tPA-S481A Prevents Neurotoxicity of Endogenous tPA in Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) is associated with loss of autoregulation due to impaired responsiveness to cerebrovascular dilator stimuli, which leads to cerebral hypoperfusion and neuronal impairment or death. Upregulation of tissue plasminogen activator (tPA) post-TBI exacerbates loss of cerebral autoregulation and NMDA-receptor-mediated impairment of cerebral hemodynamics, and enhances excitotoxic neuronal death. However, the relationship between NMDA-receptor activation, loss of autoregulation, and neurological dysfunction is unclear. Here, we evaluated the potential therapeutic efficacy of a catalytically inactive tPA variant, tPA S481A, that acts by competing with wild-type tPA for binding, cleavage, and activation of NMDA receptors. Lateral fluid percussion brain injury was produced in anesthetized piglets. Pial artery reactivity was measured via a closed cranial window, and cerebrospinal fluid (CSF) extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) was quantified by enzyme-linked immunosorbent assay (ELISA). tPA-S481A prevented impairment of cerebral autoregulation and reduced histopathologic changes after TBI by inhibiting upregulation of the ERK isoform of MAPK. Treatment with this tPA variant provides a novel approach for limiting neuronal toxicity caused by untoward NMDA-receptor activation mediated by increased tPA and glutamate following TBI.

Key words: brain injury; cerebral autoregulation; cerebral circulation; signal transduction; tissue plasminogen activator

Introduction

TRAUMATIC BRAIN INJURY (TBI) is the leading cause of injury-related death in young adults and children (Rodriguez, 1990). While the effects of TBI have been investigated extensively in adult animal models (Wei et al., 1980), less is known about it in the pediatric population. TBI can cause uncoupling of blood flow and metabolism, resulting in cerebral ischemia or hyperemia (Richards et al., 2001). Although cerebral hyperemia was historically considered the cause of diffuse brain swelling after TBI in the pediatric setting (Bruce et al., 1981), more recent evidence suggests that cerebral hypoperfusion is the dominant derangement (Adelson et al., 1997). Indeed, using a piglet model of fluid percussion injury (FPI), constriction of pial arteries and reduction of cerebral blood flow (CBF) was seen (Armstead and Kurth, 1994). The piglet offers the unique advantage of being a species with a large gyrencephalic brain with substantial white matter, thereby permitting clinically-relevant investigation of cerebral hemodynamics in the pediatric age group.

Glutamate can bind to any of three ionotropic receptor subtypes named after synthetic analogues: *N*-methyl-D-aspartate (NMDA), kainate, and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). The NMDA receptor in particular is thought to contribute to excitotoxicity (Choi, 1992). Activation of NMDA receptors elicits cerebrovasodilation, by which local metabolism is coupled to CBF (Faraci and Heistad, 1998). Glutamatergic system hyperactivity has been demonstrated in animal models of TBI, while NMDA-receptor antagonists have been shown to protect against TBI (Katayama et al., 1990; Merchant et al., 1999). Although the disposition of cerebral hemodynamics is thought to contribute to neurologic outcome, little attention has been given to the role of NMDA-mediated vascular activity in this process. This is important because we have observed that vasodilation in response to NMDA-receptor activation is reversed to vasoconstriction after FPI in the piglet (Armstead et al., 2005).

Glutamate release and activation of the NMDA receptor have long been recognized as key contributors to negative outcomes after TBI. NMDA antagonists such as MK801

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improve outcome after TBI in animal models. However, toxicity of NMDA antagonists is limiting in translating this approach to humans, though another NMDA antagonist, memantine, has shown some promise. Therefore, despite the key role of excitotoxicity in outcome after TBI, the use of NMDA antagonists for the treatment of brain injury has not been successful to date.

