Hemorrhagic Transformation After Fibrinolysis With Tissue Plasminogen Activator

Evaluation of Role of Hypertension With Rat Thromboembolic Stroke Model

Emiri Tejima, MD; Yoichi Katayama, MD, PhD; Yasuyuki Suzuki, MD; Tsuneo Kano, MD, PhD; Eng H. Lo, PhD

Background and Purpose—We used a rat model of thromboembolic stroke to evaluate whether hypertension increases the incidence of hemorrhage after fibrinolysis with tissue plasminogen activator (tPA).

Methods—In this model, a microclot suspension was injected into the middle cerebral artery territory to induce focal ischemia. Reperfusion was induced in spontaneously hypertensive rats (SHR) by administering tPA (10 mg/kg) intravenously at 2 hours or 6 hours after the onset of thromboembolic focal ischemia. In untreated control rats, saline was administered at 2 hours after ischemia.

Results—Hemorrhagic transformation was observed only in rats that received tPA at 6 hours (6 of 8 rats [75%]). Reduction of mean arterial blood pressure from 122±3 to 99±2 mm Hg with hydralazine, given to SHR for 1 week before ischemia, significantly decreased the incidence of hemorrhage in 2 of 11 rats (18%). tPA reduced infarct volumes, but cotreatment with hydralazine did not result in further protection.

Conclusions—This study demonstrates that in this rat thromboembolic model of stroke, tPA-induced hemorrhage is dependent on blood pressure and that pharmacological reduction of hypertension during fibrinolysis can reduce the risk of hemorrhagic transformation. (Stroke. 2001;32:1336-1340.)

Key Words: cerebral hemorrhage • fibrinolysis • hypertension • stroke • rats

Fibrinolytic therapy with tissue plasminogen activator (tPA) can be efficacious in the treatment of cerebral ischemic stroke.1,2 On the other hand, in a subgroup of patients, reperfusion may lead to hemorrhage and worsening of cerebral edema in the territory of ischemia, resulting in further deterioration of clinical symptoms. Factors related to an increased incidence of hemorrhage include the severity of the ischemic insult at the time of reperfusion,3–6 advanced age of the patients,7 dosage of the administered thrombolytic agent,8 and hypertension.8 Because tPA therapy remains the only approved treatment for acute ischemic stroke, methods that may reduce the risk of fibrinolysis-associated hemorrhage would be critically important. Several attempts have previously been made to use free radical scavenging and protease inhibition to reduce tPA-associated hemorrhagic risk.9–11 In the present study, we focused on the role of hypertension and tested the hypothesis that pharmacological reduction of hypertension during tPA therapy can ameliorate the occurrence of hemorrhagic transformation.

Materials and Methods

Thromboembolic Stroke Model

Male spontaneously hypertensive rats (SHR) weighing 250 to 300 g were used. Rats were anesthetized with 1.5% halothane under spontaneous respiration. Subsequent procedures for thromboembolic stroke were performed according to the methods described by Kudo et al,12 with some added modifications.6 The donor rat was anesthetized, and the femoral artery was catheterized with a 24-gauge elastic needle. Arterial blood (0.1 mL) was obtained with use of a 1.0-mL tuberculin syringe and stored at room temperature for 48 hours to induce clot formation. Saline (0.1 mL) was added into the syringe, and then the clot was fragmented by twice passing the material through a 26-gauge needle (inner diameter 270 μm). This resulted in a 0.2-mL volume of suspension of microclots of varying sizes. The recipient rat was anesthetized with 1.5% halothane under spontaneous respiration. With use of an operative microscope, the bifurcation of the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) was catheterized with a 24-gauge elastic needle. Arterial blood (0.1 mL) was obtained with use of a 1.0-mL tuberculin syringe and stored at room temperature for 48 hours to induce clot formation. Saline (0.1 mL) was added into the syringe, and then the clot was fragmented by twice passing the material through a 26-gauge needle (inner diameter 270 μm). This resulted in a 0.2-mL volume of suspension of microclots of varying sizes. The recipient rat was anesthetized with 1.5% halothane under spontaneous respiration. With use of an operative microscope, the bifurcation of the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) was surgically exposed. Care was taken to ensure that the vagus nerve and sympathetic plexus remained intact. The occipital artery and superior thyroid artery branching from the ECA and the pterigopalatine artery branching from the ICA were coagulated with bipolar forceps and...
cut. Temporary clips were applied around the CCA and the ICA. The ECA was then coagulated at a distal point within the surgical field and cut off just proximal to the coagulation site. A 5-cm length of PE-50 polyethylene tube was connected to a 21-gauge needle, and the tip of the tube was made a little thinner by pulling. The inside of the tube and the needle were filled with saline. The PE-50 tube was inserted into the ECA through the cuff site, and the tube was fixed with the use of 5.0 silk thread around the ECA. At this time, the tip of the tube within the CCA and the ICA, the normal angle of the bifurcation was maintained by holding the tube with forceps. Finally, the suspension containing microclots was injected gently into the ICA over a period of 10 seconds. During the injection, the syringe was held upright to avoid introducing air into the ICA. The clips were again placed on the ICA and CCA, the PE-50 tube was removed, and the ECA was ligated with a silk suture. During all these procedures, the rectal temperature was maintained at $37^\circ$C with a heat lamp. Blood pressure was monitored through a catheter placed in the femoral artery, and arterial blood samples were collected for blood gas analysis.

