A Rat Model of Studying Tissue-Type Plasminogen Activator Thrombolysis in Ischemic Stroke With Diabetes

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Background and Purpose—Poststroke hyperglycemia and diabetes mellitus are associated with lower thrombolytic efficacy and an increased risk of postischemic cerebral hemorrhage. We aimed to develop a rodent model of thrombolysis in diabetic stroke that mimics the clinical situation.

Method—Male 6-week Type I diabetic rats (14 weeks old) were subjected to embolic focal stroke and treated with tissue-type plasminogen activator at 1.5 hours. Reperfusion and 24-hour neurological outcomes were measured and compared with nondiabetic control rats.

Results—Diabetic rats exhibited resistance to thrombolytic reperfusion, larger infarction volumes, and increased intracerebral hemorrhage.

Conclusions—This animal model would be relevant to future studies investigating pathophysiological mechanisms and in developing new therapeutic approaches to enhance the efficacy of tissue-type plasminogen activator thrombolysis in stroke patients with diabetes or poststroke hyperglycemia. (Stroke. 2012;43:567-570.)

Key Words: diabetes ■ rats ■ thrombolytics ■ tPA

Establishing experimental animal models that closely reflect human diseases (“bedside-to-bench” research) has the potential to improve our understanding of pathophysiological mechanisms and develop new therapeutic approaches. Diabetes is a major stroke risk factor.1 Approximately 30% of patients with stroke are diabetic, and >50% develop poststroke hyperglycemia. Clinically, diabetes and poststroke hyperglycemia are associated with worse neurological outcomes,2 lower tissue-type plasminogen activator (tPA) reperfusion efficacy, and increased hemorrhagic transformation.3–6 However, there are no published studies of tPA thrombolysis in embolic clot models of focal stroke in diabetic animals. In this study, we aimed to investigate how focal embolic stroke in diabetic rats responds to tPA thrombolysis. By establishing a preclinical model that mimics clinical observations of tPA thrombolysis in patients with diabetes or poststroke hyperglycemia, we hope to stimulate further translational research in this field.

Materials and Methods

Induction of Type I Diabetes in Rats

All experiments were performed following an institutionally approved protocol in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Eight-week-old male Wistar rats (Charles River Laboratories, Wilmington, MA) with an initial body weight of 200 to 220 g were used to induce Type I diabetes by a standard intraperitoneal injection of streptozotocin (60 mg/kg; Sigma, St Louis, MO).7 Age-matching control male rats received an equal volume of citric acid buffer. Seven days after streptozotocin administration, rats with blood glucose concentrations of >280 mg/dL were retained as the diabetic rats.

Animal Models of Focal Embolic Cerebral Ischemia

We used nondiabetic male Wistar rats (controls) and 14-week-old (Type 1 diabetes for 6 weeks) streptozotocin-induced diabetic rats with blood glucose concentration 280 to 500 mg/dL. Focal embolic strokes were induced as described previously.8 A single 25-mm clot was used. Totally 58 rats were used in this study. Forty-eight rats were divided randomly into 4 treatment groups: nondiabetic rats+saline, n=12; nondiabetic rats+tPA, n=12; diabetic rats+saline, n=12; diabetic rats+tPA, n=12. Ten rats were used in experiments of microvascular perfusion examination and immunohistochemistry analysis in nondiabetic rats+tPA (n=5) and diabetic rats+tPA (n=5). Only animals surviving 24 hours after stroke were included in the outcome analysis; however, dead animals were counted in the overall mortality rate for all groups. All drug treatments were performed by an investigator blinded to the surgical groups.

Laser Doppler Flowmetry

Regional cerebral blood flow was monitored continuously by laser Doppler flowmetry as previously described.9 Regional cerebral blood flow was monitored for 1.5 hours after induction of ischemia and then continuously monitored for 1 hour after treatment.
Measurement of Cerebral Ischemic Infarction Volume and Hemispheric Swelling Rates

Rats were euthanized at 24 hours after ischemia. Seven coronal brain sections (2 mm thick) were stained with 2,3,5-triphenyltetrazolium chloride (Sigma) to quantify infarct volumes and hemispheric swelling using computer-assisted image analysis as previously described.10,11

Spectrophotometric Assay of Intracerebral Hemorrhage

At 24 hours after stroke, 2,3,5-triphenyltetrazolium chloride-stained brain sections were used for quantification of intracerebral hemorrhage volume with spectrophotometric hemoglobin assay as previously described.10

