A Comparison of Intra-arterial and Intravenous Tissue-Type Plasminogen Activator on Autologous Arterial Emboli in the Cerebral Circulation of Rabbits

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Background and Purpose The efficacy of thrombolytic therapy for treatment of embolic stroke has been a subject of both experimental and clinical examination. The aim of this study was to compare the efficacy, in regard to reduction of volume of ischemic brain, of two different modes of administration (ie, intra-arterial and intravenous) of tissue-type plasminogen activator (TPA) given 30 minutes after experimental embolic stroke in rabbits.

Methods A randomized, blinded, controlled experimental trial was undertaken. Embolic stroke was simulated in rabbits by injecting fragments of autologous arterial thrombus into one internal carotid artery. Thirty minutes after embolization, the rabbits were blindly treated with 2 mg/kg intra-arterial TPA, 2 mg/kg intravenous TPA, or saline (all n=10). Six hours after embolization the rabbits were killed. The brains were perfused with triphenyltetrazolium chloride and cut into 0.5-cm-thick coronal sections, and the areas of ischemia were measured.

Results Administration of TPA resulted in a significant reduction in the volume of ischemic cerebral injury (P<.0001): control animals sustained ischemic injury to 20.1±4.6% (mean±SD) of total brain compared with 4.6±4.1% for animals treated with intra-arterial TPA and 3.4±2.6% for those treated with intravenous TPA. The difference between intra-arterial and intravenous TPA treatment was not significant (P=.786).

Conclusions In this rabbit model of embolic stroke, administration of TPA within 30 minutes resulted in a dramatic reduction in the amount of ischemic injury, with equal efficacy for the two modes of administration. These results favor the treatment of acute embolic stroke with intravenous TPA, given the rapidity with which intravenous therapy can be established in the clinical setting. (Stroke. 1994;25:651-656.)

Key Words • carotid arteries • embolism • plasminogen activator, tissue-type • thrombolytic therapy • rabbits

See Editorial Comment, page 656

cerebral infarction has been well established.6,7 Therefore, the more rapid the return of blood flow, the greater is the potential for reducing the amount of permanent ischemic damage. Although some have advocated the administration of thrombolytic agents through a selective arterial route,8-10 this technique requires special resources that usually cannot be mobilized rapidly and undoubtedly results in a significant delay between onset of ischemia and completion of treatment. For this reason, the intravenous administration of thrombolytic agents has been advocated as the optimal method for rapid treatment. Two recent clinical reports used intravenous tissue-type plasminogen activator (TPA) in a pilot study assessing treatment instituted within 90 minutes11 or between 91 and 180 minutes2 after onset of symptoms. While this pilot study confirmed that treatment could be rapidly and safely instituted with intravenous TPA, it did not attempt to determine treatment efficacy. Further patient studies will attempt to clarify some of these issues. However, an important basic consideration is whether intravenous TPA is as efficacious as intra-arterial TPA. If intra-arterial TPA has superior efficacy, then in appropriate situations it should be considered the optimal mode of therapy. This question has previously remained unresolved.
The evaluation of thrombolytic therapy has been actively pursued with a number of animal models in which the internal carotid injection is injected with fragments of thrombus, resulting in embolization to the middle cerebral artery (MCA). This basic approach has been used most frequently with rabbits\textsuperscript{11,12} and rats.\textsuperscript{18,19}

We use an autologous arterial thrombus that has matured for 24 hours, resulting in a 'white' clot composed largely of platelets and fibrin, thereby simulating the thrombus formed on an atherosclerotic plaque.\textsuperscript{11,12} The use of an arterIALIZED thrombus in the rabbit MCA embolization model allows the investigator to more closely approximate the clinical setting. Using this animal model we compared the safety and efficacy of intravenous TPA compared with intra-arterial TPA in the treatment of acute embolic stroke.

Materials and Methods

This study was conducted in accordance with all published guidelines of the US Department of Health and Human Services and the National Institutes of Health. The protocols were approved by the Animal Welfare Committee of the Barrow Neurological Institute. This facility is approved by the American Association of Accreditation of Laboratory Animal Care.

The details of our rabbit model of embolic stroke have been described.\textsuperscript{11,12} Briefly, the day before planned embolization, New Zealand White rabbits weighing 2.5 to 3.0 kg were anesthetized with an intramuscular cocktail of ketamine (10 mL, 100 mg/mL), acepromazine (2 mL, 10 mg/mL), and xylazine (1.5 mL, 100 mg/mL) in a dose of 0.6 mL/kg (KAX cocktail). The auricular arteries of the rabbit ears were cannulated with a modified spinal needle, which was used to damage a 2-cm segment of the arterial endothelium by 'scratching' the vessel lumen. After withdrawing the needle, a 4-0 silk ligature was loosely placed proximal to the injured segment of the vessel to diminish blood flow and enhance thrombus formation. The rabbits were then allowed to recover from anesthesia and returned to their cages.