**tPA Administration**

tPA was administered at 2 hours ($n=8$) or 6 hours ($n=8$) after the induction of focal ischemia. The animals were anesthetized again, and the femoral vein was catheterized. tPA solution (Alteplase, 10 mg/kg, 1 mg/1 mL in saline) was administered by using an infusion pump over a period of 30 minutes. In an untreated control group ($n=7$), saline (10 mL/kg) was administered at 2 hours after ischemic onset. Note that a much higher dose of tPA was used in the present study compared with that used clinically because there is an 10-fold difference in fibrin-specific enzyme activity of human recombinant tPA in human versus rodent systems.$^{13}$

**Reduction of Blood Pressure**

To assess the role of hypertension, another series of experiments was conducted with the use of hydralazine to pharmacologically reduce systemic blood pressure in SHR. Hydralazine (20 mg/mL) dissolved in 30 mL of daily drinking water was given to the rats for 1 week before the induction of ischemia.$^{13}$ SHR drank almost all the amount of water daily; thus, the animals appeared to have ingested almost all the amount of the drug daily. It is reported that long-term (10-20 weeks) administration of hydralazine decreased the infarct volume after focal cerebral ischemia in SHR, presumably by improving cerebral blood flow (CBF).$^{14}$ However, such a shrinking effect on infarct volume was not observed in SHR that received short-term ($\leq$6-week) administration of the drug. Hydralazine was administered to SHR for only 1 week before ischemia; accordingly, it appears unlikely that improvement of CBF occurred in SHR. In these hydralazine-treated rats, embolic focal ischemia was induced, and then either saline was administered at 2 hours ($n=7$), or tPA was administered at 6 hours ($n=11$).

**Laser Doppler Flowmetry**

To assess the induction of focal ischemia after the injection of microclots and the restoration of perfusion after the administration of tPA, we evaluated CBF by laser Doppler flowmetry (LDF, Omega Flow, Neuroscience Inc.). A skin incision was made, and the skull was drilled to create a small dimple on the bone (1 mm posterior to the bregma and just inferior to the temporal line), and the tip of the LDF probe was placed there. This point corresponds to the somatosensory area of the frontoparietal cortex at the level of the globus pallidus, which has been demonstrated to fall within the ischemic core of this thrombembolic occlusion model.$^6$