Analysis of Neurological Deficits

At 24 hours after ischemia, rats were assessed with a 4-point neurological deficit scale that has been extensively used for rat models of stroke.12

Measurements of Microvascular Perfusion

To examine the microvascular perfusion after tPA treatment, fluorescein isothiocyanate–dextran (2×10⁶ molecular weight, Sigma; 1 mL of 50 mg/mL) was administered intravenously to the rats at 24 hours after stroke. Two coronal sections (100 μm) from each rat at bregma −0.8 and −2.8 mm were analyzed using Image Pro software. Briefly, fluorescence was digitized with a 4× objective attached to a fluorescent microscope (Nikon). The pictures were reconstructed into a whole coronal section. The data were expressed as a percentage of area (the percentage of fluorescein isothiocyanate-perfused area/total area of fields of view).13

Immunohistochemistry

To examine the cerebral microvessels, a Biotinylated Solanum Tuberosum (potato) Lectin (20 mg/mL; Vector, Burlingame, CA) was used at a titer of 1:200 at 4°C overnight. Then the tissues were reacted with Cy5-streptavidin for 2 hours at room temperature.

Statistical Analysis

Data were expressed as mean±SEM. The regional cerebral blood flow levels, infarct volumes, and hemorrhage volumes were assessed with analysis of variance followed by Tukey-Kramer tests. A value of P<0.05 was considered statistically significant.

Results

Streptozotocin-Induced Type I Diabetes in Rats

At 6 weeks after streptozotocin injection (14 weeks old), the range of blood glucose concentration was approximately 350 to 500 mg/dL, and glycosylated hemoglobin was significantly higher (9.7%±0.7%) than nondiabetic rats (3.9%±0.2%), suggesting Type I diabetes.

tPA Thrombolytics Failed to Improve Reperfusion in the Embolic Focal Stroke Model of Diabetic Rats

A dose of 10 mg/kg tPA given at 1.5 hours significantly improved regional cerebral blood flow in nondiabetic rats measured by laser Doppler flowmetry. However, tPA failed to improve the regional cerebral blood flow recovery in diabetic rats (Figure 1A).

Embolic Focal Stroke of Diabetic Rats Developed Larger Ischemic Infarction and Reduced tPA Thrombolytic Efficacy in Infarction Reduction

At 24 hours after stroke, infarction volume of saline-treated diabetic rats was significantly larger (29% increase) than nondiabetic rats. Intravenous tPA significantly reduced 52% of brain infarction in nondiabetic rats, but only 20% reduction in diabetic rats was achieved compared with saline-treated controls (Figure 1B).

tPA Thrombolytics Did Not Reduce Hemispheric Swelling in Embolic Focal Stroke of Diabetic Rats

At 24 hours after stroke, tPA thrombolysis significantly reduced hemispheric swelling in nondiabetic rats. However, embolic focal stroke in diabetic rats treated with saline developed significantly more severe hemispheric swelling.

Figure 1. Acute neurological outcomes of tissue-type plasminogen activator (tPA) thrombolytics in diabetic rats. A, Laser Doppler flowmetry monitored regional cerebral blood flow for up to 1 hour after treatment. B, Ischemic infarct volumes were quantified at 24 hours after stroke. C, Hemispheric swelling rates were quantified on 2,3,5-triphenyltetrazolium chloride-stained brain slices. D, Intracerebral hemorrhage volumes were quantified at 24 hours after stroke. Data were expressed as mean±SEM, n=10 to 12, *P<0.05 was considered statistically significant.
compared with nondiabetic rats. Although tPA thrombolytics reduced infarction volume by 20% in diabetic rats, it only slightly decreased hemispheric swelling (reduction rate 12%, not statistically significant; Figure 1C).

**Diabetic Rats Developed More Severe Intracerebral Hemorrhage and tPA Thrombolysis Potentiated Hemorrhagic Transformation**

In nondiabetic rats, tPA thrombolysis at 1.5 hours did not increase hemorrhagic transformation at 24 hours after stroke, whereas all diabetic rats showed visible intracerebral bleeding after stroke, and the hemorrhagic volume was significantly increased by tPA thrombolysis (Figure 1D).

