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ne of the major recommendations emerging from the NINDS Stroke Progress Review Group was to shift the emphasis from a purely neurocentric view of cell death toward a more integrative approach whereby responses in all brain cells and matrix are considered during cerebral ischemia (see Figure). The concept of the neurovascular unit (fundamentally comprising endothelium, astrocyte, and neuron) provides a modular framework where cell-cell signaling and cell-matrix interactions mediate the overall tissue response to stroke and its treatments. There is no doubt that reperfusion blood vessels mechanically or pharmacologically prevents cell death and rescues brain when performed in a timely manner, as it does in ischemic myocardium. However, under some circumstances, thrombolysis and reperfusion lead to cerebral hemorrhage and edema. Here, we examine the hypothesis that beneficial versus potentially deleterious outcomes after tissue plasminogen activator (tPA) stroke therapy may relate in part to matrix proteolysis within the neurovascular unit, and review recent advances in this area.

tPA: More Than Clot Lysis

More than 7 years after the major ECASS and NINDS trials, use of tPA therapy remains limited. In part, this may be due to narrow time-to-treatment windows and apparent complications of hemorrhage and brain injury. The primary action of tPA occurs inside the blood vessel. But what happens when tPA reperfusion occurs within the context of weakened vessels and perturbed neurovascular homeostasis? Although unequivocal human data are lacking, findings from animal models suggest that possibly deleterious consequences of tPA reperfusion may develop because tPA is more than just a clot buster. Tsirka, Strickland, Lipton and colleagues first demonstrated that tPA knockout mice are protected against kainic acid hippocampal injury and focal cerebral ischemia. Subsequently, it was shown that tPA may interact with the NR1 subunit of the NMDA receptor complex, thus amplifying damaging calcium currents during ischemic excitotoxicity. 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 postsynaptic cell-matrix signaling. Another recent study showed that administering a chemical antagonist or deleting the gene of the protease activated receptor-1 (PAR-1) significantly reduced infarction after focal ischemia. Although the main effect of tPA in stroke certainly occurs within the targeted vessel, these findings suggest that extravascular actions of tPA may complicate its intended role in clot lysis. Reperfusion of ischemic brain is clearly beneficial. But a deeper understanding of how tPA also affects neurovascular matrix may reveal new approaches to further improve tPA thrombolysis.

tPA and Matrix Metalloproteinases

Extracellular proteolysis in brain is an important physiological phenomenon required for cell migration, and neurite and axon extension during brain development and plasticity. Besides tPA, matrix metalloproteinases (MMPs) comprise the other major proteolytic system in mammalian brain. MMPs are important mediators in stroke because they are upregulated after ischemia, and subsequently degrade critical components of the neurovascular matrix and blood-brain barrier. Degradation of basal lamina substrates, blood-brain barrier leakage, edema, and infarction are reduced in MMP-9 knockout mice after focal cerebral ischemia. Hippocampal neuronal death is reduced in MMP-9 knockouts after transient global cerebral ischemia. Can some of the deleterious side effects of tPA be caused by tPA-induced MMP upregulation? In rat embolic stroke models, clot lysis with tPA increases ischemic brain MMP-9 levels, and co-treatment with MMP inhibitors reduced tPA-induced hemorrhagic transformation and brain injury. The “tPA-induced MMP-9” hypothesis is further supported by a study showing that after focal cerebral ischemia, MMP-9 levels are lower in tPA knockout mice compared with wild-type mice. But how does tPA amplify MMP-9? In part, it may be nonspecifically related to oxidative stress induced by reperfusion injury because the MMP-9 gene promoter possesses NFkB transcription factor sites. However, more specific signaling mechanisms may also be involved; tPA is now recognized as a cell-signaling molecule in neurons and glia. Although the precise signaling mechanisms remain to be fully elucidated, recent studies suggest a role for the low-density lipoprotein receptor related protein (LRP), a member of the large lipoprotein receptor gene family that is already implicated in vascular correlates of ApoE and amyloid processing. LRP is enriched in brain, possesses cell signaling properties, and avidly binds tPA. In human brain endothelial cells, addition of recombinant tPA upregulated...
provide a more comprehensive and realistic view of brain injury. Understanding how tPA relates to proteolytic dysregulation within the neurovascular unit may lead to novel ways of approaching stroke therapy.33,34 Recently, it was found that beta-amyloid upregulates MMP-2, MMP-9, and plasminogen activators in cerebral microvessels.35–37 Hence, it is possible that neurovascular proteolysis may also have broader implications for vascular dementia and neurodegeneration in general.

The majority of data reviewed here were derived from cell culture systems and animal models of stroke, and the concepts and hypotheses discussed will have to be carefully validated in patients. Properly titrated use of tPA is clearly beneficial to reperfuse ischemic brain and rescue compromised tissue. Approaches that optimize tPA thrombolysis may lengthen the time-to-treatment windows and render reperfusion therapy even safer and more efficacious.

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References

Targeting Neurovascular Proteolysis
The tPA–MMP-9 hypothesis may suggest new approaches for stroke therapy. In a rat model of focal stroke, co-treatment with tPA plus MMP inhibitors ameliorated reperfusion injury.28 Interferon-beta downregulates MMPs, suggesting another therapeutic combination.29 Alternatively, blocking neurotoxic properties of tPA with neuroserpin, neuronal serine protease inhibitor, increased the time-to-treatment window for thrombolysis in rat stroke models.30 Finally, there may be thrombolytic agents such as microplasmin11 or vampire bat salivary plasminogen activator32 that may not trigger MMP dysregulation or enhance excitotoxic neurodegeneration.

Over a decade of monotherapies focused on only neurons have not yielded successful stroke treatments for humans. Expanding the focus to include other cells and matrix may...


KEY WORDS: Advances in Stroke □ blood-brain barrier □ matrix metalloproteinase □ proteolysis □ reperfusion injury □ tissue plasminogen activator
tPA and Proteolysis in the Neurovascular Unit
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