Early Insulin Glycemic Control Combined With tPA Thrombolysis Reduces Acute Brain Tissue Damages in a Focal Embolic Stroke Model of Diabetic Rats

Xiang Fan, MD; MingMing Ning, MD; Eng H. Lo, PhD; Xiaoying Wang, PhD

Background and Purpose—Therapeutic effects of early insulin glycemic control for poststroke hyperglycemia in combination with tissue-type plasminogen activator (tPA) thrombolytic therapy have not yet been studied but are of great clinical interest. In this study, we tested the effects of insulin plus tPA combination in a model of focal embolic stroke in Type I diabetic rats.

Methods—Streptozotocin was used to produce Type I diabetes in male Wistar rats for 6 weeks and then embolic focal strokes were induced. All rats were treated with insulin or saline at 1 hour followed by tPA or saline at 1.5 hour after stroke. Mortality, infarction, hemispheric swelling, hemorrhagic transformation, and perfusion defects were examined at 24 hours after stroke. Total plasma plasminogen activator inhibitor-I antigen and activity levels were measured before stroke and 1.5, 3, and 6 hours after stroke by ELISA.

Results—Early insulin glycemic control alone or tPA thrombolyis alone had no significant effects on ischemic infarction. However, early insulin glycemic control combined with tPA significantly reduced brain infarction and swelling, ameliorated tPA-associated hemorrhagic transformation, and improved plasma perfusion at 24 hours after stroke. We also found that the combination significantly decreased plasma plasminogen activator inhibitor-I antigen level at 6 hours and plasminogen activator inhibitor-I activity at 1.5 and 6 hours after stroke.

Conclusions—Early insulin glycemic control may be beneficial in combination with tPA thrombolysis for ischemic stroke with diabetes mellitus or poststroke hyperglycemia. (Stroke. 2013;44:255–259.)

Key Words: focal embolic stroke ■ hyperglycemia ■ insulin glycemic control ■ rats ■ thrombolysis ■ tPA ■ Type I diabetes

Poststroke hyperglycemia is presented in all preexisting diabetes mellitus patients (about 37% of stroke patients) and 50% of nondiabetic stroke patients,1,2 and the severity of the poststroke hyperglycemia correlates with worse neurological outcomes.3,5 Furthermore, experimental and clinical investigations suggest that intravenous tissue-type plasminogen activator (tPA) in acute ischemic stroke patients with hyperglycemia may increase the risk of hemorrhagic transformation and worsen functional outcomes.4,5 Taken together, these findings provide a rationale for attempting glycemic control in hypoglycemic stroke patients in combination with tPA administration.5,6 However, hyperglycemic correction with insulin in the acute phase of stroke did not show beneficial effects.7,8 Is it possible that the lack of efficacy in these past efforts is related to the delayed institution of insulin treatments, that is, early correction of hyperglycemia is required for therapeutic benefit, in particular to tPA reperfusion therapy?7 In this preclinical study, we tested the effects of early insulin glycemic control combined with tPA thrombolysis in streptozotocin-induced Type I diabetic rats subjected to focal embolic stroke.

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 for inducing Type I diabetes by a standard intraperitoneal injection of streptozotocin (60 mg/kg; Sigma, St Louis, MO) as we previously described.9

Focal Embolic Cerebral Ischemia and Treatment Groups

After 6 weeks of diabetes mellitus, all rats were subjected to a focal embolic stroke following our previously published methods.9 This focal embolic stroke rat model was originally established by Dr Chopp’s group, its thrombolytic reperfusion time window and hemorrhagic transformation closely mimic clinical situation, which has been most commonly used for thrombolytic stroke studies.30 For rapid and sustained glycemic control, insulin (2 U per rat intravenous injection of Humulin regular insulin, combined with 4 U per rat subcutaneous injection of Humulog Mix75/25 insulin, purchased from Lilly, USA) was given at 1 hour after stroke onset. A standard...
rat dose of tPA (10 mg/kg Activase, Genentech) was intravenously injected at 1.5 hours after stroke. Blood glucose levels were measured before stroke and 1.5, 2.5, and 24 hours after stroke. Forty-four rats were blindly and randomly assigned into 4 treatment groups (n=11 per group): (1) saline at 1.5 hours, (2) tPA at 1.5 hours, (3) insulin at 1 hour plus saline at 1.5 hours, and (4) insulin at 1 hour plus tPA at 1.5 hours, after stroke. All drug treatments and outcome assessments were performed by investigators blinded to the surgical groups.

