Published in final edited form as:
Curr Pharm Des. 2012 ; 18(25): 3677–3684.

Vascular Protection to Increase the Safety of Tissue Plasminogen Activator for Stroke

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Abstract

Thrombolytic therapy with tissue plasminogen activator (tPA) remains the most effective treatment for acute ischemic stroke, but can cause vascular damage leading to edema formation and hemorrhagic transformation (HT). In this review, we discuss how tPA contributes to the pathogenesis of vascular damage and highlight evidence to support combination therapy of tPA with pharmacological agents that are vascular protective. There is an unmet need to develop therapeutic interventions which target the underlying mechanisms of vascular damage after acute ischemic stroke in order to prevent HT and improve the safety and impact of tPA.

Keywords

Ischemic stroke; vascular protection; tissue plasminogen activator (tPA); matrix metalloproteinases (MMPs); hemorrhagic transformation (HT); thrombolysis; blood–brain barrier

Ischemic stroke remains a leading cause of adult disability and death in the United States [1]. Despite massive effort and tremendous costs, the results of clinical trials for the treatment of stroke have been disappointing, with genetically engineered tissue plasminogen activator (tPA), still the only biologic agent approved by the US Food and Drug Administration (FDA) for this indication. Unfortunately, tPA is associated with a significant risk of hemorrhagic transformation (HT) and is used in less than 2–3% of all stroke victims [2, 3]. There is also evidence that tPA can increase the ultimate tissue damage caused by ischemic stroke, through neuronal and vascular toxicity. In this review, we will discuss the clinical evidence of vascular toxicity due to tPA, the proposed mechanisms of tPA-induced vascular damage, and identify potential tactics for vascular protection. We will also compare experimental treatments for their potential to modify the risk of tPA treatment.

A major research priority is to develop therapeutic interventions which can reduce tPA-induced neurovascular unit disruption in the ischemic brain through the understanding of underlying mechanisms. The failure, so far, of clinical trials of neuron protectant agents to achieve detectable tissue salvage may be explained by the vulnerability and lack of protection of essential components of the vasculature. Reperfusion with tPA has been shown to increase the expression and activity of matrix metalloproteinases (MMPs), especially

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MMP-9, and these proteases have been implicated as key regulators of the disruption of the vascular unit [4,5]. Undoubtedly, there are other mediators of the vascular toxicity of tPA and agents aimed at these targets may be able to reduce the toxicity of tPA without affecting its ability to thrombolyse.

Clinical Evidence of Vascular Damage after tPA

The main evidence guiding the clinical use of tPA in acute ischemic stroke patients comes from the NINDS tPA Stroke Trial [6] and the European Cooperative Acute Stroke Study (ECASS) III [7]. Both of these trials report a similar, and significant excess incidence of HT, when intravenous tPA is administered within 4.5 hours after the onset of stroke symptoms. Despite a net clinical benefit of tPA in treated patients, it is tempting to project a vastly improved impact if the vascular damage leading to HT and edema could be ameliorated. Identifying patient populations at increased risk of severe bleeding after tPA and testing vascular protective agents in them, may facilitate the development of new combination therapies for stroke patients.

The reluctance of some physicians to prescribe intravenous tPA for stroke can be attributed to a fear of a potential HT with tPA. Addressing this fear, there are strict guidelines regarding the inclusion and exclusion criteria for the treatment of acute stroke with tPA, most designed to minimize the risk of hemorrhagic complications. In fact, deviations from the guidelines have been shown to result in an increased risk of bleeding and worsened outcome [8]. There is a great deal of interest in identifying patient characteristics associated with an increased risk of HT after tPA. The NINDS investigators were the first to address this question through univariate and multivariate analysis of the data collected in the NINDS trial. Patients developing symptomatic intracerebral hemorrhage (sICH) within 36 hours of treatment were compared to the remaining patients and increasing age, baseline stroke severity and edema on computed tomography (CT) were significant risk factors [2]. In the multivariate analysis, however, only baseline stroke severity and edema on CT were significantly associated with this severe complication of tPA. These findings were further clarified in a retrospective study which showed that early disruption of the blood brain barrier after tPA administration, as evidenced by early Gadolinium enhancement, carries a higher risk for symptomatic HT [9]. Other patient characteristics associated with an increased risk of vascular damage and bleeding are reviewed below.

