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FULL-LENGTH ORIGINAL RESEARCH

Tissue plasminogen activator does not alter development of acquired epilepsy

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Summary

Purpose: Tissue plasminogen activator (t-PA), a proven therapy for acute ischemic stroke, is an endogenous serine protease associated with neuronal activity and synaptic plasticity in the brain. Its expression is enhanced after seizures, and is involved in seizure propagation throughout the brain. Therefore, the increased use of t-PA to treat stroke may have important implications for the development of poststroke epilepsy. Using experimental and clinical approaches, we investigated the role of t-PA in the development of epilepsy.

Methods: Mice deficient in t-PA (t-PA$^{-/-}$) or mice transgenically modified to overexpress neuronal t-PA (T4) underwent amygdala kindling, and seizure threshold and rates of kindling were compared to those in wild-type mice. For the clinical study, we recruited acute ischemic stroke patients who either received intravenous t-PA treatment on admission to hospital (n = 177; cases) or did not (n = 158; controls). We then assessed the incidence of early and late onset seizures and epilepsy in these patients.

Key Findings: T4 mice were more seizure-prone than wild-type mice, exhibiting lower seizure thresholds (p = 0.002), but there were no significant differences observed in the rate of kindling development when comparing either T4 mice, or t-PA$^{-/-}$ mice, to their wild-type controls. Furthermore, we found no significant differences between the proportion of poststroke patients experiencing early or late seizures, or developing epilepsy, between those who received t-PA and those who did not.

Significance: Overexpression of endogenous t-PA lowers seizure threshold but does not influence kindling epileptogenesis. Moreover, the therapeutic administration of t-PA in humans does not influence the development of acquired poststroke epilepsy.

KEY WORDS: Tissue plasminogen activator, Epilepsy, Ischemic stroke, Electrical kindling.

An important contributor to the long-term morbidity in stroke survivors is poststroke epilepsy (Bladin et al., 2000). Stroke has been shown to be associated with a 17-fold higher risk for epilepsy than in the general population (Ryvlin et al., 2006), with the estimated incidence of poststroke seizures ranging from 3% to 11% (So et al., 1996; Cheung et al., 2003).

Tissue plasminogen activator (t-PA) is a serine protease increasingly being used as a thrombolytic therapy for acute ischemic stroke (Adeoye et al., 2011). In addition to its thrombolytic role, t-PA is increasingly being recognized for nonfibrinolytic roles in the central nervous system, both in physiologic (Baranes et al., 1998; Horwood et al., 2001; Pawlak et al., 2003; Pang et al., 2004; Barnes & Thomas, 2008) and pathologic processes (Tsirka et al., 1995; Tsirka, 1997; Wang et al., 1998; Gingrich & Traynelis, 2000; Wu et al., 2000). An accumulating body of evidence has implicated t-PA as playing a role in the development of epilepsy (i.e., epileptogenesis): t-PA expression is increased after seizures (Qian et al., 1993), whereas t-PA$^{-/-}$ mice are resistant to chemoconvulsant-induced seizures (Tsirka et al., 1995). Further studies have demonstrated that t-PA mediates kainic acid–induced seizure propagation (Yepes et al., 2002).

Seizures can occur in an otherwise healthy brain as a result of any number of acute environmental challenges, but the development of the disease state of epilepsy itself requires pathologic reorganization of neuronal circuits to occur over time. The aforementioned studies document the influence of t-PA in modulating *seizure activity*, but do not address whether t-PA plays a role in disease development. This led us to investigate whether acute or chronic alterations in t-PA

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play a role in the development of acquired epilepsy. Combining basic science and clinical investigations, we examined the following: (1) the effect of t-PA on amygdala kindling epileptogenesis in mice; and (2) whether t-PA therapy influences the incidence of poststroke epilepsy following acute ischemic stroke.

Methods

Preclinical study

Animals

We performed two electrical amygdala-kindling experiments using mice sourced from our own colonies. The first compared adult male t-PA$^{-/-}$ mice (total n = 19) to C57Bl/6 wild-type controls (n = 16), whereas the second compared transgenic heterozygous T4 mice (overexpressing t-PA selectively in neurons (Madani et al., 1999; n = 44) to their wild-type C57Bl/6 littermates (n = 22).