Analysis of Acute Brain Tissue Outcomes
Rats were euthanized at 24 hours after ischemia, and brain slices were stained with 2,3,5-triphenyltetrazolium chloride. Ischemic infarct volumes and hemispheric swelling were assessed using computer-assisted image analysis. Thereafter, hemorrhage volume was quantified with a spectrophotometric hemoglobin assay.9

Measurements of Cerebral Perfusion
Regional cerebral perfusion of stroke rats was monitored by laser Doppler flowmetry for 1.5 hours after induction of ischemia and then continuously monitored for 1 hour after treatment (n=11 per group). At 24 hours after stroke, intravenous infusion of fluorescein isothiocyanate–dextran was used to examine the microvascular perfusion in stroke rats treated with tPA alone or insulin plus tPA as we previously described (n=5 per group).9

Measurements of Total Plasma Plasminogen Activator Inhibitor-1 Antigen and Activity Levels
Because plasminogen activator inhibitor-1 (PAI-1) is the main and potent endogenous tPA inhibitor, its plasma concentration and activity are increased in diabetes mellitus and after stroke. Both experimental and clinical investigations were indicative of a link between PAI-1 and tPA stroke therapy outcomes.11 It has been reported that insulin may lower circulating PAI-1 concentration and activity to diabetes mellitus.12,13 Thus, in this experiment, platelet-free plasma samples were collected at before ischemia and 1.5 hours (30 minutes after insulin treatment, right before tPA treatment), 3 hours, and 6 hours after ischemia. The total plasma PAI-1 antigen and activity levels were measured by ELISA kits PRAIKT-TOT and PRAIKT (Molecular Innovation, Novi, MI) according to the manufacturer’s instructions, respectively. Fifty microliters of plasma sample were applied in each well. Data were expressed as relative fold changes of prestroke levels (n=7 per group).

Statistical Analysis
Data were expressed as mean±SD. The laser Doppler flowmetry perfusion levels, infarct volumes, hemispheric swelling, hemorrhage volumes, and plasma PAI-1 antigen and activity levels were analyzed with variance followed by Tukey-Kramer tests. Plasma perfusion ratio was assessed with independent-samples $t$ test. $P<0.05$ was considered statistically significant.

Results

tEarly Insulin Combined With tPA Reduced Acute Brain Tissue Damages
Insulin significantly lowered blood glucose levels up to 24 hours after stroke compared with non–insulin-treated animals (Figure 1A). At 24 hours after stroke, early insulin glycemic control showed a significant reduction in ischemia-induced
hemispheric swelling, but a slight decrease in brain infarction (10.7% reduction, P=0.48) (Figure 1B and 1C). tPA thrombolitics alone exhibited a trend for infarct reduction (14.2% reduction, P=0.16), but as expected, significantly elevated intracerebral hemorrhage (Figure 1B and 1D). However, insulin combined with tPA significantly reduced brain infarction and swelling and ameliorated tPA-associated intracerebral hemorrhage transformation. There were also no differences in mortality; at 24 hours, mortality was 2/11 for saline alone, 2/11 for insulin plus saline, 1/11 for tPA alone, and 2/11 for insulin plus tPA.

**Early Insulin Combined With tPA Improved Cerebral Blood Perfusion**

Focal embolic ischemia resulted in comparable reductions in cerebral perfusion in all rats. No change in perfusion was detected for up to 1 hour after thrombolysis in all 4 treatment groups (Figure 2A). At 24 hours after stroke, fluorescein isothiocyanate–dextran plasma perfusion imaging demonstrated persisting areas of perfusion defects in the expected middle cerebral artery territory (Figure 2B). However, the relative fluorescein isothiocyanate–dextran plasma perfused area (ratio of ipsilateral/contralateral hemisphere) was significantly increased in the insulin plus tPA combination group compared with the tPA alone group (26% increase) (Figure 2C).

