Tissue Plasminogen Activator Cerebrovascular Thrombolysis in Rabbits Is Dependent on the Rate and Route of Administration

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Background and Purpose: The main aim of our study was to assess the cerebrovascular thrombolytic efficacy of different tissue plasminogen activator treatment protocols with Doppler ultrasound.

Methods: We occluded one internal carotid artery in 48 New Zealand White rabbits with whole blood emboli. Five minutes later the rabbits were assigned to receive one of the following tissue plasminogen activator protocols: 1) intravenous square-wave infusion in a total dose of 10 mg/kg, 2) intravenous constant infusion in a total dose of 10 mg/kg, 3) intravenous square-wave infusion in a total dose of 3 mg/kg, or 4) regional intra-arterial square-wave infusion in a total dose of 3 mg/kg. Blood flow velocities in the internal carotid arteries were continuously monitored during the study with Doppler ultrasound.

Results: In all 12 animals treated with a 10-mg/kg square-wave intravenous tissue plasminogen activator infusion, internal carotid artery blood flow was reestablished within 2 hours (57.9±33.3 minutes) after the initiation of treatment, whereas this was the case for only six (50%) of the 12 animals treated with a constant 10-mg/kg intravenous tissue plasminogen activator infusion (p<0.05, Fisher’s exact test). Internal carotid artery blood flow was restored within 2 hours (33.5±40.4 minutes) in all animals treated with a regional intra-arterial tissue plasminogen activator infusion in a total dose of 3 mg/kg and in only three (50%) of the six animals treated with the same dosage intravenously (p<0.05, signed rank test).

Conclusions: The thrombolytic efficacy of tissue plasminogen activator in the rabbit cerebral vasculature was superior when the same intravenous dose was given as a square-wave rather than a constant infusion and when the drug was given as a regional intra-arterial infusion rather than intravenously. (Stroke 1992;23:388–393)

KEY WORDS • plasminogen activator, tissue-type • ultrasonics • rabbits

Thrombolytic therapy has considerable potential in the treatment of stroke because thromboembolic occlusion is the cause of stroke in the majority of patients.1 Previous clinical experience with thrombolytic treatment has, however, been limited because the previously available drugs streptokinase and urokinase were linked with an unacceptable risk of cerebral hemorrhage.2-5 Recent developments in molecular biology have created renewed interest in thrombolytic therapy in stroke because tissue plasminogen activator (t-PA) is now available in large quantities that allow pharmacological studies. This drug is of particular interest because the fibrinolytic process induced by t-PA is relatively fibrin-specific and causes only limited systemic plasminogen activation and fibrinolysis, giving hope that t-PA will mediate cerebral arterial recanalization without an unacceptable increased risk of post-ischemic cerebral hemorrhage. This has proven to be the case in experimental animal models of stroke, in which t-PA has opened vessels occluded by clots6-11 and reduced neurological damage,12,13 and in preliminary clinical trials.14,15

The aim of thrombolytic therapy is not only to restore circulation in a previously occluded vessel but also to accomplish this in sufficient time to ensure that the procedure is clinically beneficial. Successful therapy will, therefore, depend on minimizing the time required to reestablish blood flow, rather than the absolute extent of thrombolysis. The most effective rate or route of t-PA administration needed to restore blood flow in an occluded cerebral vessel is unknown, and previous experimental studies have not addressed these issues because there is no model that can provide detailed information about temporal changes in blood flow. Serial angiography has been used mainly to determine whether clot lysis has occurred,7-11 but this method cannot be repeated frequently enough to determine the exact time of circulation restoration.

The first aim of the present study was, therefore, to develop an experimental animal model in which Doppler ultrasound could continuously monitor blood flow changes in a cerebral vessel after embolic occlusion and subsequent thrombolytic treatment. Our second aim was to carry out a pharmacological study in which this model was used to compare the rate of restoration of blood flow when t-PA was given by either alternative protocols intravenously or regional intra-arterial infusion.
carried the embolus into the ICA, causing occlusion (Figure 1).

Doppler examination of the relevant ICA was carried out with a TC2-64B (Eden Medical Electronics Inc., Kent, Wash.). The apparatus was used with an 8-MHz probe at a sample depth of 1.2 cm. The probe was held in position by a clamp over the ICA approximately 1 cm after its origin from the CCA (Figure 1). Findings were displayed in real time on a monitor and recorded on a videocassette recorder. The Doppler recording began before introduction of the embolus and was continued until flow was reestablished in the ICA or for a maximum of 2 hours after initiation of treatment. Doppler examination of the ICA was repeated 18 hours after treatment.