Surgical implantation of electrodes

Electrode implantation was performed as described previously with modifications (Salzberg et al., 2007). Briefly, mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg). Four holes were drilled into the exposed skull, facilitating the implantation of three extradural recording electrodes, and a bipolar electrode (Plastics One, Roanoke, VA, U.S.A.) into the left amygdala complex (AP: −1.5; ML: +3.5 relative to bregma; DV: −4.0 relative to the dura; Paxinos & Franklin, 2008).

Seizure threshold test

Mice were stimulated via the bipolar electrode using an Accupulser Pulse Generator/Stimulator connected to a battery-operated constant stimulus isolator (WPI, Sarasota, FL, U.S.A.). Stimulations consisted of a 1-s train of 1-msec biphasic square wave pulses at a frequency of 60 Hz. To determine the afterdischarge threshold, mice were initially stimulated with a current intensity of 60 μA. If no seizure was evoked, the current was increased by 20-μA increments until an afterdischarge of at least 6-s duration appeared on the EEG trace (Compumedics, Melbourne, Australia).

Amygdala kindling

Mice underwent either conventional amygdala kindling, consisting of bi-daily electrical stimulations with at least 4 h separating the stimulations, or sham kindling. Behavioral changes during kindling were classified according to the Racine (1972) scale, and seizure durations measured by offline analysis. Mice were kindled until they experienced five class V seizures. To assess the influence of seizures on endogenous t-PA activity (below), 1 week following completion of kindling, all mice received a final stimulation, or sham stimulation, and the brains were processed for postmortem analysis.

Amidolytic assay for determination of t-PA activity

Four hours after the final stimulation, brains were excised and the contralateral hippocampus, amygdala, and somatosensory cortex were subdissected for determination of t-PA activity using our previously described S2251 amidolytic assay (Sashindranath et al., 2011). Briefly, 0.1 M PBS+1% Triton X-100 were added to the tissue, and the samples homogenized and centrifuged at 13,000 ***g*** for 4 min. S2251, CNBr-fibrinogen, and plasminogen were added to the supernatant. Using a fluorescence plate reader (BMG Fluostar Optima, BMG Labtech, Ortenberg, Germany), absorbance at λ-405 nm was measured and second-order polynomial equations were best-fitted to each "absorbance at λ = 405 nm versus time" curve (Niego et al., 2008) using GRAPHPAD PRISM (La Jolla, CA, U.S.A.). The second-order coefficient of each best-fit polynomial equation was taken as half the rate of plasminogen activation.

Data analysis

Seizure thresholds were compared between t-PA$^{-/-}$ and wild-type, or between t-PA, T4, and wild-type, using unpaired Student's *t*-tests. Seizure durations and kindling rates were compared using analysis of variance (ANOVA). t-PA activities were analyzed using unpaired Student's *t*-test. Data were analyzed using Statistica software (Statsoft, Tulsa, OK, U.S.A.), and statistical significance was defined as $p < 0.05$.

Clinical study

Patients

Three hundred thirty-five acute ischemic stroke patients were eligible for inclusion: 177 patients who received intravenous (IV) t-PA (cases) and 158 who did not (controls). The exclusion criteria for both groups were a history of seizures or epilepsy, poststroke seizure-free patients taking antiepileptic drugs, intraarterial thrombolysis or endovascular embolectomy treatment, and a diagnosis of intracerebral hemorrhage and transient ischemic attack on presentation. The IV t-PA cases were recruited from the electronic stroke database between January 2003 and June 2009, and were consecutive acute ischemic stroke patients who fulfilled criteria for thrombolysis. The non-IV t-PA controls were recruited from July 2008 to December 2008, and were consecutive patients admitted to the Royal Melbourne Hospital Stroke Care Unit ineligible to receive t-PA.

Clinical data collection

Patient information was obtained through telephone survey and medical record review. Information on age, gender, vascular risk factors, stroke type, and severity, morbidity, and mortality were collected for each group. Stroke type and severity were classified according to clinical presentation as partial anterior circulation infarct, total anterior circulation infarct, lacunar infarct, or posterior circulation

infarct, according to the Oxfordshire Stroke Syndrome Classification (Bamford et al., 1991). Patients underwent a standardized clinical neurologic evaluation, with morbidity graded according to the 90-day modified Rankin Scale scores (van Swieten et al., 1988).