**Early Insulin Infusion Combined With tPA Reduced Total Plasma PAI-1 Antigen and Activity Levels**

Animals were treated with tPA at 1.5 hours after stroke or insulin at 1 hour combined with a followed tPA at 1.5 hours after stroke. Platelet-free plasma samples were collected at 1.5 hours (right before tPA administration) and 3 and 6 hours after stroke. The total plasma PAI-1 antigen levels were significantly elevated, and a significant reduction was detected at 6 hours after stroke in insulin plus tPA group compared with tPA alone group (Figure 3A). The total PAI-1 activity was almost neutralized by exogenous tPA at 3 hours after stroke, but it was significantly increased at 1.5 and 6 hours after ischemia in tPA alone group. However, insulin plus tPA group significantly decreased the elevated PAI-1 activity at 1.5 and 6 hours after ischemia compared with tPA alone treatment (Figure 3B).
and neuroprotective actions. But the molecular mechanism might be at least partially explained by the significantly reduced PAI-1 activity treated with early insulin infusion combined with tPA, which indicated more free form of tPA in circulation that might result in a more potent thrombolytic vascular reperfusion. In this study, we only tested relatively severer hyperglycemia of Type I diabetic rats. We are aware that the prolonged perfusion profile, long-term neurological outcomes, and the underlying mechanisms, as well as all safety aspects of the early insulin plus tPA combination, need to be further investigated in stroke animals with different severities of hyperglycemia and different types of diabetes mellitus.

In conclusion, this study suggests that early insulin glycemic control may be beneficial in combination with tPA thrombolytic therapy to ischemic stroke with diabetes mellitus or poststroke hyperglycemia.

Sources of Funding
This work was supported in part by National Institute of Health grants R01-NS065998 and U01-NS072324 (to Xiaoying Wang).

Disclosures
None.

References

Discussion
In this study, only acute brain tissue outcomes of ischemic infarction, swelling, and intracerebral hemorrhage were examined at 24 hours after focal embolic stroke in Type I diabetic rats. One caveat is that we did not investigate long-term brain tissue and neurological functional outcomes. Because a clinical report showed that at 16 hours after stroke, only 11% (from 50% at early phase) of nondiabetic and 27% (from 100% at early phase) of diabetic patients remained hyperglycemic. Additionally, posthyperglycemia has been demonstrated in contribution of accelerating infarct formation and elevating tPA thrombolysis-associated acute intracerebral hemorrhage transformation. Thus, in this proof-of-concept study, the improved acute 24-hour brain tissue outcomes by the insulin and tPA combination, especially in the context of tPA thrombolysis, that might ultimately translate into improved long-term neurological outcomes. Although the underlying mechanisms need to be further investigated, it may be assumed by the lowering blood glucose of insulin in concert with its direct and indirect antiinflammatory, antioxidative stress, antihyperthrombotic, and neuroprotective actions.

In the context of reperfusion therapy, we were unable to detect early reperfusion after tPA, even though 24-hour perfusion images showed improvements with insulin plus tPA. In part, this may be related to our relatively brief period of laser Doppler flowmetry measurements and its restricted small cortex region, but the molecular mechanism might be at least partially explained by the significantly reduced PAI-1 activity treated with early insulin infusion combined with tPA, which indicated more free form of tPA in circulation that might result in a more potent thrombolytic vascular reperfusion.

Figure 3. Effects of early insulin glycemic control combined with tPA in plasma PAI-1 antigen and activity levels. Platelet-free plasma samples were collected at prestroke and 1.5, 3, and 6 hours after stroke. A, Changes of plasma PAI-1 antigen levels over the times up to 6 hours after stroke. B, Changes of plasma PAI-1 activity levels over the times up to 6 hours after stroke. Data were expressed as mean±SD of relative fold changes of prestroke baseline. *P<0.05 vs prestroke baseline within the same treatment group. #P<0.05 between groups at the same examining time point; n=7 per group. PAI-1 indicates plasminogen activator inhibitor-1; tPA, tissue-type plasminogen activator.
(Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. *J Clin Endocrinol Metab.* 2002;87:1419–1422.


Early Insulin Glycemic Control Combined With tPA Thrombolysis Reduces Acute Brain Tissue Damages in a Focal Embolic Stroke Model of Diabetic Rats
Xiang Fan, MingMing Ning, Eng H. Lo and Xiaoying Wang

Stroke. 2013;44:255-259; originally published online November 1, 2012;
doi: 10.1161/STROKEAHA.112.663476

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/1/255

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/