Elevated Blood Pressure and HT after tPA

In patients with acute stroke, an increase in blood pressure (BP) carries a higher risk of HT, especially with tPA [10]. However, studies have shown that aggressive treatment to treat hypertension can worsen stroke outcome [11]. Data from the NINDS tPA stroke trial failed to show a relationship between elevated BP and HT but this may have resulted from the strict exclusion of severely hypertensive individuals and a protocol for BP lowering after tPA [12]. However, in a reanalysis of the NINDS data, it was shown that elevated BP resulted in a reduced likelihood of a favorable outcome in patients with increased blood glucose [13]. Also, in an observational study of 100 patients treated with tPA between 3 and 6 hours after symptom onset, severe HT was predicted by elevated systolic BP after tPA [14]. Lastly, in a retrospective study, investigators analyzed the causes of sICH in 510 patients treated with tPA. They found that violations in the blood pressure protocol were independently associated with higher risk of sICH [15].

Although elevated BP has been associated with increased vascular damage and bleeding in stroke patients treated with tPA, the recommended management is not evidence-based. Further understanding of the mechanisms of vascular damage due to elevated BP may lead to improved patient outcomes in these patients.

Hyperglycemia and Hemorrhage after tPA

In a prospective observational study of 138 patients treated with tPA within 3 hours of onset, only serum glucose was an independent predictor of symptomatic HT, with a substantial increase (2.5 ×) when blood glucose levels were above 200 mg/dl (11.2 mmol/L) [13]. This was replicated in the PROACT II trial, where symptomatic HT occurred in 35% of patients with serum glucose values greater than 200 mg/dL [16]. Most recently, of 1098 patients treated with tPA within 3 hours of onset, hyperglycemia (as defined by serum glucose greater than 144 mg/dL) was associated with increased risk of sICH (RR = 1.69), decreased risk of favorable outcome (RR=0.69) and increased risk of death (RR=1.64) [17]. Although the evidence supports an increased risk of hemorrhage due to tPA in patients with hyperglycemia, the mechanism of this effect and the consequence of glucose reduction is unknown and under investigation.

Mechanisms of Vascular Damage due to tPA

tPA is an endogenous compound, found mainly in the blood, that plays an important role within the fibrinolytic system [18–20]. In addition, tPA is also widely expressed in the developing and mature brain [21]. The ability of cultured endothelial cells from a variety of vascular sources and species to produce tPA, suggests that tPA production is a function of all endothelium. tPA is also expressed by the neurons and microglia [22] and is believed to mediate neuronal death and microglial activation following excitotoxic injury [23].

Emerging data suggests that tPA, a proteolytic enzyme, possesses neurovascular toxic properties, making its administration after ischemic stroke fraught with risk [24–28]. Exogenous administration of tPA after vascular occlusion is associated with the breakdown of the blood brain barrier (BBB), increasing endothelial permeability and brain edema [27]. In addition to its effects in the intravascular compartment, exogenous tPA has the ability to cross the intact or injured blood brain barrier, reaching the brain parenchyma, where it exerts neurotoxic effects [26, 28, 29]. Preserving the integrity of the blood brain barrier is important in reducing tPA neurotoxicity and that is the focus of this review.

Mediators of tPA-induced Vascular Toxicity

The deleterious effects of tPA on the ischemic cerebral vasculature are well appreciated but the molecular mediators of this effect have been less discussed. It is important to identify targets aimed at providing vascular protection for patients receiving tPA for stroke. Some of the most likely targets for pharmacologic intervention are:

1. Matrix metalloproteinases (MMPs)

MMPs are a group of zinc endopeptidases that are synthesized and released in an inactive “pro” form. They are subsequently proteolytically cleaved to their active form and can degrade all components of the mammalian central nervous system. Neurons, endothelial cells, microglia, and astrocytes, as well as infiltrating inflammatory cells, can all express MMPs after stroke [20]. Although their basal level is barely detectable under normal conditions, several MMPs are upregulated and activated following cerebral ischemia, such as MMP-2, MMP-3, MMP-7 and MMP-9 [30–32]. The role played by MMPs after cerebral ischemia is time-dependent. In the early stage (hours to days), MMPs degrade the tight junction and basal lamina proteins, resulting in blood brain barrier disruption, brain edema, neuronal cell death and hemorrhage [33, 34]. Such effects were reduced in MMP-9 knockout mice following focal cerebral ischemia [35]. Also the hippocampal neuronal cell death was reduced in the same knockouts following transient global cerebral ischemia [36]. Stroke patients with elevated plasma levels of MMP-9 have greater brain injury and worsened

neurological outcome [37] and those who suffer cerebral hemorrhage after tPA therapy have higher plasma MMP-9 levels that those who do not [38]. In the late stage, however, MMPs play a beneficial role, by mediating neurovascular remodeling [34]. Emerging evidence suggests that inhibition of MMP-9 during the late stage is associated with more severe brain injury and worsened functional outcome [39].