Patients were followed for 2 years following stroke onset by telephone interview, or until death. Patients who developed seizures within 2 years poststroke had the date of the first seizure recorded, together with the type of seizures and any recurrence. Patients who experienced recurrent seizures (two or more seizures) were considered to have epilepsy. A cut-off point of 2 weeks after stroke onset distinguished between early- and late-onset seizures. Patients who died before 2 years following their stroke were identified, and their date of death was recorded. The questionnaire used to generate this information is included as Data S1.

Statistical analysis

Data analysis was performed using SPSS (IBM, Armonk, NY, U.S.A.) and SAS (Cary, NC, U.S.A.), with a two-tailed p-value < 0.05 considered statistically significant. Chi-square testing assessed whether intravenous t-PA is associated with increased incidence of seizures or epilepsy following acute ischemic stroke. Kaplan-Meier survival curves were constructed whereby patients who died seizure-free before 2 years poststroke, or were lost to follow up, were censored (Bland & Altman, 1998). The primary end point was defined as the occurrence of a poststroke seizure, and this was compared between the cases and controls using the log rank method. Multivariate analysis was performed using a Cox proportional hazards model. Variables in the analysis for cases and controls were determined based on results of univariate analysis. In the multivariate analysis for risk of any seizure, variables used were age, hemorrhagic transformation, and stroke severity. In the multivariate analysis for risk of recurrent seizures, the same variables were used but with the addition of late-onset seizures.

Standard protocol approvals

All animal work was approved by the University of Melbourne Animal Ethics committee, and all human experimentation was approved by the Melbourne Health Human Ethics committee. Informed consent was sought from all patients, and in cases where informed consent could not be sought, relatives or caretakers were interviewed.

Results

Preclinical study

Electrically evoked limbic seizures increase t-PA activity in discrete brain regions

t-PA–mediated plasminogen activation was not detected in t-PA$^{-/-}$ mice after seizure, or sham seizure (Fig. 1). In wild-type mice, electrically evoked seizures significantly

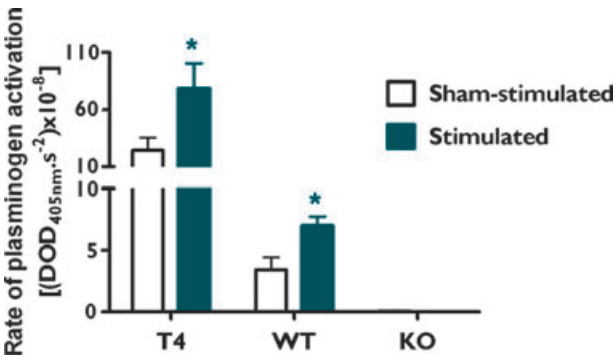

Figure 1.
Electrically evoked seizures increase t-PA activity in brain regions remote from the site of seizure initiation. Plasminogen activation was detected in the amygdala of T4, wild-type (WT), and t-PA$^{-/-}$ mice 30 min following an electrically stimulated tonic–clonic seizure (green-blue bars), or sham seizure (open bars). Sample sizes: T4 seizure = 9, sham = 7; WT seizure = 11, sham = 6; t-PA$^{-/-}$ seizure = 4, sham = 3. *p < 0.05 compared to sham-stimulated control. Data represent group mean + standard error of the mean (SEM).
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enhanced t-PA activity compared to sham-stimulated controls, with a 168% increase in t-PA activity in the amygdala (p = 0.011). Despite the higher baseline activity of t-PA in T4 mice, electrically evoked seizures further increased t-PA levels by 117%. Increases in t-PA activity were also observed in the hippocampus of both wild-type and T4 mice following a seizure (117% and 36%, p = 0.001). Smaller nonsignificant increases were also seen in the cortex of wild-type and T4 mice (74% and 47%) after seizure. These results demonstrate that t-PA activity is acutely enhanced after kindled seizures throughout the brain, validating this