Twenty-four hours after endothelial injury, the rabbits were returned to the laboratory and anesthetized as described above. The injured segments of the auricular artery were then resected. The thrombus was harvested from the artery using a dissecting microscope. The artery was opened longitudinally, and the thrombus was separated from the vessel wall. The thrombus was suspended in Dulbecco's phosphate-buffered saline and sharply divided into 0.5x0.5-mm segments. Three thrombus segments were individually aspirated into a 20-gauge angiocatheter attached to a 1-mL syringe filled with buffered saline.

The right auricular artery was cannulated proximal to the location of the arteriotomy with a 24-gauge angiocatheter to monitor blood pressure. The arterial line was connected to a continuous infusion of heparinized saline (1 IU/mL) run at 1 mL/h. An auricular vein was cannulated with a 22-gauge angiocatheter and connected to an infusion of 0.9% normal saline run at 4 mL · kg\textsuperscript{−1} · h\textsuperscript{−1}. The blood glucose, pH, PCO\textsubscript{2}, PO\textsubscript{2}, and hematocrit were monitored via the arterial line. The rabbits were then placed in the supine position, and a tracheostomy was performed. Ventilation was maintained with a volume-cycled ventilator with a tidal volume of 20 mL/kg and an FIO\textsubscript{2} of 21%. The ventilator rate was adjusted to maintain the Pco\textsubscript{2} between 35 and 45 mm Hg. The rabbits were paralyzed with 0.5 mg/kg tubocurarine followed by an infusion of 0.75 mg/h. Anesthesia was maintained with hourly intramuscular injections of the KAX cocktail (0.6 mL/kg). A rectal probe was placed for monitoring core temperature; normothermia (37.0±0.1°C) was maintained by adjusting a heating blanket.

The left common (CCA), internal (ICA), and external (ECA) carotid arteries were exposed and isolated using standard microsurgical techniques. Several modifications of the original model\textsuperscript{12} were incorporated into this experimental protocol (Figure). The ICA was exposed up to the point of entry into the skull base, and all branching vessels were temporarily excluded from the ICA with temporary vessel clips. A 22-gauge angiocatheter was inserted retrogradely into the main trunk of the ECA and secured with a 4-0 silk ligature. This was used for administration of intra-arterial drug or placebo treatment. A continuous infusion of saline run at 1 mL/h was used to maintain patency of the catheter before and after treatment. Temporary aneurysm clips were placed on the proximal CCA and the ECA (Figure). A 2-inch, 20-gauge angiocatheter was inserted into the CCA and fed into the proximal ICA. The vessel was flushed with 1 mL of heparinized saline (4 IU/mL). Direct cannulation of the ICA eliminated the possibility of retrograde passage of emboli into the systemic circulation. The three catheters containing the arterialized thrombus were set into the hub of the CCA-ICA catheter (Figure), and the three emboli were gently delivered, under direct vision, into the ICA. The CCA-ICA catheter was then removed, and the small arteriotomy in the CCA was isolated with temporary aneurysm clips and repaired with 10-0 nylon suture. All vessel clips were removed, and normal circulation through the CCA to the ICA was reestablished.

Treatment was initiated 30 minutes after embolization. Rabbits were randomly assigned to one of three treatment options: intravenous TPA (0.5 mg/kg), intra-arterial TPA (0.6 mL/kg), or placebo (1 mL of buffered saline).

Schematic drawing of embolization procedure in rabbits. Autologous arterialized thrombus is injected through a catheter that has been advanced through the common carotid artery (CCA) into the internal carotid artery (ICA). Note the temporary aneurysm clips on the proximal external carotid artery (ECA) during the embolism procedure. This is done to ensure the passage of the embolus into the ICA. After repair of the CCA arteriotomy with a 10-0 nylon suture, the clip is removed, and circulation to the ICA is restored. The catheter in the ECA was used for intra-arterial drug administration.
groups. Group 1 (control, n=10) received infusions of saline. Group 2 (intra-arterial TPA, n=10) was treated with intra-arterial TPA solution (1 mg/mL), an initial bolus of 1 mg followed by infusion of 0.5 mg · kg⁻¹ · h⁻¹ for 2 hours. Group 3 (intravenous TPA, n=10) was treated with intravenous TPA solution (1 mg/mL), an initial bolus of 1 mg followed by infusion of 1 mg · kg⁻¹ · h⁻¹ for 2 hours. The dosage of TPA used in this protocol had been effective in previous studies in our laboratory and is similar to that used in the rabbit by other authors. Laboratory personnel were blinded to the animal treatment groups. All animals received simultaneous intra-arterial and intravenous infusions of identical volume at the same rates using Harvard infusion pumps (Harvard). Intra-arterial infusions were delivered through the catheter secured in the main trunk of the left ECA (Figure); intravenous infusions were piggybacked into the auricular vein catheter using an adapter that attached directly to the angiocatheter hub. Solutions containing TPA or saline for infusion were prepared in identical vials labeled only with identification codes.