Evidence suggests that tPA can upregulate and activate various members of the MMP family (especially MMP-3 and MMP-9). In fact, the "tPA-induced MMP-9" hypothesis is generally accepted and supported by experimental evidence [40]. In vivo experiments have shown that tPA thrombolytic therapy following embolic experimental stroke increases brain MMP-9 levels and that the co-administration of MMP inhibitors reduces the HT and brain injury [41, 42]. Moreover, tPA knockout mice had significantly lower MMP-9 levels following focal cerebral ischemia compared to WT mice [43]. Primary human cerebral microvascular endothelial cell culture showed a time- and dose- dependent elevation of the active forms of MMP-2 and MMP-9 [43]. Taken together, it is clear that tPA induces MMP-9, although the exact mechanism is not fully understood.

Oxidative stress is believed to play a role in tPA-induced MMP-9 upregulation. Reperfusion injury secondary to the tPA therapy increases oxidative stress, which in turn can upregulate the MMP-9 gene whose promotor possesses NFKB transcription factor sites [40]. More specific mechanisms have also been studied. Low density lipoprotein receptor related protein (LRP) binds tPA and such binding is believed to play a role in MMP upregulation. Treatment of endothelial cells with inhibitory RNA to suppress LRP blunted the tPA-induced MMP-9 upregulation [43]. Moreover, LRP antagonists ameliorated the BBB disruption after direct intraventricular injection of tPA [44]. In addition, tPA plays a role in the MMP activation cascade. tPA catalyzes the conversion of the zymogen plasminogen into plasmin, the active serine protease that dissolves the fibrin clot. Plasmin can also cleave numerous substrates and is involved in several pathways including MMP activation. Plasmin can activate both pro-membrane type-1 MMP (proMT1-MMP) and proMMP-3, which in turn activates MMP-2 and MMP-9 respectively. In addition, tPA activates proMMP-9 via plasmin-independent pathways through its action on low density lipoprotein receptor-related protein (LRP) and protease activated receptor 1 (PAR1) [20].

2. LDL receptor–related protein (LRP)

The low density lipoprotein receptor-related protein (LRP) is a member of the LDL receptor (LDLR) family, which is a highly conserved gene family. In addition to their primary role, receptor-mediated endocytosis, they serve multiple biological functions, including regulation of proteinase and proteinase inhibitor activity, activation of lysosomal enzymes, regulation of calcium entry, transport and activation of steroid hormones, signal transduction, neurotransmission and long term potentiation among others [45, 46]. The 600 KDa LRP is a multifunctional receptor that is involved in lipid metabolism and embryonic development. However, the full spectrum of LRP biological functions are not yet clear [46].

Yepes and colleagues found that tPA upregulation following cerebral ischemia and the subsequent blood brain barrier disruption can occur independently of plasminogen and MMPs. tPA injected into the cerebrospinal fluid in the absence of ischemia induced a dose dependent increase in vascular permeability, an effect that was not blunted in plasminogen deficient (plg−/−) mice, suggesting the involvement of players other than plasminogen and MMPs. Anti-LRP antibodies and LDL receptor family antagonist (RAP) abolished tPA's effect, indicating that tPA acts through LDL receptor related protein (LRP) [44].

3. Caspase-8

Although glutamate antagonists are neuroprotective as monotherapy, studies suggest that tPA toxicity does not require a direct action on the NMDA receptors. MK-801, an NMDA receptor blocker, did not reduce tPA-induced cell injury in ischemic human brain endothelial cells. It was suggested that tPA potentiates apoptosis in NMDA-treated brain endothelial cells and neurons via shifting the apoptotic signal from the intrinsic to the extrinsic pathway. In mice, tPA-induced injury was reduced by the intracerebral administration of a caspase-8 inhibitor but not a caspase-9 inhibitor [25].