Tissue plasminogen activator (tPA) can enhance excitotoxic neuronal cell death through interactions with the NMDA receptor by causing excessive increases in intracellular calcium, leading to apoptosis and necrosis (Nicole et al., 2001; Wang et al., 1998). However, the latter actions of NMDA-receptor activation may not necessarily represent the only reversible component of toxicity. In the context of the neurovascular unit, for example, impaired cerebral hemodynamics are thought to contribute to neuronal cell necrosis. tPA upregulation contributes to impaired cerebral hemodynamics, including disturbed cerebral autoregulation during hypotension, and cell damage after FPI (Armstead et al., 2006,2009,2011a). tPA contributes to impaired NMDA-mediated cerebrovasodilation via upregulation of mitogen-activated protein kinase (MAPK; Armstead et al., 2011b), a family of at least three kinases (extracellular signal-regulated kinase [ERK], p38, and Jun N-terminal kinase [JNK]) that are critically important in regulating hemodynamics after TBI (Armstead et al., 2009).

The release of excitatory amino acids such as glutamate and the activation of the NMDA receptor also contribute to impaired cerebral autoregulation (Armstead, 2002). Recent approaches to limit elevation of glutamate after TBI in the mouse and pig using glucagon post-insult prevent brain tissue damage and preserve autoregulation by blunting tPA upregulation (Armstead et al., 2011a; Fanne et al., 2011). Based on these studies, we posit that glutamate and tPA act in concert to induce neurotoxicity. In the absence of tPA (tPA-null mice), even high levels of CNS glutamate occurring after brain injury are weakly neurotoxic. In addition, exogenous tPA is not neurotoxic when glutamate levels are kept low. Based on this, we propose that tPA and glutamate create a vicious cycle, wherein tPA increases the toxicity of glutamate by increasing the sensitivity of NMDA receptors to tPA, and glutamate increases the neurotoxicity of tPA by signal transduction through NMDA receptors that have been activated by tPA (Armstead et al., 2011b). Furthermore, neurotoxicity induced by tPA increases cerebrospinal fluid (CSF) levels of glutamate (Fanne et al., 2011), and neurotoxicity induced by glutamate increases the levels of tPA (Armstead et al., 2011a), which further exacerbates injury. The corollary of this proposed feed-forward cycle is that preventing activation of NMDA receptors by tPA will decrease the toxicity of glutamate as well.

We hypothesize that a mutant tPA which is able to bind but not activate NMDA receptors would improve outcome after TBI. The variant tPA-S481A is predicted to compete with wild-type tPA for binding to the NMDA receptor, and thus protect it from activation by high levels of endogenous wild-type tPA that occur post-TBI. Indeed, tPA-S481A binds to the R1 subunit of NMDA and prevents its activation in the lung (Nassar et al., 2011a,2011b,2011c). Use of this tPA variant in the present study is proposed as a novel approach to limiting neurotoxicity by excessive NMDA-receptor activation associated with the robust increase of glutamate and tPA that occurs within the brain following TBI (Armstead et al., 2005;

Katayama et al., 1990). We minimize the importance of the prevailing dogma that NMDA-receptor toxicity is directly mediated through calcium-dependent neuronal apoptosis and necrosis. Alternatively, we suggest that the vicious cycle created by tPA and NMDA and its effect on CBF is key in determining outcome after brain injury. Breaking this vicious cycle with tPA-S481A is proposed to prevent reduction in pial artery diameter, impairment of autoregulation, and neuronal cell necrosis after TBI. These studies hold the potential to re-engineer the standard of care for TBI.

Methods

tPA-S481A

To generate tPA-S481A, a mutation was introduced into wild-type tPA by polymerase chain reaction (PCR) using the QuickChange Mutagenesis kit (Stratagene, La Jolla, CA), and the complete sequence was verified (Nassar et al., 2011a, 2011b, 2011c). The protein contains two extra amino acids, RS-, at the NH₂ terminus, resulting from the introduction of the Bgl II cloning site. Proteins were expressed in the S2 Drosophila Expression System (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol, and purified by antibody affinity chromatography using anti-tPA coupled to CN-Br-activated Sepharose.

Closed cranial window and brain injury procedures

Newborn pigs (1–5 days old, 1.0–1.4 kg) of both sexes were studied. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. The animals were anesthetized with isoflurane (1–2 MAC), and maintained with α -chloralose (30–50 mg/kg supplemented with 5 mg/kg/h IV).