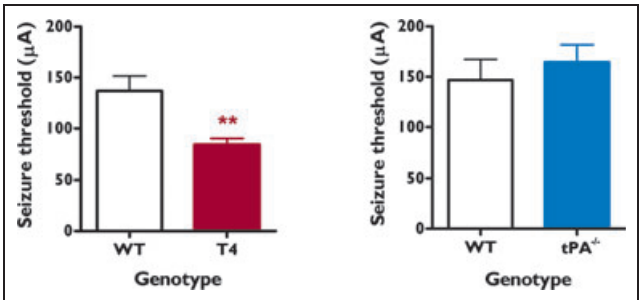

Figure 2.
Overexpression of t-PA lowers the threshold for electrically induced seizures. Threshold for electrically evoked seizures is significantly reduced in T4 mice (red bar), but is not altered in t-PA$^{-/-}$ mice (blue bar), compared to respective wild-type (WT) controls. Sample sizes: T4 = 15, WT littermates = 13; t-PA$^{-/-}$ = 18, WT control = 12. **p < 0.01. Data represent group mean + SEM.
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model as one in which epileptogenesis could be influenced by increased expression of this enzyme.

Overexpression of t-PA lowers seizure threshold

T4 mice required significantly less current to induce a seizure compared to wild-type littermates ($p < 0.01$) (Fig. 2), suggesting an inherent brain hyperexcitability in T4 mice. In contrast, the afterdischarge threshold did not differ between t-PA$^{-/-}$ mice and wild-type ($p > 0.05$) (Fig. 2), indicating that the absence of this enzyme does not influence electrically induced seizure threshold.

Tissue plasminogen activator does not influence amygdala kindling epileptogenesis

Kindling progressed in all mice as expected, evidenced by increases in seizure severity and duration induced by additional stimulations. However, no significant differences were observed in kindling rates ($p > 0.05$) (Fig. 3A) or seizure durations ($p > 0.05$) (Fig. 3B) when comparing t-PA$^{-/-}$ and wild-type mice, or when comparing these outcomes in T4 versus wild-type mice (kindling rate: $p > 0.05$; seizure duration: $p > 0.05$) (Fig. 3C,D). This implies that neither t-PA deletion or overexpression, nor seizure-induced t-PA, influences kindling epileptogenesis.

Clinical study

Patient characteristics

Of the 335 patients, 38 (11.3%) were unable to be included in the analysis because follow-up data were unavailable. This comprised 18/177 IV t-PA cases and 20/158 non-IV t-PA controls. A comparison of baseline characteristics showed that the nature of stroke differed significantly between cases and controls, with patients receiving t-PA more likely to have a total anterior circulation infarction (TACI) ($p < 0.001$) and less likely to have lacunar infarctions (LACIs) ($p = 0.047$) or posterior circulation infarctions (POCIs) ($p < 0.001$) (Table 1). Those receiving t-PA more often had a severe stroke ($p < 0.001$), anterior circulation stroke ($p < 0.001$), and a higher mean diastolic blood pressure ($p = 0.012$). Stroke outcomes also differed between the groups, with t-PA-treated patients more likely to have hemorrhagic transformation ($p = 0.006$) and a poorer modified Rankin scale (mRS) ($p = 0.004$), compared to the non–t-PA control group.

Tissue plasminogen activator administration for acute ischemic stroke does not alter the incidence of seizures

The cumulative incidence of a seizure over 2 years poststroke, whether early ($\chi^2 = 0.001$; $p = 0.973$) or late ($\chi^2 = 0.179$; $p = 0.672$), was not different between the t-PA treated and nontreated groups. Early onset seizures (i.e., within 2 weeks) occurred in 8 of 159 IV t-PA–treated patients (5.0%) and late-onset seizures occurred in 16 (10.1%). In comparison, 8 (5.8%) and 11 (8.0%) of 138 non–t-PA treated controls experienced an early- and late-onset seizure, respectively. The odds ratio of experiencing any seizure after IV t-PA compared to controls was 1.114 (95% confidence interval [95% CI] 0.581–2.134). The Kaplan-Meier survival curve also indicated no significant difference in any seizure development between t-PA–treated cases and untreated controls ($\chi^2 = 0.432$; $p = 0.511$; Fig. 4).

