Tissue plasminogen activator is an endogenous fibrin-specific serine protease with potent thrombolytic activity. We investigated the efficacy of tissue plasminogen activator in reducing cerebral infarct size after thromboembolic stroke in a rabbit model. Seventeen rabbits were randomized to receive either tissue plasminogen activator (2.5 mg/kg, n=6) or vehicle control (n=11). We controlled mean arterial pressure, hematocrit, and arterial blood gases before and after the intracarotid embolization of an autologous clot. Cerebral blood flow (cm³/100 g/min) (mean±SEM) was immediately reduced from 55.2±7.7 to 8.5±2.5 in the control group and from 61.8±14.8 to 10.0±3.5 in the treated group after embolization. Cerebral blood flow recovered significantly within 60 minutes of thrombolytic therapy and attained a value of 59.6±10.0 cm³/100 g/min 4 hours after embolization, whereas cerebral blood flow in control animals demonstrated only a minimal recovery to 15.3±8.9 cm³/100 g/min. Cerebral infarct size (percent of hemisphere) was reduced from 34.4±5.6% in control animals to 8.8±5.6% in treated animals (mean±SEM, p<0.01). These results suggest that tissue plasminogen activator may be efficacious in restoring cerebral blood flow and thus limiting infarct size in acute thromboembolic stroke. (Stroke 1990;21:1705-1709)

Tissue plasminogen activator reduces brain injury in a rabbit model of thromboembolic stroke

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There are 400,000 new strokes annually in North America, with thrombotic or embolic occlusion accounting for up to 80% of them. Many of the early pharmacologic efforts to restore vessel patency using fibrinolytic agents such as streptokinase and double-chain urokinase failed because of systemic and intracranial hemorrhages as well as reperfusion injury. However, an improved understanding of the pathophysiology of tissue reperfusion, the development of clot-specific fibrinolytic agents, and their successful clinical application in coronary artery thrombosis have generated renewed interest in thrombolytic therapy for stroke.

Tissue plasminogen activator (t-PA) is a serine protease that recently has been produced by recombinant DNA technology. Tissue plasminogen activator is fibrin specific, which allows thrombolysis while obviating a systemic lytic state. This fibrin specificity is felt to be responsible for the improved vascular patency rates in clinical trials after acute myocardial infarction. Its short half-life (2–8 minutes) and lack of antigenicity afford improved control regarding the timing and frequency of thrombolytic therapy when compared with other thrombolytic agents currently under investigation.

Most experimental studies to date investigating the efficacy of t-PA after embolic stroke have focused on the time required for recanalization to be achieved or the impact on the neurologic exam. There is limited data to date regarding the efficacy of t-PA in limiting cerebral infarction after embolic stroke. In the present study, we sought to determine if the administration of t-PA after embolic stroke in rabbits would result in salvage of ischemic brain, as measured by infarct size, and to correlate these results with changes in regional cerebral blood flow (CBF).

Materials and Methods

New Zealand White rabbits (2.7–3.2 kg) of either sex were anesthetized with a solution of acepromazine (20 mg, Ayerst Lab, Fort Dodge, Iowa) and ketamine (50 mg/kg, Parke-Davis Co., Morris Plains, N.J.). The right femoral artery was cannulated with PE90 tubing (BD Co., Parsippany, N.J.) for arterial blood gas sampling and blood pressure monitoring. The right femoral vein was cannulated for drug infusion. Whole blood was aspirated into PE90 tubing and incubated for 4 hours at 37°C for later use as a thromboembolus.

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A platinum–iridium electrode (Medwire Co., Mt. Vernon, N.Y.) was inserted into the left femoral artery and advanced to the aortic arch to monitor aortic hydrogen concentration, used in determining CBF by the hydrogen clearance technique.

The skull was exposed through a midline scalp incision, and a diamond burr was used to create an 8-mm-long epidural trough (2 mm wide) positioned 10 mm lateral to the midline originating at the coronal suture ipsilateral to the hemisphere to be embolized. A single 2-mm-diameter skull perforation was created similarly over the contralateral hemisphere. The dura was left intact and 30-gauge platinum–iridium electrodes were inserted 2 mm into the cortical mantle through the skull perforations for monitoring regional CBF. Three electrodes were spaced evenly in the trough, and a single electrode was placed in the hemisphere contralateral to the embolus as a control. A 4-mm burr hole was placed over the frontal lobe ipsilateral to the side to be embolized for placement of a fiberoptic epidermal intracranial pressure (ICP) monitor (Ladd Research Industries, Inc., Burlington, Vt.). All perforations of the skull then were hermetically sealed with fast-setting epoxy.