The closed cranial window technique for measuring pial artery diameter and collection of CSF for enzyme-linked immunosorbent assay (ELISA) analysis has been previously described (Armstead and Kurth, 1994). The method used to induce brain FPI has been described previously (Armstead and Kurth, 1994). A device designed by the Medical College of Virginia was used. A small opening was made in the parietal skull contralateral to the cranial window. A metal shaft was sealed into the opening on top of the intact dura and fluid coupled to the brain injury device. The intensity of the injury was 1.9–2.2 atm with a constant duration of 19–23 msec.

Protocol

Pial small arteries (resting diameter 120–160 μ m) were examined. Typically, 2–3 mL of artificial CSF were flushed through the window over a 30-sec period, and excess CSF was allowed to run off through one of the needle ports. For sample collection, 300 μ L of the total cranial window volume of 500 μ L was collected by slowly infusing artificial CSF into one side of the window, and allowing the CSF to drip freely into a collection tube on the opposite side.

Five experimental groups were studied (all $n = 5$): (1) sham control, (2) FPI, vehicle pre-treated, (3) FPI post-treated with tPA-S481A (1 mg/kg IV), (4) FPI post-treated with tPA (1 mg/kg IV), and (5) FPI post-treated with the ERK MAPK antagonist U 0126 (1 mg/kg IV). Hypotension was induced by the rapid withdrawal of either 5–8 or 10–15 mL blood per kilogram to induce moderate or severe hypotension

(decreases in mean arterial blood pressure of 25% and 45%, respectively; Armstead et al., 2011a). Such decreases in blood pressure were maintained constant for 10 min by titration of additional blood withdrawal or blood reinfusion (Armstead et al., 2011a). The vehicle for all agents was 0.9% saline, except for the MAPK inhibitor, for which we used dimethyl sulfoxide (100 μ L) diluted with 9.9 mL 0.9% saline. In sham-control animals, responses to hypotension (moderate and severe), papaverine, NMDA, glutamate (10^{-8} and 10^{-6} M), and prostaglandin E_2 (PGE_2) and PGI_2 (1 and 10 ng/mL), were obtained initially, and then again 1 h later in the presence of the agent vehicle. In drug post-treated FPI animals, drugs were administered (randomized) 30 min before FPI, and the responses to hypotension and papaverine were obtained at 1 h after injury.

CBF was measured in the cerebral cortex and hippocampus using radioactively-labeled microspheres (Armstead et al., 2011a). A catheter was placed in the left ventricle via the right carotid artery to inject the microspheres. In the present study, 15- μ m microspheres (300,000–800,000 spheres), containing a known amount of radioactivity, was injected into the left ventricle. Reference blood was withdrawn from the femoral artery. After each experiment, the pig was sacrificed and the brain removed and weighed. CBF was determined by counting cerebral cortex brain tissue samples in a gamma counter. Brain tissue sample size varied from 100–500 mg; there were at least 400 spheres/g of tissue. There was an adequate number of spheres to accurately determine CBF during hypotension, as shown previously (Armstead et al., 2011a). The energy from each nuclide was separated by differential spectroscopy. Up to five different isotope CBF determinations were made in each piglet. Aliquots of the microsphere solutions injected were used for overlap calculations. The count in each milliliter per minute of blood flow was determined by dividing the counts in the reference withdrawal by the rate of

reference withdrawal. Thus blood flow can be calculated as $Q = C \times R \times CR^{-1}$, where Q is brain blood flow (in mL/min), C is counts per minute (cpm) in the tissue sample, R is the rate of withdrawal of the reference blood sample (in mL/min), and CR is the total counts in the reference blood sample. CBF so determined reflects flow to the cerebral cortex and hippocampus both ipsilateral and contralateral to the injury site.

ELISA

Commercially-available ELISA kits (Assay Designs, Ann Arbor, MI) were used to quantify CSF ERK MAPK concentration. Phosphorylated ERK MAPK enzyme values were normalized to total form and then expressed as percent of the control condition.

Statistical analysis

Pial artery diameter and CSF ERK MAPK values were analyzed using analysis of variance (ANOVA) for repeated measures. If the value was significant, the data were then analyzed by Fisher's protected least significant difference test. An α level of <0.05 was considered significant in all statistical tests. Values are represented as mean $\pm$ standard error of the mean (SEM) of the absolute value or as percentage changes from control values.