Five minutes after embolic occlusion of the ICA, the rabbits were assigned to receive one of four t-PA treatment protocols or equal volumes of saline given intravenously or by regional intra-arterial infusion. Treatment protocols for the t-PA (provided by Burroughs Wellcome Co., Research Triangle Park, N.C.) were as follows:

1. Intravenous t-PA in a total dose of 10 mg/kg given as a square-wave infusion, with 20% given as a loading dose and the remainder over 30 minutes by constant infusion (n = 12). This infusion schedule was calculated to produce a square wave from a biological half-life of 3 minutes.12

2. Intravenous t-PA in a total dose of 10 mg/kg given as a constant infusion over 60 minutes (n = 12).

3. Intravenous t-PA in a total dose of 3 mg/kg given as a square-wave infusion, with 20% given as a loading dose and the remainder over 30 minutes by constant infusion (n = 6).

4. Regional intra-arterial t-PA in a total dose of 3 mg/kg given as a square-wave infusion, with 20% given as a loading dose and the remainder over 30 minutes by constant infusion (n = 6).

Twelve animals treated with saline intravenously (n = 6) or by regional intra-arterial infusion (n = 6) served as controls. The intravenous infusions of t-PA or saline were made through an ear vein and the regional intra-arterial infusions of t-PA or saline through the indwelling external carotid artery catheter (Figure 1).

Eighteen hours after treatment, the neurological status of each animal was rated on a three-point scale: 1, normal activity; 2, stroke (with symptoms including head tilt, circling, and hemiparesis); and 3, death. Doppler examination of the relevant ICA was made under halothane anesthesia, and the animals were then killed with an overdose of barbiturates. The brain of each animal was immediately removed and carefully examined for the presence of visible clots in the major intracranial vessels. The brains were placed in 10% phosphate buffered formalin and, after 7 days’ fixation, each brain was sliced into coronal 5.0-mm thick blocks and examined for macroscopic evidence of infarction or hemorrhage. Macroscopic evidence of infarction included tissue swelling, loss of anatomic definition, and cavitation. The size of each hemorrhagic lesion seen was estimated as punctate (barely visible to the unaided eye), small (<1.0 mm diameter, as seen on one or two block faces), or large (>1.0 mm, as seen on more than two block faces). The clinical and neuropathological

Materials and Methods

We used 48 New Zealand White rabbits weighing between 2.5 and 3.0 kg in the study. The animals were anesthetized with halothane during surgery and for the first 2 hours of the study, and their body temperatures were maintained at 37°C with a heat lamp during this time. We performed a lateral neck dissection to expose the bifurcation of the common carotid artery (CCA) and placed a temporary clip at the origin of the external carotid artery. A transverse arteriotomy was performed on the external carotid artery, and a PE-60 catheter with injection cap filled with 0.2 ml heparinized saline was introduced and advanced to the clip at the origin of the external carotid artery. The clip was then removed to allow introduction of an embolus into the CCA without disturbing flow there or in the internal carotid artery (ICA) (Figure 1).

Whole blood from a donor rabbit was allowed to clot at 37°C for 2 hours. The clot was sliced into small cubes with a razor blade, and each cube was weighed. We selected cubes weighing 2.3–2.7 mg based on our experience that clots of this size reliably occlude the proximal ICA of the rabbit. The emboli were suspended in 100 μl calcium-free Dulbecco’s phosphate-buffered saline.

To introduce an embolus into the ICA, we removed the injection cap and allowed the animal’s own blood to fill the catheter to remove the heparin solution. The catheter was clamped and the clot placed into the injection cap with forceps. The cap was replaced, the clamp was removed, and the clot was slowly advanced into the CCA by injection of normal saline under careful observation to avoid air bubbles. The blood stream then

FIGURE 1. Illustration showing Doppler probe over internal carotid artery (ICA) downstream from embolus introduced through catheter shown in external carotid artery (ECA). CCA, common carotid artery.
examinations were performed by investigators blinded to the rabbits' treatment.

Results

Emboli introduction caused occlusion of the proximal ICA with cessation of blood flow in all 48 rabbits (Figure 2). The size of the emboli did not differ significantly in the six groups: intravenous square-wave t-PA infusion (10 mg/kg), 2.52±0.10 mg (mean±SD); intravenous constant t-PA infusion (10 mg/kg), 2.53±0.10 mg; intravenous square-wave t-PA infusion (3 mg/kg), 2.60±0.04 mg; intra-arterial square-wave t-PA infusion (3 mg/kg), 2.63±0.03 mg; intravenous saline controls, 2.46±0.10 mg; and intra-arterial saline controls, 2.57±0.04 mg.