4. Degranulation of mast cells

Strbian and colleagues demonstrated the role of mast cells in mediating brain hemorrhage and reperfusion injury after tPA treatment. In vitro experiments have shown that tPA directly stimulates mast cell degranulation, thus releasing their preformed vasoactive substances, proteolytic enzymes, anticoagulants and chemotactic factors. Both pharmacological mast cell stabilization and genetic mast cell deficiency protected against tPA-induced brain edema and hemorrhage [47].

Combination therapy

The mechanism of the vascular unit disruption after ischemia and reperfusion with tPA has long been a major research priority. Combination therapies for brain injury are gaining momentum over monotherapies because of the complexity and the systemic nature of injury process, and the limitation of monotherapy, which targets only a single mechanism implicated in the stroke injury cascade. It is hoped that combination therapy using tPA and a neuroprotective agent can achieve better outcomes than using either drug alone. Therefore, many neuroprotective agents have been tested in combination with tPA, and, while some of them have been able to protect the vascular unit and inhibit HT, others may increase HT (Table 1). Careful review of these experimental studies may provide clues as to which tactics to pursue as combination therapy with tPA and which to avoid.

Increased hemorrhage

Several recent studies have reinforced the observation that some drugs may increase HT when combined with thrombolytics. Lapchak, et al. [48], found that caffeinol does not improve functional recovery and may increase the incidence of hemorrhagic stroke after embolization and tPA. In another study, Hoyt, et al. [49], also showed that caffeinol increases hemorrhage when combined with tPA. The increase in tPA-induced HT with caffeinol may be due to the prohemorrhagic effects of ethanol [48].

Nitrite adjuvant therapy with delayed tPA administration did not reduce infarct volume and showed evidence of HT at 48 h after MCAO compared to saline treatment at 6 or 2 h of suture MCAO in rats [50]. More recently, Haelewyn et al. [51], demonstrated that nitrous oxide inhibits tPA-induced thrombolysis and subsequent reduction of ischemic brain damage, whereas postischemic nitrous oxide reduces ischemic brain damage, increases brain hemorrhage and disruption of the BBB after thromboembolic stroke in rats. As predicted, Pfeilschifter et al., [52], reported that warfarin, an oral anticoagulant, when administered with tPA, exacerbates the risk of thrombolysis-associated HT in a mouse embolic model of ischemic stroke.

The most recent surprising finding was that of erythropoietin (EPO) in combination with tPA. Although a promising vascular protective agent alone [53], a recent clinical trial identified an increased in sICH and mortality when EPO was administered in combination with tPA [54]. Subsequently, it was reported that EPO promotes extracellular matrix

degradation and edema formation in animals treated with tPA and this was associated with an increased activity of MMP-9 [55], The EPO development story points to the importance of preclinical combination investigations prior to clinical trials, in order to identify potential negative interactions of treatments.

Decreased hemorrhage

Fortunately, some neuroprotective agents have been shown to reduce the vascular damage or HT when combined with tPA.

Free Radical Scavengers

Free radicals, the fundamental mediators of reperfusion injury [56] and reperfusion-related HT [57], are generated soon after vessel occlusion, with explosive propagation after reperfusion. Several free radical scavengers have shown protective effects in combination with tPA in animal stroke models. Edaravone, a free radical scavenger, in combination with tPA, significantly attenuated tPA extravasation and appears to be a reasonable strategy for diminishing the negative effects of tPA [58]. Yamashita et al.[59], 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. Combination therapy with the free radical spin trap alpha-phenyl tert butyl nitrone (alpha-PBN) treatments reduced the severity of delayed (6 h) administration tPA-induced hemorrhage and brain injury in an embolic stroke model of spontaneously hypertensive rats [60]. In another investigation, Lapchak et al. [61] showed that 2,2,6, 6-tetramethylpiperidine-N-oxyl (TEMPO), another spin trap agent, when combined with tPA, was similarly effective as PBN/tPA in reducing the incidence of hemorrhage without affecting infarct volume.