Results

tPA-S481A prevents loss of neurons in the CA1 and CA3 hippocampus, and reductions of CBF and pial artery diameter after FPI

FPI was associated with marked neuronal cell loss in the CA1 and CA3 hippocampus (Armstead et al., 2006), which was prevented by tPA-S481A (1 mg/kg IV) administered 30 min post-injury (Fig. 1). FPI is known to reduce CBF and

FIG. 1. Influence of fluid percussion injury (FPI) on the number of degenerating hippocampal neurons in the absence and presence of tPA-S481A (1 mg/kg IV, 30 min after FPI; $n=3-5$). At left is a typical CA1 section (A, control; B, FPI; C, FPI + tPA-S481A). At right (D) is summary data for CA1 and CA3 (* $p < 0.05$ compared to corresponding control; + $p < 0.05$ compared to FPI alone).

pial artery diameter (Armstead and Kurth, 1994), but tPA-S481A blocked this injury-induced hypoperfusion in the parietal cortex and hippocampus (Fig. 2). tPA-S481A had no effect on pial artery diameter or CBF in sham (uninjured) animals (124 ± 10 versus $128 \pm 11 \mu\text{m}$ for pial artery diameter in the absence and presence of tPA-S481A, respectively; $n=5$). In the context of the neurovascular unit, preservation of CBF is thought to maintain neuronal cell integrity. This concept is supported by our previous work in which up-regulated plasminogen activator expression, which contributed to histopathology after FPI, was ameliorated by EEIIMD, a peptide derived from PAI-1 that blocks tPA-mediated vasoconstriction post-TBI (Armstead et al., 2005,2009). The present data confirm and extend this concept, in that tPA-S481A

prevented both hypoperfusion and loss of neurons in the hippocampus associated with FPI, thereby demonstrating a cause-and-effect relationship between cerebral hemodynamics and histopathology post-injury. These data support the use of this variant as a novel approach to limit toxicity from excessive NMDA-receptor activation caused by the robust increase in tPA and glutamate within the brain following TBI. Future research will be directed at determining if hippocampal neuronal cell protection by tPA-S481A has functional significance in the prevention of impairment of behavioral indices of outcome such as learning and memory. We next asked if the reductions in NMDA-receptor activation and neuronal cell loss are mediated through the loss of auto-regulation post-TBI.

FIG. 2. Influence of fluid percussion injury (FPI) on cerebral blood flow (CBF) in the parietal cortex (A) and hippocampus (B), and on pial artery diameter (C). In the parietal cortex and hippocampus CBF was obtained in controls (C), and in those with moderate and severe hypotension (MH and SH) in sham animals, and those with FPI, and FPI + tPA-S481A (1 mg/kg IV, 30 min post-FPI; $n=5$; $*p<0.05$ compared to corresponding sham animals in panels A and B, and untreated FPI animals in panel C; $^{\dagger}p<0.05$ compared to corresponding control values in panels A and B; $^{\#}p<0.05$ compared to corresponding untreated post-FPI values).

tPA-S481A prevents impairment of cerebral autoregulation during hypotension after FPI

CBF was unchanged during hypotension (moderate caused 25% and severe caused 45% reductions in mean arterial pressure) in sham control animals, indicating intact cerebral autoregulation (Fig. 2). CBF was reduced after FPI and reduced further during hypotension, but these reductions in CBF were prevented by tPA-S481A (Fig. 2). FPI blunts pial artery dilation during hypotension (Armstead, 2002), but the response is preserved by tPA-S481A (1 mg/kg IV, 30 min post-injury; Fig. 3). In contrast, pial artery dilation induced by papaverine was unchanged by FPI (Armstead et al., 2011a) and tPA-S481A, indicating the specificity of the variant on endogenous tPA (Fig. 3). Previously, we observed that the NMDA antagonist MK801 prevented impairment of cerebral autoregulatory pial artery dilation after FPI (Armstead, 2002). This suggests that protection of cerebral autoregulation by tPA-S481A likely relates to its amelioration of glutamate toxicity in the setting of TBI. Clinically, autoregulation during hypotension is often impaired after TBI, and the degree of impairment correlates with Glasgow Outcome Scale scores in pediatric TBI (Freeman et al., 2008). These data, when coupled with those shown in Figure 1, indicate that tPA-S481A's ability to protect autoregulation likely involves a cause-and-effect relationship in preventing injury to CA1 and CA3 cells. We next explored the mechanism by which tPA-S481A prevents impairment of cerebral autoregulation post-TBI.

