Mechanisms of Hemorrhagic Transformation After Tissue Plasminogen Activator Reperfusion Therapy for Ischemic Stroke

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Abstract—Reperfusion therapy with tissue plasminogen activator (tPA) is a rational therapy for acute ischemic stroke. Properly titrated use of tPA improves clinical outcomes. However, there is also an associated risk of hemorrhagic transformation after tPA therapy. Emerging data now suggest that some of these potentially neurotoxic side effects of tPA may be due to its signaling actions in the neurovascular unit. Besides its intended role in clot lysis, tPA is also an extracellular protease and signaling molecule in brain. tPA mediates matrix remodeling during brain development and plasticity. By interacting with the NMDA-type glutamate receptor, tPA may amplify potentially excitotoxic calcium currents. At selected concentrations, tPA may be vasoactive. Finally, by augmenting matrix metalloproteinase (MMP) dysregulation after stroke, tPA may degrade extracellular matrix integrity and increase risks of neurovascular cell death, blood–brain barrier leakage, edema, and hemorrhage. Understanding these pleiotropic actions of tPA may reveal new therapeutic opportunities for combination stroke therapy. (Stroke. 2004;35[suppl I]:2726-2730.)

Key Words: blood–brain barrier ■ cerebral ischemia ■ hemorrhage ■ neuroprotection

A rational approach to treat acute ischemic stroke is to rapidly reperfuse the affected brain tissue. Evidence from controlled clinical trials suggests that thrombolysis of the occluding clot with recombinant human tissue plasminogen activator (tPA) can successfully reperfuse ischemic brain.1,2 However, tPA therapy may work best if administered within a narrow 1- to 3-hour therapeutic window after stroke onset.3,4 Although some intriguing evidence exists to suggest that some patients may also benefit from later tPA treatments,5 the criteria for identifying these patient subsets need to be precisely defined.

To date, only 3% to 5% of patients receive tPA therapy for acute ischemic stroke. In part, this may be related to the elevated risks of symptomatic intracranial hemorrhage, and the resulting narrowed therapeutic time windows to lessen these complications of hemorrhagic transformation. Thus, the clinical problem at hand is how to increase the time window for tPA, decrease or eliminate the risks of cerebral hemorrhage, and ultimately to increase the overall efficacy of tPA reperfusion therapy. In this short review, we propose that tPA is a pleiotropic molecule. In addition to its intended role in clot lysis, tPA may also possess important signaling and protease actions in the neurovascular unit after stroke, some of which would be responsible for mediating hemorrhagic transformation in affected brain (Figure 1). We will discuss some accumulating evidence to support this hypothesis, with the hope that delineating some of these pathways may reveal new therapeutic opportunities for combination stroke therapies.

Pleiotropic Actions of tPA in Brain

tPA plays a central role in maintaining homeostatic control in the blood coagulation cascade. By cleaving the precursor molecule plasminogen, it produces the active enzyme plasmin, which then dissolves fibrin-based clots in focal cerebral ischemia. When given to the right patients, tPA effectively reperfuses ischemic brain and rescues tissue. However, in addition to the intended clot lysis effects in brain, tPA may also have other signaling properties.

tPA is intimately involved in extracellular matrix remodeling during brain development.6,7 By modifying the parenchymal matrix, tPA may mediate neuronal precursor migration, as well as neurite and axonal extension.8,9 In adult brain, tPA may play a critical role in long-term potentiation,10,11 possibly by microproteolysis of extracellular space that allows dynamic remodeling at the synaptic and dendritic levels.12 Knockout mice deficient in endogenous tPA show perturbed forms of long-term potentiation,13 whereas transgenic mice overexpressing tPA show enhanced long-term potentiation and learning.14 In a pathologic context, tPA may...
exacerbate deleterious learning, such as that which may occur during drug addiction. A recent study demonstrated that tPA may mediate the rewarding effects of morphine by regulating dopamine release in the affected nucleus accumbens.\textsuperscript{15} Matrix remodeling may also contribute to neuronal plasticity during delayed recovery from stroke and nervous tissue injury. TPA knockout mice show reduced morphological and functional recovery after sciatic nerve crush.\textsuperscript{16,17}

One of the initial pieces of evidence that tPA may participate in stroke pathophysiology came from studies by Tsirka and colleagues, who showed that tPA knockout mice were resistant to kainate-induced excitotoxic lesions in the hippocampus.\textsuperscript{18} Subsequently, Lipton and colleagues showed that tPA knockout mice were similarly resistant to brain infarction after focal cerebral ischemia.\textsuperscript{19} It should be acknowledged, however, that these findings were somewhat dependent on model systems. In rat models, others have found that infusion of exogenous tPA did not increase infarcts after stroke.\textsuperscript{20,21} Nevertheless, the mechanisms by which tPA may amplify ischemic injury have been recently clarified. In cell culture systems, tPA may interact with the NR1 subunit of the NMDA receptor complex, thus amplifying damaging calcium currents during ischemic excitotoxicity\textsuperscript{22} (Figure 2). Furthermore, tPA (and plasmin) may also target nonfibrin substrates in brain extracellular matrix. For example, tPA amplifies excitotoxic neuron death in the hippocampus by degrading interneuronal laminin and disrupting prosurvival cell–matrix signaling.\textsuperscript{23} Finally, a recent study showed that clinically relevant concentrations of tPA can be potently vasoactive; at low 1 nmol/L concentrations tPA inhibits phenylephrine-induced vasoconstriction, whereas at high 20 nmol/L concentrations tPA augmented phenylephrine’s effects on vessel tone.\textsuperscript{24} Taken together, these data suggest important nonclot lysis roles for tPA and possibly other serine proteases in brain.