A midline neck incision then was made and the animal tracheostomized (BD Co., 3.0-mm i.d.) and mechanically ventilated (Bird Electronic Corp., Palm Springs, Calif.). The common carotid artery (CCA) and the internal (ICA) and external (ECA) carotid arteries were isolated, and the ECA was ligated. After a 30-minute equilibration period, baseline measurements of serum glucose, hematocrit, ICP, arterial blood gases, mean arterial pressure, temperature, and regional CBF were obtained and recorded. Each reading was repeated to ensure reproducibility; core temperature, mean arterial pressure, and ICP were recorded continuously. Temporary vascular clips then were applied to the proximal ICA and CCA. A microarteriotomy was made in the CCA and the artery cannulated with PE90 tubing containing a 3.5-cm-long segment of the autologous incubated clot. The clot–containing catheter was advanced to the origin of the ICA. The ICA clip was momentarily opened, the clot embolus injected into the ICA under direct microscopic visualization, and the temporary clip then re-applied. The cannula was withdrawn and the artery immediately repaired with interrupted 10-0 nylon sutures. Both vascular clips then were removed and blood flow restored to the CCA.

Mean arterial pressure was maintained at 50–60 mm Hg with homologous citrated blood. Core temperature was maintained within 1° C of the animals’ initial temperature with a heating blanket. Blood pH, Pco₂, and Po₂ were maintained within the physiologic ranges by controlling rate and depth of ventilation and/or by the use of intravenous sodium bicarbonate (Sigma Chemical Co., St. Louis). Blood gas determinations were made using a Corning 168 blood gas analyzer (Ciba-Corning Diagnostics Corp., Medfield, Mass.). Regional cerebral blood flow determinations were made within 30 minutes of embolization. A reduction in regional CBF to 15 cm³/100 g/min or less in at least one electrode in the embolized hemisphere was the established criterion for ischemic intensity. This value was chosen based on the critical reduction in CBF thought to be necessary to produce a cerebral infarct.25,36 Once it was established that the criterion had been met, t-PA therapy was initiated. Only those electrodes reflecting the critical reduction in regional CBF were used in the calculations for determining CBF throughout the remainder of the experiment. The experiment was terminated if an animal failed to reach critical reduction in CBF after embolization (n=3).

The animals were supported for 4 hours from the time of the embolic event. This allowed sufficient time for infarct delineation. Recordings of all measurements previously described under baseline readings were performed at five time points: at baseline and at 30, 90, 180, and 240 minutes after embolization.

At the end of the experiment, the animal was killed with sodium pentobarbital (150 mg/kg i.v., Anthony Products Co., Arcadia, Calif.). A calvarieotomy then was performed and the brain harvested. The brain was cut in a breadloaf fashion into 2-mm slices and incubated with 1.5% buffered triphenyltetrazolium chloride to delineate the infarct. Infarct size was calculated planimetrically from photographs and recorded as a percentage of the total hemispheric area. The use of triphenyltetrazolium chloride to distinguish between viable and infarcted tissue in various organs is well-documented.27–32 Seventeen rabbits were used in this study, randomly assigned to receive either t-PA or Tween buffer as the vehicle control. A total of 2.5 mg/kg of t-PA was administered, 20% as a bolus and the remainder over 2 hours by continuous intravenous infusion. This dose is based on extrapolation from human data in which 1.0–1.5 mg/kg is used, as well as from the observation that rabbits possess approximately 60% of the activity of clot lysis with t-PA when compared with humans.

The hydrogen clearance method was used to allow for rapid and multiple determinations of regional CBF.33 Cerebral blood flow was determined by supplying 5% hydrogen to the inspired gas mixture for 3–5 minutes until saturation was achieved. The hydrogen washout curves then were recorded on a Nicolet digital oscilloscope (model 4094, Nicolet Instrument Corp., Madison, Wis.) and the blood flows were immediately calculated by the T₁/2 method; raw data then was stored on disk. The disappearance of hydrogen from the aortic blood, as indicated by the platinum–iridium electrode positioned in the aortic arch, determined the start point for the T₁/2 measurements of the cortical hydrogen washout curves. No intracranial hemorrhages have been associated with
**Results**

Arterial blood gases (Pao2 and Paco2) were maintained in physiologic range throughout the experiment without significant intergroup or intragroup differences (127.7±5.8 and 31.7±1.7 mm Hg, respectively). The only significant difference between the control and t-PA-treated groups was the arterial pH immediately after the embolic event. At this time, a moderate acidosis (pH 7.36±0.02 relative to the control value of 7.46±0.02) was noted in the t-PA-treated group. The reason for this difference is not clear, and the acidosis was rapidly reversed with sodium bicarbonate administration.