tPA-S481A preserves cerebrovasodilation mediated by NMDA-receptor activation and prostaglandins

NMDA-receptor activation elicits pial artery vasodilation, which is converted to vasoconstriction by FPI in the newborn pig (Armstead et al., 2005). The release of endogenous tPA in the setting of FPI aggravates NMDA-receptor-mediated vasoconstriction (Armstead et al., 2005). Because the NMDA antagonist MK801 prevents impairment of pial artery dilation during hypotension after FPI (Armstead 2002), these data indicate that reversal of NMDA-receptor-mediated dilation

and its aggravation by tPA likely contributes to cerebral dysregulation in the setting of TBI.

Pial artery dilation during hypotension is due in part to the release of dilator prostaglandins, such as PGE₂ and PGI₂ (Armstead, 2005). FPI impairs PGE₂- and PGI₂-mediated pial artery dilation (Armstead, 1998), which probably contributes to disturbed cerebral autoregulation after TBI. The relationship between NMDA and tPA in the context of impaired PG-mediated vasodilation after TBI has not to our knowledge previously been explored.

First, we observed that glutamate-mediated and NMDA-receptor-mediated pial artery dilation was reversed after FPI to vasoconstriction. tPA-S481A (1 mg/kg IV) given 30 min post-insult not only prevented excitatory amino acid-induced pial artery vasoconstriction, but promoted stimulus-induced pial artery dilation, as seen during physiological conditions (Fig. 4). PGE₂- and PGI₂-induced pial artery dilation was blunted by FPI, which was also prevented by tPA-S481A (Fig. 5). Taken together, these data suggest that tPA-S481A protects against cerebrovascular dysregulation after TBI, at least in part by preventing impairment of NMDA-receptor-, PGE₂-, and PGI₂-induced pial artery vasodilation. We next investigated the mechanism by which tPA-S481A prevented impairment of NMDA-receptor-, PGE₂-, and PGI₂-induced pial artery vasodilation post-TBI.

tPA-S481A inhibits upregulation of ERK MAPK

MAPK, a family of kinases (ERK, p38, and JNK), is a post-receptor signaling system that contributes to setting of vascular tone, and is upregulated after TBI (Armstead et al., 2009; Otani et al., 2003). The release of ERK MAPK after FPI in the pig contributes to reducing CBF and pial artery diameter, and worsens histopathology (Armstead et al., 2009). Additionally, tPA aggravates the reversal of NMDA-receptor-mediated dilation to vasoconstriction after FPI by upregulating ERK and JNK MAPK (Armstead et al., 2011b). Since impairment of NMDA-receptor-mediated pial artery dilation contributes to the impairment of cerebral autoregulation after FPI, we hypothesized that tPA-S481A, by competing with wild-type tPA

FIG. 3. Influence of (A) hypotension (moderate and severe), and (B) papaverine, on pial artery diameter before (Control) and after fluid percussion injury (FPI), and after FPI in animals treated 30 min post-injury with tPA-S481A (1 mg/kg IV; $n=5$; * $p<0.05$ compared with corresponding controls; + $p<0.05$ compared to FPI alone).