A summary of the results is shown in Table 1. In all 12 rabbits treated with a 10-mg/kg square-wave i.v. t-PA infusion, ICA blood flow was reestablished within 2 hours (57.9±33.3 minutes, mean±SD) after start of treatment (Table 1 and Figure 3), whereas this was the case for only six (50%) of the 12 animals treated with a constant 10-mg/kg i.v. t-PA infusion. This difference was statistically significant by Fisher's exact test (p<0.05).

ICA blood flow was restored within 2 hours (33.5±40.4 minutes) in all six rabbits treated with a regional intra-arterial t-PA infusion. This was observed in only three (50%) of the six animals treated with the same dosage intravenously. This difference was statistically significant by the signed rank test (p<0.05). None of the 12 control rabbits developed blood flow in the relevant ICA during the first 2 hours.

In eight (67%) of the 12 rabbits in the 10-mg/kg square-wave i.v. t-PA infusion group, ICA blood flow was reestablished after the infusion had ended, and this was also the case for four of the six rabbits (67%) in the 10-mg/kg constant i.v. infusion group. Blood flow was reestablished in the relevant ICA in five (83%) of the six animals treated with regional intra-arterial t-PA during the infusion, whereas this was the case for none of the animals in the 3-mg/kg i.v. t-PA infusion group (p<0.05).

Two rabbits in the 10-mg/kg square-wave i.v. t-PA and three in the constant 10-mg/kg i.v. t-PA infusion groups died during the first 18 hours, whereas there were no deaths in the other t-PA treatment or control groups (Table 2). Doppler examination at 18 hours revealed blood flow in the relevant ICA in all 10 surviving rabbits in the 10-mg/kg square-wave i.v. t-PA infusion group. Eight of the nine surviving rabbits in the 10-mg/kg constant i.v. infusion group had ICA blood flow at this time. Blood flow in the relevant ICA was also present at 18 hours in all six animals treated with regional intra-arterial t-PA and in four of the six animals treated with a 3-mg/kg square-wave i.v. t-PA infusion. One (17%) of the six intravenous control rabbits and two (33%) of the intra-arterial saline control rabbits had blood flow in the ICA at 18 hours.

The clinical and autopsy findings are summarized in Table 2. In 23 (64%) of the 36 t-PA-treated rabbits, all

Table 1. Reestablishment of Internal Carotid Artery Blood Flow After Treatment in 48 Rabbits

<table>
<thead>
<tr>
<th>Time after treatment start</th>
<th>Square-wave t-PA infusion, 10 mg/kg i.v. (n=12)</th>
<th>Constant t-PA infusion, 10 mg/kg i.v. (n=12)</th>
<th>Square-wave t-PA infusion, 3 mg/kg i.v. (n=6)</th>
<th>Square-wave t-PA infusion, 3 mg/kg i.a. (n=6)</th>
<th>Control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>12 (100%)</td>
<td>6 (50%)</td>
<td>3 (50%)</td>
<td>6 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>18 hours</td>
<td>10* (100%)</td>
<td>8† (89%)</td>
<td>4 (67%)</td>
<td>6 (100%)</td>
<td>1 (17%)</td>
</tr>
</tbody>
</table>

t-PA, tissue plasminogen activator; i.v., intravenous; i.a., intra-arterial; n, number of rabbits.

*2 deaths; †3 deaths.
of which had restoration of ICA circulation, we found evidence suggesting that treatment had resulted in downstream movement of the embolus from the ICA to the intracranial vessels. The evidence included symptoms consistent with stroke, visible clots in the middle cerebral artery (MCA), or MCA territory infarction. The incidence of these findings did not differ significantly among the four t-PA treatment groups, but was significantly higher in t-PA-treated rabbits as a group compared with controls (Fisher's exact test, p<0.05). An embolus in the MCA was visible in 16 (44%) of the 36 t-PA-treated rabbits, including all five who died during the first 18 hours. There were no visible emboli in the other major intracranial arteries on the ipsilateral or contralateral side. Eleven (31%) treated rabbits had macroscopic infarction in the MCA perfusion territory, and seven of these infarcted areas contained a large hemorrhage.

The clinical and autopsy findings in the t-PA-treated rabbits contrasted with those in the control groups. One (8%) intra-arterial control animal with a recanalyzed ICA at 18 hours showed a MCA embolus, MCA territory infarction, and a large intracerebral hemorrhage. In another intra-arterial control rabbit and one intravenous control rabbit had clinical signs of stroke at 18 hours, but Doppler examination revealed that the relevant ICA in each animal remained occluded. Autopsy in each of these two rabbits showed thrombosis of the entire ICA and ipsilateral circle of Willis, and infarction in the MCA perfusion territory. None of the remaining control rabbits had macroscopic evidence of hemorrhage.

Discussion

We found that the thrombolytic effect of t-PA in the rabbit cerebral vasculature was dependent on both the infusion rate used and the route of administration.