**Assessment of Infarction and Hemorrhage**

At 24 hours after the injection of microclots, all rats were euthanized by injecting an overdose of sodium pentobarbital (100 mg/kg IP). The brains were rapidly removed, and coronal brain slices of 2 mm in thickness were cut from the frontal pole to the edge of the cerebellum. All slices were examined under a surgical microscope to evaluate the presence of hemorrhagic transformation. Slices were then immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 30 minutes. The resultant TTC-stained sections were examined by using a standard computer-assisted image analysis system. Infarct areas and regions of hemorrhage were visually identified and outlined manually, and areas were then integrated to yield total volumes. Lesions assessed by TTC staining may sometimes evolve for the first few days after ischemia, and lesion volume evaluated at 24 hours may not reflect the final lesion volume, especially after very mild transient focal ischemia. However, in our hands, this model appears to be somewhat severe, and lesions even at 24 hours appear rather stable. The animal procedures that were used received approval by the Animal Care and Use Committee at Nihon University School of Medicine. A Fisher exact probability test or an ANOVA followed by post hoc $t$ tests with corrections for multiple groups was performed to compare the various outcomes between the untreated control rats and tPA-treated rats. Differences with a value of $P<0.05$ were considered significant.

**Results**

**Systemic Variables**

In all rats, arterial blood pH, $P_{CO_2}$, and $P_{O_2}$ values remained within normal range before and after ischemia: pH was 7.42±0.01 and 7.39±0.02, respectively; $P_{CO_2}$ was 38±2 and 40±1 mm Hg, respectively; and $P_{O_2}$ was 144±9 and 140±4 mm Hg, respectively (values are mean±SEM). Mean arterial blood pressures in these SHR were in the range of 122±3 mm Hg (before ischemia) and 119±5 mm Hg (after ischemia). In hydralazine-treated control SHR, mean arterial blood pressure was significantly ($P<0.05$) and consistently reduced to 99±2 mm Hg before ischemia and 98±2 mm Hg after ischemia. These levels are close to those measured in normotensive Sprague-Dawley rats in our previous study under similar conditions of anesthesia.$^6$ Data for blood gases were not different significantly among groups.

**LDF Measurements of CBF**

Just after the injection of microclots, successful induction of focal ischemia was achieved in all rats, as demonstrated by a reduction in CBF to levels <20% of the preischemic baselines (Figure 1). At the time of tPA or saline infusion (2 or 6 hours post-ischemia), control SHR with hydralazine (Hy) alone (normotensive control), and SHR treated with tPA at 2 hours or 6 hours after the onset of ischemia, control SHR with hydralazine (Hy) alone (normotensive control), and SHR treated with tPA at 6 hours after treatment with Hy. $^7$ $P<0.05$ vs preischemic baseline.
hours), CBF had slightly increased but remained <40% of the baseline in all cases. At 1 hour after the onset of tPA administration, CBF significantly recovered to almost the same levels of preischemia (Figure 1). In saline-infused control SHR, reperfusion was not achieved, and CBF remained <40% of baseline (Figure 1).

Hemorrhagic Transformation
Hemorrhagic transformation was not observed in any of the control SHR, which were infused with saline, or in SHR that received early treatment with tPA at 2 hours after ischemia. In contrast, hemorrhagic transformation was present in 6 of 8 (75%) SHR treated with tPA at 6 hours. Confluent hemorrhagic infarction was consistently found in the ischemic core of this model, involving the somatosensory cortex and/or lateral caudoputamen (Figure 2). Measurement of hemoglobin content in brain tissue may be a more quantitative means to evaluate the volume of cerebral hematomas. However, in the present study, the type of hemorrhagic transformation that we observed in the ischemic core was consistently hemorrhagic infarction, not petechial hemorrhage or parenchymal hematoma. Accordingly, we think that our method of assessing hemorrhagic transformation may reasonably reflect the degree of hemorrhagic severity. Hemorrhagic tissue constituted 43±10 mm³, or ≈25%, of the total infarction volume. By reducing blood pressure with hydralazine, the occurrence of hemorrhagic transformation was markedly decreased. Hemorrhage was observed in only 2 of 11 (18%) SHR treated with tPA at 6 hours (P<0.05 compared with SHR treated with tPA at 6 hours without reduction of blood pressure).

Infarction Area and Volume
In the control SHR, cerebral infarction was noted in the cerebral cortex and in the striatum, corresponding to the territory of the middle cerebral artery. Infarction areas at various bregma levels are shown in the Table. Infarction volume in the control SHR was 228±44 mm³ (mean±SEM) (Figure 3). Treatment with tPA at 2 hours or 6 hours after ischemia reduced mean infarction volumes to 163±39 and 174±25 mm³, respectively. However, these reductions did not reach statistical significance. The addition of hydralazine to reduce blood pressure did not result in any further changes in infarction volumes (Figure 3).