FIG. 4. Influence of (A) *N*-methyl-D-aspartate (NMDA), and (B) glutamate, on pial artery diameter before (Control), after fluid percussion injury (FPI), and after FPI in animals post-treated (30 min) with tPA-S481A (1 mg/kg IV; $n=5$; $*p<0.05$ compared with corresponding controls; $^+p<0.05$ compared to corresponding FPI alone).

for binding to the NMDA receptor, would limit ERK MAPK upregulation after FPI. Indeed, FPI elevated CSF ERK MAPK, an effect potentiated by wild-type tPA, but blunted by tPA-S481A (Fig. 6). Administration of the ERK MAPK antagonist U 0126 (1 mg/kg IV) prevented impairment of pial artery dilation during hypotension (Armstead et al., 2010). These biochemical data support the pharmacological data, and suggest that tPA-S481A prevents impairment of cerebral autoregulation after FPI by inhibiting upregulation of ERK MAPK. In the neurovascular unit, normalization of cerebral hemodynamics, including protection of autoregulation, is a key therapeutic target, since CBF contributes to outcome.

Discussion

tPA-S481A protects against cerebral injury caused by endogenous tPA in the setting of TBI. While tPA is well known to aggravate excitotoxic neuronal cell death, little is known about the role of NMDA-receptor-mediated vascular activity

in neuropathologic outcomes. Activation of NMDA receptors elicits cerebrovasodilation and may represent one of the mechanisms that couple local metabolism to blood flow (Faraci and Heistad, 1998). In healthy brain, tPA is critical for the full expression of the flow increase evoked by activation of the mouse whisker barrel cortex (Park et al., 2008). tPA promotes nitric oxide (NO) synthesis during NMDA-receptor activation by modulating the phosphorylation state of neuronal nitric oxide synthase (Park et al., 2008). These findings suggest that tPA is a key mediator linking NMDA-receptor activation to NO synthesis and functional hyperemia.

In contrast, in the injured brain our recent studies show that tPA release is upregulated (Armstead et al., 2005), and aggravates FPI-induced reversal of NMDA-receptor-mediated pial artery vasodilation to vasoconstriction by upregulating the ERK and JNK isoforms of MAPK (Armstead et al., 2005,2011b). A potential explanation for the differential role of tPA in the normal and injured brain could relate to increased superoxide production after FPI (Kulkarni and Armstead,

FIG. 5. Influence of (A) prostaglandin E₂ (PGE₂), and (B) PGI₂, on pial artery diameter before (Control), after fluid percussion injury (FPI), and after FPI in animals post-treated (30 min) with tPA-S481A (1 mg/kg IV; $n=5$; $*p<0.05$ compared with corresponding controls; $^+p<0.05$ compared to FPI alone).

FIG. 6. Influence of fluid percussion injury (FPI) on phosphorylated extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) in the absence and presence of tissue plasminogen activator (tPA; 1 mg/kg IV), and tPA-S481A (1 mg/kg IV) treatment 30 min after FPI ($n=5$; * $p < 0.05$ compared with FPI alone; + $p < 0.05$ compared with FPI + tPA).

2002), which together with increased NO generates excessive peroxynitrite. Once formed, peroxynitrite could impair cerebrovasodilator systems post-injury. However, the severity of the constriction observed with NMDA after FPI + tPA is substantial, and probably not the sole result of loss of a dilator, such as NO scavenging by superoxide, but is also due to production of a vasoconstrictor. While the identity of this vasoconstrictor is not known with certainty, endothelin may play a role, since it has been found to be upregulated and to contribute to the impaired dilation induced by NMDA-receptor activation after FPI (Armstead, 2001).

Reversal of NMDA-induced vasodilation to vasoconstriction is important, since administration of the NMDA antagonist MK801 protects against impairment of cerebral autoregulation after FPI in the pig (Armstead, 2002). How-

ever, the toxicity of MK801 has limited the use of this approach in humans, though another NMDA antagonist, memantine, has shown some promise. Therefore, despite the key role of excitotoxicity in outcomes after TBI, the use of NMDA antagonists for the treatment of brain injury has not been successful to date.

Administration of the tPA variant tPA-S481A post-injury prevented hypoperfusion, the loss of autoregulation during hypotension, and limited histopathologic changes produced by FPI in the piglet (Fig. 7). However, this variant had no effect on pial artery diameter or CBF in the absence of brain injury. This compound is a catalytically-inactive tPA variant with a single mutation in the active site that maintains its "docking site," through which it binds to the NMDA receptor (Nassar et al., 2011a,2011b,2011c), but cannot cleave and thereby activate the receptor. Therefore, tPA-S481A competes with wild-type tPA for binding to the NMDA receptor, and protects it from activation by wild-type tPA (Fig. 7). These data indicate that docking alone does not necessarily cause toxic signaling. This tPA variant provides a novel approach to limiting toxicity of the NMDA receptor hyperactivation associated with the robust increase in glutamate and tPA seen within the brain following TBI. Thus, the present data not only reveal a novel therapeutic target for TBI, but also provide a potential reason for the failure of previous studies to show efficacy using NMDA-receptor antagonists in the clinical setting.