Chen et al. [62], showed that melatonin (5 mg/kg) adjuvant therapy with tPA (10 mg/kg) at 6 h postinsult of photothrombotic occlusion of the distal MCA did not significantly affect brain infarction compared with controls, but significantly attenuated BBB permeability and the risk of HT. NXY-059 in combination with tPA had a 47% incidence of hemorrhage which was improved when compared to tPA alone (67%) in rabbit embolic stroke [63]. Lapchak et al., [64, 65], in other studies showed that the combination of these drugs improved the behavioral outcome or clinical rating early (5 min) and late (6 h) after an embolic stroke. In a large clinical trial, NXY-059, initially appeared to reduce disability after stroke (the first Stroke-Acute-Ischemic-NXY-Treatment trial, SAINT I), especially in tPA-treated patients [66], but this effect could not be reproduced (SAINT II) [67]. The mechanism by which these scavengers exert their salutary effect on tPA-induced HT remains obscure, but one possible mechanism is that these agents may inhibit the activation of MMP-9 by scavenging the tPA-induced free radicals generartion or by trapping the free radicals derived from ischemia/reperfusion injury. However, other mechanisms are possible, and additional studies are needed to understand it in detail.

MMP inhibitors

Emerging evidence indicates that elevated expression and activation of MMPs, especially MMP-9, play a critical role in disruption of BBB and HT [21]. MMPs following cerebral ischemia also lead to increased infarct size, vascular permeability and hemorrhage [68, 69]. In contrast, inhibition of MMP-9 is associated with attenuation of infarct size and reduced risk of hemorrhagic complications [39, 42]. In addition, MMP-9 expression, infarct size, and

brain edema in a tPA knock-out mouse were significantly lower than in wild-type mice [70].Thus, pharmacological inhibition of MMPs (at an appropriate time) may hold promise as a safe and effective adjunct therapy for tPA in acute ischemic stroke. In a Lapchak et al., [71] study, rabbits were embolized with radiolabeled blood clots, followed five minutes later by administration of the broad spectrum MMP inhibitor, BB-94, or vehicle in combination with tPA (3.3 mg/kg tPA) at 60 minutes. The results showed that when the combination of BB-94 and tPA was administered, there was only a 41% incidence of hemorrhage (compared with 77% in the tPA group). Also, HT was decreased when rats were treated with the p-aminobenzoyl-gly-pro-D-leu-D-ala-hydroxamate, an MMP inhibitor, at 3 or 6 h of ischemia and then reperfused with tPA at 6 h [72]. A recent study from our group, examining the utility of minocycline as an MMP inhibitor in rat stroke model showed that tPA treatment was associated with augmented MMP-9 levels in brain. The ability of minocycline to protect against tPA associated HT was correlated with reductions in MMP-9 expression and activity [68]. A study by Murata et al. [73], also demonstrated that addition of minocycline treatment to animals treated with tPA 6 h after the onset of stroke reduced infarct size to those similar to animals treated with tPA after 1 hour. Similarly, HT, which was increased in animals only treated with tPA after 6 h, was significantly reduced. Through reduction of MMP levels, minocycline reduces the risk of HT increased by tPA administration following ischemic stroke and extends the therapeutic time window of tPA. Therefore, minocycline is likely to be more successful than other studied MMP inhibitors in that it has been shown to also be protective at an extended time window in experimental animals. To date, minocycline has been shown to be safe and feasible in stroke patients, alone or in combination with tPA [74]. These encouraging findings support further development of this combination.

LRP antagonist

Use of the LRP antagonist, receptor-associated protein (RAP), showed that in an intact BBB, LRP mediates endothelial translocation of tPA. Interestingly, thrombolytic agents seem to compete for LRP in the in vitro BBB model [75]. Moreover, direct intraventricular injections of tPA into mouse brain increased BBB permeability, and this response was ameliorated by LRP antagonists [44]. These data suggest that a specific receptor signaling pathway may trigger dysregulated proteolysis in the neurovascular unit after tPA treatment. Furthermore, inhibition of LRP by RAP suppressed both MMP-3 induction in endothelial cells and the increase in intracranial hemorrhage by tPA treatment after experimental stroke. These findings indicate that tPA promotes intracranial bleeding via MMP-3 induction in endothelial cells, which is regulated through the LRP pathway [76]. It remains to be shown whether LRP antagonists, through impairment of both MMP-3 and MMP-9 induction, may have the potential to suppress HT in patients with stroke.