Administration of t-PA for acute ischemic stroke does not alter the incidence of poststroke epilepsy

The cumulative incidence of epilepsy in the 2 years poststroke showed no statistical difference between the groups ($\chi^2 = 0.103$; $p = 0.748$). Epilepsy occurred in 13 (8.2%) of

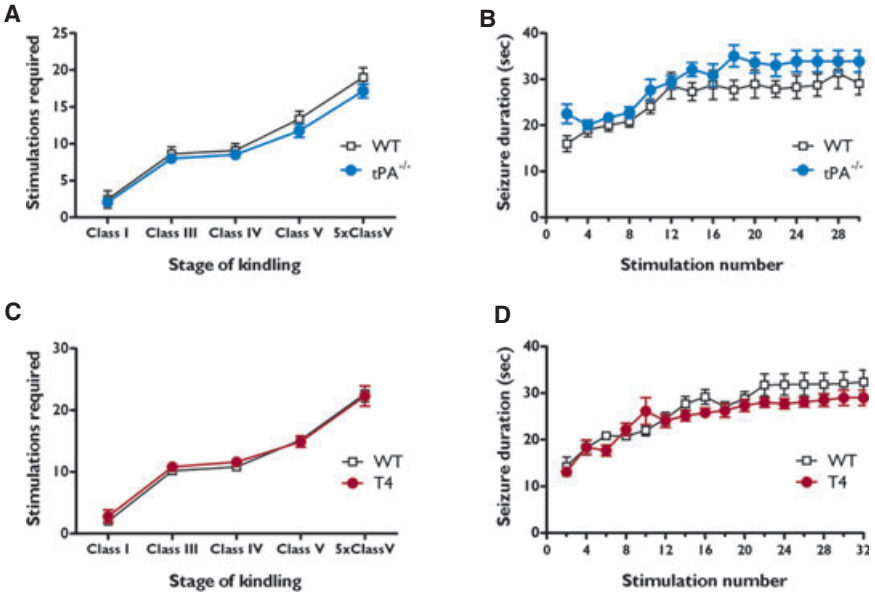

Figure 3.
Kindling epileptogenesis is not influenced by the absence or overexpression of t-PA. Kindling rates, as measured by the number of stimulations required to experience 5 lass V seizures (left panels), and the seizure durations elicited by subsequent stimulations (right panels), did not differ between t-PA$^{-/-}$ animals and wild-type (WT) controls (upper panels) or between T4 overexpressing and WT controls (lower panels). Sample sizes: T4 = 10, WT littermates = 11; t-PA$^{-/-}$ = 13, WT control = 11; data represent group mean ± SEM.

Table 1. Baseline characteristics of stroke patients treated with IV t-PA (cases) or not treated (controls)

Variable	t-PA cases (n = 159)	Non–tPA controls (n = 138)
Patient demographics		
Age (years), mean ± SD	71.51 ± 14.7	69.99 ± 15.6
Female sex, no. (%)	66 (41.51)	73 (52.90)
Pre-mRS score		
0–1, no. (%)	127 (79.87)	93 (69.40)
2–6, no. (%)	32 (20.13)	41 (30.60)
Vascular risk factors		
Hypertension, no. (%)	118 (74.21)	95 (68.84)
Smoking history, no. (%)	52 (32.70)	51 (36.96)
Diabetes, no. (%)	39 (24.53)	42 (30.43)
Hypercholesterolemia, no. (%)	77 (48.43)	51 (36.96)
Ischemic heart disease, no. (%)	61 (38.36)	41 (29.71)
Atrial fibrillation, no. (%)	41 (25.95)	34 (24.82)
Previous stroke, no. (%)	21 (13.21)	30 (21.74)
Stroke presentation		
Oxfordshire Community Stroke Project (OCSP)		
PACI, no. (%)	82 (51.57)	78 (56.52)
TACI, no. (%)	66 (41.51)##	14 (10.14)
LACI, no. (%)	5 (3.14)	12 (8.70)
POCI, no. (%)	6 (3.77)##	34 (24.64)
Stroke severity		
Moderate, no. (%)	93 (58.49)##	124 (89.86)
Severe, no. (%)	66 (41.51)	14 (10.14)
Admission glucose, mean ± SD	7.78 ± 3.51	7.67 ± 3.09
Admission systolic, mean ± SD	150.52 ± 30.24	150.61 ± 29.20
Admission diastolic, mean ± SD	83.62 ± 14.36*	79.40 ± 14.25
Outcomes		
mRS score		
0–1, no. (%)	114 (71.70)##	59 (49.58)
2–6, no. (%)	45 (28.30)	60 (50.42)
Hemorrhagic transformation, no. (%)	30 (18.87)#	10 (7.25)

PACI, partial anterior circulation infarction; TACI, total anterior circulation infarction; LACI, lacunar infarction; POCI, posterior circulation infarction; mRS, modified Rankin scale.
##p < 0.001, #p < 0.01, and *p < 0.05 indicate significantly different from control.