Pial artery dilation during hypotension is due in part to the release of dilator prostaglandins, such as PGE₂ and PGI₂ (Armstead, 2005). FPI impairs PGE₂- and PGI₂-mediated pial artery dilation (Armstead, 1998), which probably contributes to disturbed cerebral autoregulation after TBI. We believe the data presented here are the first to explore the relationship between NMDA and tPA in the context of impaired PG-mediated vasodilation after injury. These results indicate that tPA-S481A limits impairment of PG-mediated vasodilation after FPI, suggesting another mechanism by which tPA-S481A protects cerebral autoregulation in the setting of TBI.

There are several experimental caveats to the present study design. One is the lack of consideration of the time window

FIG. 7. Schematic diagram summarizing our concept relating tissue plasminogen activator (tPA) variant administration and mechanisms such as mitogen-activated protein kinase (MAPK) to outcome, including cerebral hemodynamics and histopathology, in the setting of fluid percussion injury (FPI). Arrow thickness reflects the relative degree to which this action occurs (NMDA-R, N-methyl-D-aspartate receptor; ERK, extracellular signal-regulated kinase; CBF, cerebral blood flow).

during which tPA-S481A can be given and still yield protection of cerebral hemodynamics and prevention of histopathology. The time point chosen for administration (30 min post-FPI) was arbitrary, and based on the need to demonstrate the proof of principle that a drug that maintains docking but not cleavage of the NMDA receptor would be efficacious as a neuroprotectant. A second caveat relates to the lack of consideration of the ability of tPA-S481A to improve measures of neural function, such as behavior. However, a study design incorporating an approach to identifying the time window of efficacy for neuroprotection and/or an index of neural outcome would make the present study unwieldy in size. Such milestone-driven investigations are best left to future researchers. A third limitation of the present study design is the quantification of ERK MAPK in CSF, but not measurement of this signaling molecule in brain tissue (particularly in the hippocampus) to correlate it with histopathology. We have recently measured ERK MAPK in the parietal cortex after FPI via immunohistochemistry, and observed that such changes were reflected in the CSF, where it was quantified by ELISA (Armstead et al., 2010), which in turn were qualitatively correlated with the degree of histopathology via hematoxylin and eosin staining (Armstead et al., 2009). However, we did not directly determine this relationship quantitatively for the hippocampus in the present study. Thus, the ERK MAPK pathway may be correlational and not causal in relating CBF to hippocampal tissue injury. Finally, while some studies have suggested that ERK promotes damage, others have indicated that ERK MAPK may in fact be neuroprotective, particularly in ischemic preconditioning. The reasons for this duality of function are uncertain, and will be investigated in future studies.

Notably, the present data also identify another potential mechanistic target (MAPK) for the prevention of tPA- and NMDA-receptor-mediated loss of autoregulation. This observation may lead to a multipronged interventional paradigm aimed at mitigating the toxicity of this pathway in order to prevent impairment of cerebral autoregulation, improve cerebrovascular hemodynamics, preserve the neurovascular unit, and improve outcome after TBI (Fig. 7). By suggesting that blood flow is key, the present data argue against the prevailing dogma that NMDA-receptor toxicity is directly mediated through calcium-dependent neuronal apoptosis and necrosis. We propose that, in the context of the neurovascular unit, the vicious cycle created by tPA and NMDA and its effect on CBF is key in determining outcome after brain injury. Breaking this vicious cycle with tPA-S481A is proposed to prevent reductions in pial artery diameter, impairment of autoregulation, and neuronal cell necrosis after TBI. These studies hold the potential to re-engineer the standard of care for TBI.

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Author Disclosure Statement

No competing financial interests exist.

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