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. 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.

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. Subsequently, Lipton and colleagues showed
that tPA knockout mice were similarly resistant to brain
infarction after focal cerebral ischemia. It should be ac-
knowledged, 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. 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 (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
degradating interneuronal laminin and disrupting prosurvival
cell–matrix signaling. 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 ef-
facts on vessel tone. 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 neurovascu-
lar matrix. The candidate mediators are matrix metalloproteinases (MMPs), which comprise a large family of zinc
endopeptidases responsible for remodeling almost all matrix
substrates in brain. MMP-9 knockout mice are protected
against brain trauma, spinal cord injury, and focal cerebral
ischemia. Blood–brain barrier leakage is reduced in
these knockout mice after injury. Furthermore, brain edema
after intracerebral hemorrhage is reduced by MMP inhib-
itors. 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 and severity 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 (Figure 3). Correspondingly, ischemic brain
MMP-9 levels were reduced in tPA knockout mice. 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 neuro-
logic outcome, and MMP-9 levels are increased in patients
who receive tPA. Finally, patients with higher plasma
MMP-9 levels are more likely to undergo hemorrhagic
transformation after tPA.

How does tPA amplify MMP-9? Because the MMP-9 gene
promoter possesses activator protein-1 and nuclear factor-κB
transcription factor sites, this may be nonspecifically related

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**Figure 1.** Schematic outlining potential pleiotropic actions of tPA in the context of ischemic stroke.

**Figure 2.** Amplification of NMDA calcium currents by tPA in neuronal cultures. Reprinted with permission from Nature Med. Copyright 2001, Nature Publishing Group.
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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