Discussion
Although reperfusion with tPA therapy has been found to be beneficial in acute ischemic stroke, there remains the difficult issue of the elevated risk of hemorrhagic transformation. Hypertension has been implicated as a major risk factor for cerebral hemorrhage. Therefore, it is reasonable to hypothesize that hypertension may be an important contributing factor to hemorrhagic transformation after tPA therapy. In the present study, we used an embolic stroke model involving rats in which a microclot suspension was injected into the middle cerebral artery to induce focal ischemia. In hypertensive SHR, delayed administration of tPA resulted in a high rate of hemorrhagic transformation. Our main finding was that reduction of systemic blood pressure with hydralazine significantly reduced the incidence of hemorrhagic transfor-

### Infarct Areas at Various Levels From the Frontal Pole

<table>
<thead>
<tr>
<th>Area, mm²</th>
<th>At 2 mm</th>
<th>At 4 mm</th>
<th>At 6 mm</th>
<th>At 8 mm</th>
<th>At 10 mm</th>
<th>At 12 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.9±4.1</td>
<td>23.4±5.0</td>
<td>33.3±5.3</td>
<td>25.1±3.4</td>
<td>14.4±3.0</td>
<td>7.7±2.1</td>
</tr>
<tr>
<td>tPA at 2 h</td>
<td>6.4±3.2</td>
<td>15.3±4.8</td>
<td>23.6±4.4</td>
<td>17.0±3.9</td>
<td>12.9±3.7</td>
<td>5.3±1.7</td>
</tr>
<tr>
<td>tPA at 6 h</td>
<td>5.9±1.8</td>
<td>17.2±3.2</td>
<td>26.1±3.3</td>
<td>19.8±4.3</td>
<td>11.7±3.1</td>
<td>6.4±1.2</td>
</tr>
<tr>
<td>Control with Hy</td>
<td>8.2±3.3</td>
<td>25.8±4.6</td>
<td>34.6±6.0</td>
<td>29.3±4.4</td>
<td>15.2±3.6</td>
<td>7.6±2.3</td>
</tr>
<tr>
<td>tPA at 6 h with Hy</td>
<td>6.3±3.0</td>
<td>14.9±4.5</td>
<td>24.0±4.9</td>
<td>17.5±3.7</td>
<td>13.4±2.9</td>
<td>4.0±2.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Hy indicates hydralazine.
possible that the different rates of hemorrhage are not due to effects manifested during reperfusion but rather to the differing severity of ischemic injury. Compared with SD rats, SHR have a less developed cerebral collateral circulation, so ischemic severity during occlusion may have been greater even before reperfusion.14,20

Reduction of blood pressure during reperfusion therapy may reduce the risk of hemorrhagic transformation. However, it is clearly important to recognize that excessive lowering of blood pressure may result in additional cerebral ischemia. The brain responds to an ischemic challenge with compensatory vasodilatation and recruitment of collaterals. Lowering blood pressure may significantly decrease the efficacy of these alternate supply routes. Indeed, it is interesting to note that although hydralazine reduced the incidence of hemorrhage in our model, there were no effects on final infarction volumes. Further studies are warranted to carefully assess the range of blood pressures that can ameliorate the risk of hemorrhage without having a negative impact on the levels of CBF in the ischemic brain.

Acknowledgments
This research was supported in part by grants from the National Institutes of Health (R29-NS32806, R01-NS37074, and R01-NS38731). IPA was obtained as a generous gift from Kyowa Hakko Kogyo Co, Japan. The authors thank Minoru Asahi for helpful discussion and advice.

References


Hemorrhagic Transformation After Fibrinolysis With Tissue Plasminogen Activator: Evaluation of Role of Hypertension With Rat Thromboembolic Stroke Model
Emiri Tejima, Yoichi Katayama, Yasuyuki Suzuki, Tsuneo Kano and Eng H. Lo

Stroke. 2001;32:1336-1340
doi: 10.1161/01.STR.32.6.1336

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/32/6/1336