Mast cell stabilization

It has recently been found that mast cell stabilization decreases ischemic brain swelling and neutrophil infiltration [77] and reduces hematoma volume, brain swelling, and mortality after intracerebral hemorrhage [78]. The same group further reported that combination treatment with cromoglycate, a classic inhibitor of MC degranulation, with tPA provided vascular protection and better neurological outcome and lower mortality after 24 h in a rat model of MCAO [47]. This finding revealed a proinflammatory mechanism that may provide a novel pharmacological target if confirmed in the clinical setting. Mast cell stabilization therefore deserves further study as an adjuvant to thrombolysis.

Platelet inhibition

Platelet activation and deposition in brain microvessels appear to be key events in the pathogenesis of ischemia-induced neuronal degeneration and behavioral deficits. Therefore,

pharmacological predministration with cilostazol for 7 days significantly suppressed the risk and severity of cerebral hemorrhage after injection of tissue-type plasminogen activator in mouse embolic stroke [79]. Administration of the novel nonpeptide glycoprotein IIb/IIIa receptor antagonist, SM-20302, in combination with tPA significantly reduced tPA-induced intracerebral hemorrhage [80].

Other neuroprotective agents in combination therapy

Albumin: Albumin is the main protein of plasma, and *Tang* et al. [81], reported that combination treatment using tPA with albumin improved neurological deficits, BBB permeability, confirmed by Evans blue extravasation, and reduced brain edema significantly.

Annexin 2: Recently, Annexin 2, a tissue plasminogen activator administered in conjunction with low-dose tPA (2.5 mg/kg) significantly enhanced fibrinolysis, attenuated mortality, brain infarction, and HT, even when administered at 4 h post-ischemia in a rat focal embolic stroke model [82].

Activated protein C (APC): APC, a plasma serine protease with systemic anticoagulant, anti-inflammatory and antiapoptotic activities, and direct vasculoprotective and neuroprotective activities, blocks tPA-mediated HT after transient brain ischemia and embolic stroke in rodents [83]. Also, Cheng et al. [84], showed that activated protein C (APC) inhibits a pro-hemorrhagic tPA-induced, NF-kappa B-dependent MMP-9 pathway in ischemic brain endothelium in vivo and in vitro by acting through protease-activated receptor in embolic stroke. These findings suggest that APC may improve thrombolytic therapy for stroke, in part, by reducing tPA-mediated HT.

Estrogen: More recently, 17β-estradiol (E2) co-therapy with thrombolysis resulted in significantly reduced neurological deficits, MMP-9 activity, BBB permeability and HT after embolic stroke in ovariectomized female Wistar rats when compared with tPA alone [85]. Moreover, E2 combination therapy with tPA attenuated the expression and activation of uPA, MMP-2, and MMP-9 [86].

Neuroserpin: Neuroserpin is a serine protease inhibitor (serpin) that selectively inhibits tPA within the central nervous system (CNS) and has neuroprotective effects in animal models of ischemic stroke. Zhang et al. [87], showed that administration of neuroserpin in combination with tPA (10 mg/kg, IV), 4 h after embolic stroke, reduced BBB leakage, brain edema, and ischemic lesion volume compared with rats treated with tPA alone, although ischemic lesion volumes were the same in both groups before the treatment.

*Tacrolimus (*FK506*):* The immunosuppressant tacrolimus (FK506, Prograf®), an agent widely used to prevent allograft rejection in clinical organ transplantation, showed promising neuroprotective effects in animal models of cerebral ischemia. Combined treatment of tPA with tacrolimus reduced the tPA-induced BBB dysfunction and HT in the ischemic hemisphere after thrombotic cerebral ischemia [88].

Summary

Although several mediators of the vascular damage due to tPA after acute ischemic stroke have been identified, pharmacologic agents approaching these targets are few. The most studied of the approaches to date has been MMP inhibition. Broad spectrum MMP inhibitors such as BB-94 and minocycline have consistently shown a reduction in HT after tPA in numerous models and laboratories. Also in support of this strategy is the increased HT and mortality experienced when patients received EPO and tPA, potentially due to an increase in MMP 9 expression and activity. Other agents that either decrease or increase HT after tPA

in experimental stroke have a less well defined mechanism of action or require further preclinical investigation prior to advancing to clinical trials.

Conclusion

Ischemic stroke patients at increased risk of sICH after tPA therapy, either through the presence of high stroke severity, hyperglycemia or excessive elevation of blood pressure, may benefit from vascular protective therapy. Broad spectrum MMP inhibition, administered acutely, may reduce the risk of hemorrhagic complications, thereby increasing the positive impact of reperfusion in these patients. Other treatment strategies, targeting the molecular mediators of tPA-induced vascular damage, are under development.