The hematocrit (32.0±1.5%), mean arterial pressure (55.0±3 mm Hg), and ICP (7.2±0.8 mm Hg) all remained at baseline values throughout the experiment in both control and experimental groups without significant differences between groups. The lack of raised ICP is correlated with the finding of no gross intracranial hemorrhages and our previous work with a similar model in which an acute rise in ICP is seen only in those animals in which infarct size is greater than 45% of the hemisphere.

The regional CBF in both control and t-PA-treated groups was very similar before and immediately after embolization (see Figure 1); specifically, regional CBF was reduced from 61.8±14.8 to 9.9±3.2 cm³/100 g/min in the t-PA-treated group and from 55.2±7.7 to 7.9±1.8 cm³/100 g/min in the control group (n=6 and 11, respectively). Within 60 minutes of the initiation of t-PA therapy, regional CBF was significantly elevated to 48.5±14.0 cm³/100 g/min and demonstrated a further increase to 63.3±14.2 cm³/100 g/min, a value slightly higher than the baseline mean regional CBF before embolization. This increase in regional CBF was sustained throughout the remainder of the 4-hour experiment. The control group, however, sustained only very modest increases in regional CBF, attaining a maximal value of 15.3±8.9 cm³/100 g/min at the final time point.

Cerebral infarct size, expressed as a percentage of the total area of the embolized hemisphere, was significantly reduced in the t-PA-treated group when compared with the vehicle control group. Infarct size in the control group was 34.4±5.6% of the hemisphere, whereas in the t-PA-treated group, infarct size was 8.8±5.6% of the hemisphere (n=11 and 6, respectively; p=0.006).

One of the t-PA-treated animals demonstrated a small residual clot at the ICA bifurcation; however, this was not associated with an infarct, nor was it expected because there was rapid restoration of CBF after the initiation of t-PA therapy in this animal.

**Discussion**

We have demonstrated a significant reduction in cerebral infarct size and restoration of CBF after t-PA administration in a rabbit model of thromboembolic stroke. Neuronal vulnerability in stroke is felt to be related to both the intensity and the duration of the ischemia. The present study demonstrates that control of the intensity and the duration of the ischemia with a fixed reduction in CBF (<15 cm³/100 g/min) for a defined period of time will result in a reproducible yet potentially reversible infarct.

Factors thought to be related to successful outcome after thrombolytic therapy include the time interval from arterial occlusion to recanalization, the severity of the ischemia, the volume of the ischemic region, the type and dosage of the thrombolytic agent used, and the presence of any underlying risk factors such as hypertension, hyperglycemia, and hemorrhage. The repeated demonstration that vessel patency and neurologic outcome is improved with early initiation of t-PA makes timely therapy the single most important factor governing successful use of thrombolytics in reperfusing ischemic brain. The threshold for ischemic compromise of microvascular integrity and the blood–brain barrier may very well be the overriding determinant to the success of thrombolytic therapy, certainly as it relates to reperfusion injury if not actual neuronal salvage.

Our finding of rapid restoration of CBF after initiation of t-PA administration is in agreement with Papadopoulos et al. Furthermore, as demonstrated in several models of acute ischemia followed by reper-
fusion,25,42 we noted a relative hyperemia after t-PA administration. This t-PA effect may be localized in the microcirculation, the conduction system, or both. It is possible that thrombolytic therapy reduces stasis thrombosis in the capillary circulation. It is also possible that the progressive recanalization of the conduction vessels may result in the recruitment of collateral circulation. Additionally, this hyperemia may reflect a disordered autoregulation or simply a response to the metabolic debt incurred during ischemia.

The reduction in infarct size seen in our model is in agreement with the results of Chehrazi et al,35 in which the administration of t-PA immediately after the embolus resulted in near complete salvage of the ischemic area. However, it is unclear in their study as to the equivalency of the insult between groups or even within a group because many of the physiologic parameters known to affect CBF and infarct size such as mean arterial pressure, hematocrit, and blood gases were not monitored.

In summary, the administration of t-PA after thromboembolic stroke in rabbits is associated with the rapid restoration of CBF and a significant reduction in infarct size. Further studies are ongoing to determine and modify the therapeutic window for t-PA therapy.

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References


**KEY WORDS:** cerebral blood flow • plasminogen activator, tissue-type • rabbits
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