**tPA Thrombolytics Did Not Improve Neurological Function Deficits in Embolic Focal Stroke of Diabetic Rats**

At 24 hours after stroke, tPA thrombolytics significantly decreased neurological function deficits in nondiabetes (2.67±0.17 versus 1.44±0.29, n=10) assessed with a 4-point scale, but not in diabetic rats (2.91±0.21 versus 2.82±0.26, n=10). Additionally, diabetic rats had 16.7% (2 of 12) mortality in both saline and tPA treatments. No animal died in the nondiabetes groups within 24 hours after stroke. In addition, physiological parameters measured before ischemia, 1.5 hours after ischemia, and 2.5 hours after ischemia remained within the normal range in all groups (data not shown).

**Embolic Focal Stroke of Diabetic Rats Attenuated tPA Thrombolytics-Induced Cerebral Microvascular Perfusion**

At 24 hours after stroke, nondiabetic rats treated with tPA showed significant microvessel perfusion in the ischemic area examined by fluorescein isothiocyanate–dextran plasma perfusion imaging analysis. There was a slight decrease (16%) of the fluorescein isothiocyanate–dextran plasma perfused area in the ipsilateral hemisphere compared with the contralateral hemisphere in nondiabetic rats. However, there was a significantly larger reduction (45%) of fluorescein isothiocyanate–dextran plasma-perfused area in the ipsilateral hemisphere compared with the contralateral hemisphere of diabetic rats treated with tPA (Figure 2A–B). The red frame on Figure 2A indicates the region of high magnified images shown here with lectin staining for microvessels were clearly present in the nonfluorescein isothiocyanate-perfused area (Figure 2C). Thrombus was clearly present in the origin of the right middle cerebral artery and anterior cerebral artery of all diabetic rats treated with or without tPA and nondiabetic rats treated with saline, but not in nondiabetic rats treated with tPA, indicating incomplete clot lysis in diabetic rats (Figure 2D). Together with the earlier cerebral blood flow measurements, these data suggested that tPA thrombolytics had impaired clot lysis and reperfusion in embolic focal stroke of diabetic rats.

**Discussion**

In this study, using a common embolic focal stroke rat model, we aimed to test neurological outcomes after tPA.
thrombolysis in diabetic rats and estimated the translational relevance of this animal model for future investigations. The Type I diabetic rat model has been well established. These rats exhibit hyperglycemia, cerebrovascular inflammation, and coagulation dysfunction\textsuperscript{2,3,4} that contribute to an increased risk for stroke. Clinically, these mechanisms may be associated with a lower tPA thrombolytic efficacy and higher hemorrhagic transformation rates. We started this study with 12-week Type I diabetic rats (20 weeks old); however, the mortality rates were unacceptably high (approximately 70%, data not shown). Therefore, we used 6-week Type I diabetic rats (14 weeks old) in the present study. Our results closely mimic the clinical situation\textsuperscript{2,3,4} with worse neurological outcomes observed after tPA thrombolysis in diabetic rats. We are aware that it would be very important to see long-term outcomes and how age and gender differences might impact results of this model in future investigations. In conclusion, we believe this animal model would be of considerable relevance of this animal model for future investigations. The Type I diabetic rat model has been well established. These rats exhibit hyperglycemia, cerebrovascular inflammation, and coagulation dysfunction\textsuperscript{2,3,4} that contribute to an increased risk for stroke. Clinically, these mechanisms may be associated with a lower tPA thrombolytic efficacy and higher hemorrhagic transformation rates. We started this study with 12-week Type I diabetic rats (20 weeks old); however, the mortality rates were unacceptably high (approximately 70%, data not shown). Therefore, we used 6-week Type I diabetic rats (14 weeks old) in the present study. Our results closely mimic the clinical situation\textsuperscript{2,3,4} with worse neurological outcomes observed after tPA thrombolysis in diabetic rats. We are aware that it would be very important to see long-term outcomes and how age and gender differences might impact results of this model in future investigations. In conclusion, we believe this animal model would be of considerable relevance of this animal model for future investigations. The Type I diabetic rat model has been well established. These rats exhibit hyperglycemia, cerebrovascular inflammation, and coagulation dysfunction\textsuperscript{2,3,4} that contribute to an increased risk for stroke. Clinically, these mechanisms may be associated with a lower tPA thrombolytic efficacy and higher hemorrhagic transformation rates. We started this study with 12-week Type I diabetic rats (20 weeks old); however, the mortality rates were unacceptably high (approximately 70%, data not shown). Therefore, we used 6-week Type I diabetic rats (14 weeks old) in the present study. Our results closely mimic the clinical situation\textsuperscript{2,3,4} with worse neurological outcomes observed after tPA thrombolysis in diabetic rats.

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**Disclosures**
None.

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