Acknowledgments

Funding: NIH-NINDS RO1063965 (SCF) and VA Merit Review BX000891 (SCF)

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Table 1

Combination treatment for stroke

Combination drugs with t-PA	Stroke model	Results	Citation
Increased hemorrhage			
caffeinol	Rabbit small clot embolic strokes(RSCEM)	Intracerebral hemorrhage↑	[48], [49]
Nitrite	Rat suture stroke model	Nitrite is not a protective adjuvant therapy to delayed rt PA administration after ischemic stroke in rats B.B.B damage↑	[50]
Nitrous oxide	Rat embolic stroke model	inhibits tPA-induced thrombolysis and subsequent reduction of ischemic brain damage, postischemic nitrous oxide reduces ischemic brain damage increases brain hemorrhages and disruption of the blood-brain barrier	[51]
Warfarin	Mice embolic stroke model	Increased risk of hemorrhage transformation	[52]
Erythropoietin(EPO)	Mice embolic stroke model	B.B.B permeability↑	[55]
Decreased hemorrhage			
Edaravone(free radical scavenger)	Rat embolic stroke model	Extravasation of tPA↓	[58], [59]
Alpha –PBN (free radical spin trapping)	Rat embolic stroke model in spontaneously hypertensive rats	Hemorrhage volume↓	[60]
PBN+TEMPO	Rabbit embolic stroke model	incidence of hemorrhage↓	[61]
Melatonin	Mice suture MCAO model	BBB permeability↓ Hemorrhage transformation↓	[62]
NXY-059	Rabbit embolic stroke model	incidence of hemorrhage↓ hemorrhage volume↓	[63]
NXY-059	Rabbit embolic stroke model	Behavior rating↑ Combination therapy was not statistically better than either drug alone.	[64]
NXY-059	Rabbit embolic stroke model	Additional behavioral improvement↑	[65]
BB-94(metalloproteinase inhibition)	Rabbit embolic stroke model with radiolabeled blood clots	t-PA induced hemorrhage ↓	[71]
MMP inhibitor p-aminobenzoyl-gly-pro-D-leu-D-alahydroxamate	Rat embolic stroke model	Brain edema↓ Risk and gravity of intracerebral hemorrhage↓ Neurological outcome↑ Extend the therapeutic window for thrombolysis	[72]
minocycline	Rat suture MCAO stroke model	Incidence of hemorrhage↓ Neurological outcome↑ mortality↓	[68]

Combination drugs with t-PA	**Stroke model**	**Results**	**Citation**
minocycline	Embolic stroke model in spontaneously hypertensive rats	Plasma MMP-9↓ Infarction size↓ Brain hemorrhage↓ Extend t-PA treatment time window	[73]
cromoglycate	Rat suture MCAO model MC-deficient rats	Hemorrhage transformation↓ Neurological outcome↑	[47]
Cilostazol	Mice embolic stroke model	the risk and severity of cerebral hemorrhage↓ mmp-9 activity↓	[79]
SM-20302 (nonpeptide glycoprotein IIb/IIIa platelet receptor antagonist)	Rabbit embolic stroke model	Intracerebral hemorrhage↓	[80]
Albumin	Suture MCAO stroke model	Neurological outcome↑ Brain edema↓ BBB permeability↓	[81]
Annexin A2	Rat embolic stroke model	Time window for stroke reperfusion prolonged brain infarction↓ hemorrhage transformation↓ fibrolysis↑ mortality↓	[82]
APC(activated protein C)	Rat MCAO and embolic stroke model	t-PA induced hemorrhage ↓	[84]
17{beta}-estradiol	Embolic stroke model in in ovariectomized female Wistar rats	matrix metalloproteinase-9 activity, blood-brain barrier permeability, and hemorrhagic transformation	[85]
17{beta}-estradiol	Suture MCAO stroke model in ovariectomized female rats	Infarct volume↓ MMP-2,MMP-9 ,u-PA ↓	[86]
Neurosepin	Rat embolic stroke model	BBB leakage↓ Brain edema↓ Ischemic lesion volume↓ Widen the therapeutic window for thrombolysis	[87]
FK506(tacrolimus)	Photochemical embolic stroke model in spontaneously hypertensive rats	Hemorrhagic transformation↓	[88]