tPA–Matrix Metalloproteinase Interactions and Cerebral Hemorrhage

Accumulating data suggest that hemorrhagic transformation after tPA therapy in ischemic stroke may be related to dysregulated extracellular proteolysis within the neurovascular matrix.\textsuperscript{25,26,27} The candidate mediators are matrix metalloproteinases (MMPs), which comprise a large family of zinc endopeptidases responsible for remodeling almost all matrix substrates in brain.\textsuperscript{28,29} MMP-9 knockout mice are protected against brain trauma, spinal cord injury, and focal cerebral ischemia.\textsuperscript{30,31,32} Blood–brain barrier leakage is reduced in these knockout mice after injury.\textsuperscript{33} Furthermore, brain edema after intracerebral hemorrhage is reduced by MMP inhibitors.\textsuperscript{29} All of these data are consistent with the hypothesis that aberrant MMP-9 activity may be responsible for degrading neurovascular substrates in the neurovascular basal lamina.

The connection between tPA and MMP-9 comes from various pharmacologic, genetic and clinical studies. Broad-spectrum MMP inhibitors significantly reduce the incidence\textsuperscript{34} and severity\textsuperscript{35} of tPA-associated cerebral hemorrhage in animal models of clot-based embolic stroke. Administration of exogenous tPA significantly amplified the ischemic levels of MMP-9 in hypertensive rats after transient focal cerebral ischemia\textsuperscript{35,36} (Figure 3). Correspondingly, ischemic brain MMP-9 levels were reduced in tPA knockout mice.\textsuperscript{37} Emerging clinical data support this hypothesis as well. Although the indirect confounds of stroke severity versus causality will need to be clarified, stroke patients with elevated plasma levels of MMP-9 have greater brain injury and poor neurologic outcome,\textsuperscript{38} and MMP-9 levels are increased in patients who receive tPA.\textsuperscript{39} Finally, patients with higher plasma MMP-9 levels are more likely to undergo hemorrhagic transformation after tPA.\textsuperscript{40}

How does tPA amplify MMP-9? Because the MMP-9 gene promoter possesses activator protein-1 and nuclear factor-\textsuperscript{\textgamma}B transcription factor sites,\textsuperscript{37} this may be nonspecifically related
to oxidative stress induced by reperfusion injury. However, emerging data suggest that more specific signaling mechanisms may also be involved. tPA is now recognized as a cell signaling molecule in brain. As discussed earlier, tPA-mediated modification of N-methyl-D-aspartate (NMDA) calcium currents may contribute to cell signaling. In neurons and astrocytes, tPA is capable of activating intracellular protein kinase cascades. Recent studies also suggest a role for the low density lipoprotein receptor–related protein (LRP). LRP is a member of the large lipoprotein receptor gene family that is expressed in brain endothelial cells, neurons, and astrocytes. LRP is a receptor for tPA, possesses cell signaling properties, and is already implicated in vascular correlates of ApoE and amyloid processing. In human brain endothelial cell cultures, recombinant tPA upregulated MMP-9 mRNA and protein, whereas this tPA-induced MMP-9 response was decreased in endothelial cells treated with RNA interference to suppress LRP (Figure 4). These data suggest that LRP-mediated upregulation of MMPs after tPA may degrade neurovascular matrix after stroke, thus leading to hemorrhagic transformation. Furthermore, endothelial cell death may also be triggered by the ensuing disruption of cell–matrix homeostasis. Cerebral endothelial cells underwent anoikis-like death after hypoxia-reoxygenation, and this cell death was mediated by MMP-induced degradation of fibronectin matrix and prosurvival integrin signaling. Hence tPA-induced MMPs may also induce cell death within the neurovascular unit. It should be acknowledged that, in keeping with tPA’s pleiotropic actions, multiple signaling pathways may ultimately contribute. Indeed, it has been shown that direct intraventricular injections of tPA into mouse brain may bind LRP and also increase blood–brain barrier permeability in an MMP-independent manner as well.

Targeting tPA–MMP interactions may suggest new approaches for combination stroke therapy. In a rat model of focal stroke, cotreatment with tPA plus MMP inhibitors...
ameliorated reperfusion injury.46 Interferon-β downregulates MMPs, suggesting yet another therapeutic combination.47 Alternatively, blocking neurotoxic properties of tPA with neuroserpin (ie, neuronal serine protease inhibitor) increased the time-to-treatment window for thrombolysis in rat stroke models.48 Finally, there may be thrombolytic agents such as microplasmin49 or vampire bat salivary plasminogen activator50 that may not trigger MMP dysregulation or enhance excitotoxic neurodegeneration. Any approach to optimize tPA thrombolysis may reduce risks of hemorrhage, lengthen the time-to-treatment windows, and enhance the efficacy and safety of reperfusion therapy for stroke.

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