159 IV t-PA patients and 9 (6.5%) of 138 controls. Cox proportional hazards analysis showed that the only risk factor for epilepsy for both IV t-PA (hazards ratio [HR] 138.561; 95% CI 23.472–817.972; $\chi^2 = 19.516$; $p < 0.001$) cases and non-IV t-PA controls (HR 6.179; 95% CI 1.086–35.158; $\chi^2 = 4.214$; $p = 0.040$) was the occurrence of a late poststroke seizure (i.e., >2 weeks following the stroke).

Occurrence of poststroke seizures or epilepsy is associated with a poorer functional outcome but does not alter mortality

Patients with either a seizure or epilepsy were observed to have a poorer functional outcome (10.8% mRS 0–1; 89.2% mRS 2–6; $\chi^2 = 10.355$; $p = 0.001$) compared to seizure-free patients (37.8% mRS 0–1; 62.2% mRS 2–6), despite no significant difference in pre-mRS scores ($\chi^2 = 1.076$; $p = 0.300$). However, the presence of seizures was not associated with an increased risk of mortality: 4 (9.3%) and 12 (27.9%) of 43 seizure or epilepsy patients died within 30 days and 2 years, respectively, compared with 41 (16.1%; $\chi^2 = 0.859$; $p = 0.354$) and 81 (31.9%; $\chi^2 = 0.118$; $p = 0.732$) of 254 seizure-free patients (Table S1).

Discussion

Abnormal neuronal and axonal remodeling is thought to be a fundamental component of the pathogenesis of acquired epilepsy following a brain insult, such as postischemic stroke. These neuroplastic events could be influenced by synaptic remodelers, such as serine proteases, centrally acting molecules that can regulate neuronal plasticity (Wang et al., 2008). t-PA is upregulated after seizures (Qian et al., 1993), is involved in seizure propagation through the brain (Yepes et al., 2002), and is involved in excitotoxicity and axonal remodeling (Tsirka et al., 1995), properties that could potentially mediate a vulnerability to acquired epilepsy following a brain insult. Despite the fact that T4 mice, which overexpress t-PA in neurons, exhibit lower thresholds for electrically evoked seizures, we found no evidence that an excess or deficiency of t-PA influenced the development of epilepsy (i.e., epileptogenesis). t-PA deletion or overexpression did not influence the rate of electrical amygdala kindling in mice, a well-validated model of limbic epileptogenesis; in addition, acute treatment with t-PA following ischemic stroke did not alter the occurrence of poststroke epilepsy.

The lower seizure threshold for electrically evoked seizures observed in T4 mice is perhaps unlikely to be due to elevated circulating t-PA levels at the time of stimulation, since, compared to t-PA$^{-/-}$, wild-type mice also display a relative elevation in t-PA levels but without a difference in threshold. It is therefore possible that this effect is instead reliant on preexisting alterations in brain cytoarchitecture caused by chronic exposure to elevated t-PA. As a protease, several substrates for t-PA exist, which could potentially mediate such alterations. Plasminogen, the predominant substrate, promotes plasmin-dependent fibrinolysis, and this action underlies its clinical utility as a treatment for acute ischemic stroke (Cesarman-Maus & Hajjar, 2005). However, plasmin also mediates *neurodegeneration* under certain conditions (Skrzypiec et al., 2009), and activates the complement system (Goldberger & Colten, 1980), both of which could be related to changes in seizure threshold. In addition to plasminogen, other substrates for t-PA exist, some of which have been demonstrated to mediate effects of t-PA in the brain. For example, t-PA has been reported to interact with the NR1 subunit of NMDA receptors to potentiate NMDA receptor–mediated neurotransmission (Nicole

