	Rat hemorrhagic cerebral infarction model	Yamashita *et al.* [23]

enger, edaravone, in preventing HT. Administration of tPA alone significantly worsened the survival rate compared with those rats treated with vehicle. On the other hand, treatment with edaravone plus tPA significantly increased the survival rate, improved motor function, and dramatically decreased HT. We also demonstrated that treatment with edaravone suppressed MMP-9 expression at and around cerebral microvessels, inhibited the degradation of basement membrane protein, and prevented the microvessels from dissociating. These results suggested that edaravone can protect cerebral microvascular integrity, because it safeguards the basement membrane from excess free radicals and MMP-9, leading to a subsequent decrease in HT and improvement in the survival rate and neurological outcome [23].

In a clinical trial, edaravone attenuated the resulting disability in humans 90 days after acute ischemic stroke without serious adverse events [24], and it has been used clinically in Japan as a neuroprotective agent for acute stroke patients since 2001. In a large clinical trial, another free radical scavenger, NXY-059, initially seemed to reduce disability after stroke [25], but this effect could not be reproduced [26]. Nonetheless, one other characteristic of NXY-059 was its potential to inhibit symptomatic HT after tPA treatment [25]. NXY-059 is water soluble (octanol/water partition coefficients; cLog $p = -2.09$). In contrast, edaravone has a biphasic, water-soluble, and lipid-soluble nature (cLog $p = 1.33$), and it has been reported that it is able to easily pass through the BBB to enter the brain parenchyma and cerebral fluid [27]. As the site most vulnerable to free radical damage is on the outer side of the vascular endothelium (*e.g.*, the basal membrane), this unique chemical property of edaravone might be an advantage for its delivery to the basement membrane. Therefore, combination therapy with edaravone and tPA is a promising therapeutic strategy for acute stroke patients, not only in reducing infarct size but also in minimizing catastrophic HT.

In this article, we briefly highlighted recent progress in the development of new therapeutic strategies for vascular protection in the post-stroke brain. To realize more effective therapies for patients suffering from stroke, it is important to combine these strategies in the acute phase following a stroke.

References

1. The national institute of neurological disorders and stroke rt-pa stroke study group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med (1995) 333: 1581–1587.
2. The ninds t-pa stroke study group: Intracerebral hemorrhage after intravenous t-pa therapy for ischemic stroke. Stroke (1997) 28: 2109–2118.
3. del Zoppo GJ: Stroke and neurovascular protection. N Engl J Med (2006) 354: 553–555.
4. Adibhatla RM and Hatcher JF: Tissue plasminogen activator (tpa) and matrix metalloproteinases in the pathogenesis of stroke: Therapeutic strategies. CNS Neurol Disord Drug Targets (2008) 7: 243–253.
5. Jin R, Yang G and Li G: Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. J Leukoc Biol (2010) 87: 779–789.
6. Justicia C, Panes J, Sole S, Cervera A, Deulofeu R, Chamorro A and Planas AM: Neutrophil infiltration increases matrix metalloproteinase-9 in the ischemic brain after occlusion/reperfusion of the middle cerebral artery in rats. J Cereb Blood Flow Metab (2003) 23: 1430–1440.
7. McColl BW, Rothwell NJ and Allan SM: Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice. J Neurosci (2008) 28: 9451–9462.
8. Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, Fini ME and Lo EH: Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J Neurosci (2001) 21: 7724–7732.
9. Lee CZ, Xue Z, Zhu Y, Yang GY and Young WL: Matrix metalloproteinase-9 inhibition attenuates vascular endothelial growth factor-induced intracerebral hemorrhage. Stroke (2007) 38: 2563–2568.
10. Yang Y, Estrada EY, Thompson JF, Liu W and Rosenberg GA: 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.
11. Clark AW, Krekoski CA, Bou SS, Chapman KR and Edwards DR: Increased gelatinase a (mmp-2) and gelatinase b (mmp-9) activities in human brain after focal ischemia. Neurosci Lett (1997) 238: 53–56.
12. Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribo M, Quintana M and Alvarez-Sabin J: Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation (2003) 107: 598–603.
13. Castellanos M, Sobrino T, Millan M, Garcia M, Arenillas J, Nombela F, Brea D, Perez de la Ossa N, Serena J, Vivancos J, Castillo J and Davalos A: Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: A multicenter confirmatory study. Stroke (2007) 38: 1855–1859.
14. Chan PH: Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab (2001) 21: 2–14.
15. Maier CM, Hsieh L, Crandall T, Narasimhan P and Chan PH: Evaluating therapeutic targets for reperfusion-related brain hemorrhage. Ann Neurol (2006) 59: 929–938.
16. Goto H, Fujisawa H, Oka F, Nomura S, Kajiwara K, Kato S,