Six hours after embolization, the rabbits were killed with an overdose of intra-arterial KAX cocktail. A large midline thoracotomy was immediately performed, and the ascending aorta was cannulated with a 16-gauge angiocatheter through the left ventricle, after which the catheter was secured in position with a clamp across the left ventricle. The descending aorta was clamped, and the right atrium was opened. The animals were then perfused through the aortic catheter, over 1 to 2 minutes, with 50 mL of warmed (37°C) 2% triphenyltetrazolium chloride (TTC) solution. The animals were then left covered with core temperature maintained at 37°C to 38°C for 30 minutes, after which the brains were removed and placed in 10% phosphate-buffered formalin. One week later the brains were sliced into five coronal sections, each approximately 5 mm thick. The stained sections were examined for evidence of hemorrhage, and the areas of brain injury were measured. Transparent plastic sheets were placed over each section, and the total area of the brain slice and the area of ischemic damage (as outlined by TTC staining) were traced on the overlay. The tracings were digitized and analyzed with a computer program (PC3D, Jandel Scientific). The percentage of whole brain ischemic injury was calculated for each rabbit as (sum of ischemic areas/sum of brain slice areas)×100%

All evaluations and statistical comparisons were performed without knowledge of each animal's treatment group. Statistical significance was determined by χ² test or Fisher's exact test for attribute data. Physiological parameters and the areas of cerebral injury were assumed to be parametric variables and were compared using one-way ANOVA, with Scheffe's test for multiple comparisons between groups. Differences were considered to be significant at the level of P<.05. All statistical calculations were performed using the software program STATA, version 3.0 (Computing Resource Center).

Results

There was no significant change in temperature, blood pressure, blood glucose, or arterial blood gas parameters in the three groups during the monitoring period.

One-way ANOVA of infarct size showed significant differences (P<.0001) between the two TPA groups and the control group. However, the minor difference between the intra-arterial and the intravenous TPA groups was not significant (P=.79). The percentage of area of whole brain ischemic damage averaged 20.1±4.6% (range, 12.7% to 27.4%) in the control group, 4.6±4.1% (range, 0% to 12.1%) in the intra-arterial TPA group, and 3.4±2.6% (range, 0% to 6.9%) in the intravenous TPA group (Table). In addition, whereas all control animals experienced ischemic injury, two animals in each of the TPA treatment groups had no evidence of ischemic injury. However, this difference was not statistically significant (χ² analysis, P=.32).

There was no evidence of intracerebral hemorrhage in any rabbit. Although there was no significant difference in the hematocrit for the three groups during the experiment, a trend (not statistically significant) toward less of a decrease in hematocrit was noted in the controls (mean decrease, 1.1%) compared with the two TPA treatment groups (decrease, 3.7% to 3.8%). However, no difference in the incidence of bleeding from the wound margins was noted.

Discussion

In this randomized, blinded, controlled trial, thrombolytic therapy for acute embolic stroke was found to significantly reduce infarct size when treatment was initiated 30 minutes after embolism. Furthermore, treatment was equally efficacious whether administered by intra-arterial or intravenous routes. Both modes of administration resulted in reduction of infarct size of approximately 500% (P<.0001), without any evidence of intracerebral hemorrhage (Table). The findings in this experimental model add support to the rationale of using intravenous TPA in clinical trials for the treatment of acute stroke.

The failure to demonstrate differences between the efficacy of intra-arterial and intravenous routes of TPA administration in this model of embolic stroke is somewhat at odds with clinical experience suggesting that recanalization rates are better with intra-arterial thrombolytic therapy. There are a number of possible explanations for this apparent discrepancy. First, infarct size, and not the recanalization rate, was the primary measure of efficacy in this report. Previous work with this model suggests that salvage of ischemic cortex is associated with thrombolysis of arterial emboli, but direct evidence of lysis/recanalization was not sought in this series of experiments. Several factors favor thrombolysis in this model: the emboli are all approximately 24 hours old (the thrombus becomes increasingly resis-
tant to lysis as it ages, so that after 72 hours thrombolytic agents are much less effective), and an extensive collateral blood supply is available to help ensure delivery of the thrombolytic agent to the site of embolism.