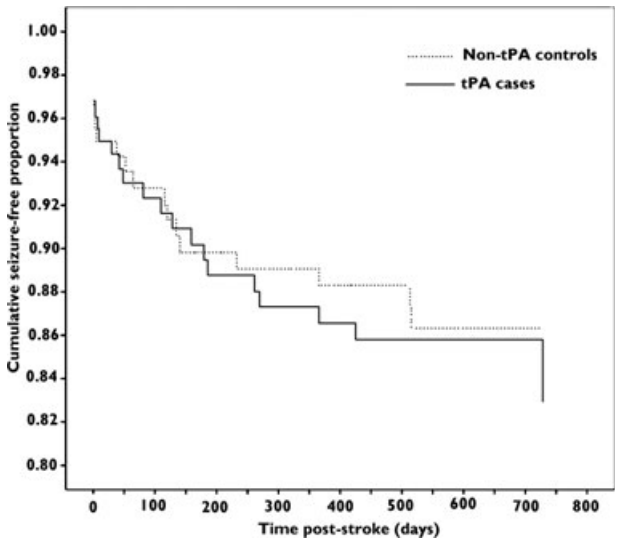

Figure 4.
Administration of t-PA for acute ischemic stroke does not alter the incidence of seizures. Kaplan-Meier survival curve describes the proportions of stroke patients either treated with IV t-PA (t-PA cases – solid line) or not (Non–t-PA controls – hashed line) which are seizure-free as time following stroke extends.
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et al., 2001), although this finding remains controversial (Samson et al., 2008; Medcalf, 2011). Alterations in NMDA receptor–mediated neurotransmission, such as might be expected in T4 mice, may lead to neuronal hyperexcitability and result in a lowered seizure threshold. Alternatively, excessive cleavage of pro-brain-derived neurotrophic factor into its mature form (Pang et al., 2004) that may be caused by t-PA overexpression would also be expected to lower seizure threshold, since this growth factor is heavily implicated in seizures (Ernfors et al., 1991) and epilepsy (Binder et al., 2001; He et al., 2004). These speculative mechanisms require further elucidation to determine how excessive t-PA results in reduced threshold for electrically evoked seizures. It is also interesting to note that t-PA–deficient mice did not demonstrate a raised threshold for seizures, even though other seizure induction paradigms utilizing chemoconvulsants indicate seizure protection in t-PA$^{-/-}$ mice (Tsirka et al., 1995). This discrepancy either indicates the existence of some biologic mechanisms that compensate for the loss of t-PA to maintain normal physiology, or that the electrical method of inducing seizures relies on parameters different from chemoconvulsant models.

The observation that altering t-PA levels does not alter susceptibility to kindling epileptogenesis was somewhat surprising, given the role of t-PA in seizure spread (Yepes et al., 2002) and its role in seizure-induced pathologic mossy fiber sprouting (Wu et al., 2000). However, the results of this study strongly indicate that seizure-induced increases in t-PA expression (Qian et al., 1993) and activity (Sashindranath et al., 2011) do not play a role in the subsequent kindling epileptogenic process. Consistent with this, mice that do not express t-PA kindle at the same rates as wild-type mice.

As far as comparisons can be made between the different paradigms and treatment regimens, the results of the clinical study were consistent with those of the animal study. It is postulated that neuroplastic changes in the brain that occur during recovery from ischemic stroke, with sprouting and growth of new axons and synaptic connections into territories that they do not normally innervate, create aberrant synaptic networks that may play a role in lowering the seizure threshold (Jefferys, 2010). Therefore, given the recognized role of t-PA in neuronal plasticity and the accumulating number of studies demonstrating the association between t-PA and seizures in experimental animals, we asked whether the use of t-PA for acute ischemic stroke would increase the incidence of poststroke seizures and epilepsy. However, we demonstrated that acute treatment with IV-t-PA does not alter the incidence of poststroke seizures and epilepsy compared with nontreated patients. Our findings agree with the results of the only other clinical study that investigated the association between t-PA and seizures. This study recruited three groups of patients (38 IV t-PA patients, 269 anticoagulant patients, and 769 antithrombotic patients) (De Reuck & Van Maele, 2010), demonstrating no association between t-PA and seizures, although the small sample size for the treatment group and the less rigorous method of follow-up used (i.e., reliance on patient readmission following a possible seizure event) limits the strength of conclusions drawn from this study.

In conclusion, our study demonstrates that overexpression of t-PA lowers limbic seizure threshold in mice, but that t-PA itself—either exogenously applied poststroke, or increased or reduced endogenous expression by genetic manipulation—does not appear to influence to the development of the disease state of epilepsy.

Acknowledgment

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Disclosure

The authors declare no conflicts of interest, financial or otherwise, associated with this work. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Questionnaire.

Table S1. Baseline characteristics of seizure and seizure-free patients.

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