Fujii M, Maekawa T and Suzuki M: Neurotoxic effects of exogenous recombinant tissue-type plasminogen activator on the normal rat brain. J Neurotrauma (2007) 24: 745–752.
17. Tsuji K, Aoki T, Tejima E, Arai K, Lee SR, Atochin DN, Huang PL, Wang X, Montaner J and Lo EH: Tissue plasminogen activator promotes matrix metalloproteinase-9 upregulation after focal cerebral ischemia. Stroke (2005) 36: 1954–1959.
18. Herz J and Strickland DK: Lrp: A multifunctional scavenger and signaling receptor. J Clin Invest (2001) 108: 779–784.
19. Polavarapu R, Gongora MC, Yi H, Ranganthan S, Lawrence DA, Strickland D and Yepes M: Tissue-type plasminogen activator-mediated shedding of astrocytic low-density lipoprotein receptor-related protein increases the permeability of the neurovascular unit. Blood (2007) 109: 3270–3278.
20. Benchenane K, Berezowski V, Ali C, Fernandez-Monreal M, Lopez-Atalaya JP, Brillault J, Chuquet J, Nouvelot A, MacKenzie ET, Bu G, Cecchelli R, Touzani O and Vivien D: Tissue-type plasminogen activator crosses the intact blood-brain barrier by low-density lipoprotein receptor-related protein-mediated transcytosis. Circulation (2005) 111: 2241–2249.
21. Wang X, Lee SR, Arai K, Tsuji K, Rebeck GW and Lo EH: Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med (2003) 9: 1313–1317.
22. Cheng T, Petraglia AL, Li Z, Thiyagarajan M, Zhong Z, Wu Z, Liu D, Maggirwar SB, Deane R, Fernandez JA, LaRue B, Griffin JH, Chopp M and Zlokovic BV: Activated protein c inhibits tissue plasminogen activator-induced brain hemorrhage. Nat Med (2006) 12: 1278–1285.
23. Yamashita T, Kamiya T, Deguchi K, Inaba T, Zhang H, Shang J, Miyazaki K, Ohtsuka A, Katayama Y and Abe K: Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. J Cereb Blood Flow Metab (2009) 29: 715–725.
24. Edaravone Acute Infarction Study Group: Effect of a novel free radical scavenger, edaravone (mci-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. Cerebrovasc Dis (2003) 15: 222–229.
25. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, Grotta J, Lyden P, Shuaib A, Hardemark HG and Wasiewski WW; Stroke-Acute Ischemic NXY Treatment (SAINT) Trial Investigators: Nxy-059 for acute ischemic stroke. N Engl J Med (2006) 354: 588–600.
26. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, Diener HC, Ashwood T, Wasiewski WW and Emeribe U; SAINT Ⅱ Trial Investigators: Nxy-059 for the treatment of acute ischemic stroke. N Engl J Med (2007) 357: 562–571.
27. Yamamoto Y KT, Watanabe K, Watanabe K: Antioxidant activity of 3-methyl-l-phenyl-2-pyrazolin-5-one. Redox Rep (1996) 2: 333–338.
28. Su EJ, Fredriksson L, Geyer M, Folestad E, Cale J, Andrae J, Gao Y, Pietras K, Mann K, Yepes M, Strickland DK, Betsholtz C, Eriksson U and Lawrence DA: Activation of pdgf-cc by tissue plasminogen activator impairs blood-brain barrier integrity during ischemic stroke. Nat Med (2008) 14: 731–737.
29. Hung YC, Chen TY, Lee EJ, Chen WL, Huang SY, Lee WT, Lee MY, Chen HY and Wu TS: Melatonin decreases matrix metalloproteinase-9 activation and expression and attenuates reperfusion-induced hemorrhage following transient focal cerebral ischemia in rats. J Pineal Res (2008) 45: 459–467.
30. Sumii T and Lo EH: Involvement of matrix metalloproteinase in thrombolysis-associated hemorrhagic transformation after embolic focal ischemia in rats. Stroke (2002) 33: 831–836.
31. Murata Y, Rosell A, Scannevin RH, Rhodes KJ, Wang X and Lo EH: Extension of the thrombolytic time window with minocycline in experimental stroke. Stroke (2008) 39: 3372–3377.
32. Lapchak PA, Araujo DM, Song D, Wei J, Purdy R and Zivin JA: Effects of the spin trap agent disodium-［(tert-butylimino) methyl］benzene-1, 3-disulfonate n-oxide (generic nxy-059) on intracerebral hemorrhage in a rabbit large clot embolic stroke model: Combination studies with tissue plasminogen activator. Stroke (2002) 33: 1665–1670.