An additional factor that must be considered in evaluating this apparent discrepancy is that much of the data supporting the increased efficacy of intra-arterial treatment over intravenous treatment involve thrombolytic agents other than TPA. There are clear differences in the efficacy of the various agents used for thrombolytic therapy, particularly when they are administered by an intravenous route. In patients receiving intravenous thrombolytic therapy for acute myocardial infarction, angiography 90 minutes after the initiation of treatment has demonstrated that effective thrombolysis is achieved less frequently with streptokinase (43% to 64%) or urokinase (53% to 66%) compared with standard-dose TPA (63% to 79%).

Recent studies have shown that accelerated intravenous dosing regimens of TPA has produced superior results, with 90-minute patency rates ranging from 82% to 91% (mean, 85%) in five studies of a combined total of more than 500 patients. Thus, for TPA the thrombolytic efficacy of intra-arterial and intravenous routes may be nearly equal.

Of course, it is possible that a small difference in infarct size existed between the intra-arterial and intravenous treatment groups in this study but was not detected (type II error). The small number of animals in each group increases the risk of this type of error; however, the large probability value of .79 and the fact that the variance of the infarct size measurements was small (and similar for both treatment groups) tend to argue against this.

The modifications that have been made in our original model of embolic stroke have resulted in a significant improvement in reliability. A key factor in this achievement was the identification and isolation of all extracranial ICA branches. Unlike the situation in the primate, in which the cervical ICA has no branches, the cervical ICA in the rabbit is a frequent origin of the occipital artery. In a review of the cervical carotid anatomy in 105 rabbits, the occipital artery originated directly from the ICA in 25.7% of cases, and the site of origin was quite distal (near the skull base) in 13.3% of these animals. Temporal occlusion of all the extracranial branches of the cervical ICA with microvascular vessel clips and direct cannulation of the ICA origin ensure that all emboli pass directly to the intracranial circulation. In our previous report the incidence of stroke in the control group was 85%, with a percentage of whole brain ischemic injury of 12.7% (mean ± SD), compared with the results in this report of a 100% incidence of stroke in control animals and a percentage of whole brain ischemic injury of 20.1 ± 4.6%. The improved reliability should be an advantage in evaluating new treatment strategies for the management of acute embolic stroke.

Anesthesia in the rabbit can be problematic; these animals poorly tolerate many of the usual anesthetic agents. We have used an intramuscular cocktail of ketamine, acepromazine, and xylazine with good success. In addition to its anesthetic properties, ketamine is a known N-methyl-d-aspartate receptor antagonist. As a class, this group of drugs has been shown to prevent delayed ischemic neuronal injury, particularly in selected regions of the hippocampus. Because the animals in this protocol are all approximately the same age and size, anesthesia is standardized and the effects of ketamine on all animals should be similar. Nevertheless, there remains a possibility that ketamine might enhance the salvage of ischemic tissue in the treatment groups by preventing the delayed ischemic injury that may be associated with successful reperfusion. The short duration of this protocol (all animals are killed 6 hours after embolization) and the extensive distribution of ischemia outside the hippocampus should minimize this effect.

The results in our rabbit model of embolic stroke demonstrate that intravenous and intra-arterial administration of TPA are equally effective when initiated within 30 minutes of the onset of ischemia. Both methods of TPA administration produced similar dramatic reductions in stroke volume. Ultimately, however, the clinician is faced with the time necessary to initiate therapy as a critical factor in the treatment of stroke. Under the best of circumstances supraselective catheterization of the cerebral vessels could be expected to add 2 hours of delay to the initiation of thrombolytic therapy for the patient presenting to the emergency department with acute stroke. The lack of general availability of expertise with these specialized techniques might further negatively influence treatment.

While the initiation of intravenous treatment with TPA within 30 minutes of embolism, as described in this laboratory study, is presently not achieved in most clinical settings, recent reports have demonstrated that with community education and careful planning it is possible to deliver intravenous TPA therapy to patients within 30 to 90 minutes of the onset of stroke symptoms. Given the rapidity with which intravenous therapy can be instituted, we believe that these experimental data support continued clinical investigation into the efficacy of intravenous TPA in the treatment of acute stroke.

References
A comparison of intra-arterial and intravenous tissue-type plasminogen activator on autologous arterial emboli in the cerebral circulation of rabbits.

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