When the same dosage of t-PA was given intravenously, thrombolytic efficacy was superior when the drug was given as a 30-minute square-wave infusion rather than a 60-minute constant infusion. This was presumably due to the more rapid increase in blood levels after bolus administration, which were then maintained throughout the square-wave infusion.12

Experimental t-PA thrombolysis was also dependent on the route of administration. The thrombolytic efficacy of t-PA was improved when the same amount of drug was given as a regional intra-arterial infusion rather than intravenously. The increased efficacy of t-PA when injected directly into an occluded vessel is probably due to a higher concentration of the drug gaining access to the clot. After intravenous administration, the amount of drug reaching the clot is presumably dependent on blood flow through collateral channels. In addition, there is a lower concentration of t-PA in the circulation owing to rapid inactivation and removal from the circulation12 and a relatively large volume of distribution.

The doses of t-PA used in this study were based on our experience that relatively high doses of t-PA are needed to consistently lyse clots of a size sufficient to occlude the proximal ICA of the rabbit. Although the t-PA doses used in this experimental study are higher than those in current clinical studies, it would seem reasonable to suggest that the thrombolytic effect of t-PA in human cerebral vasculature is also dependent on the injection rate and route of administration. This may have important therapeutic consequences because the amount of cerebral injury after a thromboembolic stroke is dependent on the duration of ischemia.16-18

The most extensive clinical experience with intravenous t-PA has been in the treatment of coronary vascular thrombosis, in which a bolus is now recommended at the onset of treatment.19,20 The results of our study suggest that if t-PA is given intravenously, a square-wave infusion should be given careful consideration when planning further clinical stroke studies since this may improve thrombolytic efficacy while keeping the total dose relatively low.

Experience with the intra-arterial administration of thrombolytic agents in acute stroke is limited.21-23 This is especially true for t-PA, for which there are only a few case reports.24,25 Current clinical practice in the treatment of peripheral arterial occlusion involves using low-dose regional intra-arterial thrombolytic agents, including t-PA.26-33 It is thought that regional infusion has a higher reperfusion success rate and decreased incidence of bleeding complications elsewhere. However, there have been no prospective, direct comparative studies of regional and intravenous t-PA administration in peripheral arterial occlusion to substantiate the opinion that regional treatment is superior.

Regional intra-arterial administration of t-PA may have several advantages in acute stroke. An intra-
arterial injection of t-PA may deserve consideration if clinical studies show that intravenous administration does not reestablish blood flow quickly enough to prevent tissue damage. We anticipate that the frequency of side effects will be related to the total dose of t-PA used. If regional administration has a higher thrombolytic potency than an equivalent intravenous dose, smaller amounts may be given intra-arterially to obtain faster clot lysis with fewer side effects. There may, therefore, be situations in which treatment delays caused by arterial catheterization will be counterbalanced by improved thrombolysis of large clots with fewer side effects. It is also possible that successful treatment will involve intravenous and then intra-arterial administration of t-PA. Alternatively, intra-arterial administration may be reserved for those patients in whom intravenous administration has failed. A final decision on which method to use can only be made, however, after prospective controlled clinical trials have been carried out.

In our study, ICA blood flow was restored in the majority of treated rabbits after the t-PA infusion was completed. This is of interest because the half-life of t-PA in rabbits is approximately 3 minutes, followed by rapid elimination from the blood. However, it has been previously demonstrated in rabbits and humans that the thrombolytic effect of t-PA is sustained beyond its time of clearance from the circulation. This is thought to be due to binding of t-PA to fibrin in the clot and subsequent protection from inhibitors while thrombolysis continues.

The experimental model used in this study was designed not to induce stroke and neurological damage but to allow accurate pharmacological studies of the thrombolytic efficacy of different t-PA protocols. Because of the excellent circle of Willis, occlusion of the ICA in rabbits does not normally produce a stroke. Many animals treated with t-PA, however, developed symptoms or had autopsy findings suggesting cerebral ischemia, presumably due to migration of the ICA embolus into the intracranial vessels. The most likely mechanism is partial clot lysis with size reduction allowing clot migration into an intracranial terminal vessel such as the MCA. These observations suggest caution in the use of thrombolytic therapy in patients with minor symptoms caused by ICA occlusion.

In conclusion, we have used Doppler ultrasound in an experimental animal model to assess the thrombolytic efficacy of t-PA in the cerebral vasculature. We found that the thrombolytic effect of t-PA in the rabbit cerebral vasculature was dependent on both the injection route and the rate of administration. In restoring ICA blood flow after embolic occlusion, a square-wave intravenous infusion of t-PA was superior to a constant intravenous infusion, and a regional intra-arterial infusion of t-PA was superior to a square-wave intravenous infusion.

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