Effect of Intra-arterial Antifibrinolytic Agents on Autologous Arterial Emboli in the Cerebral Circulation of Rabbits

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We conducted a randomized, blinded controlled trial to test the efficacy of fibrinolytic therapy with tissue plasminogen activator and urokinase in the treatment of acute embolic stroke. Embolic stroke was simulated in rabbits by injecting three 0.5×0.5 mm fragments of autologous arterial thrombus harvested from a traumatized auricular artery. Thirty minutes after embolization the rabbits were blindly treated with tissue plasminogen activator (n=21), urokinase (n=20), or 0.9% saline (n=20). At 6 hours the rabbits were sacrificed, and the cerebral vasculature was inspected for the location and number of emboli. The brain was then cut into 0.5-cm-thick coronal sections and stained with triphenyltetrazolium chloride to define areas of infarction. Treatment with either tissue plasminogen activator or urokinase significantly reduced the number of emboli present in the cerebral circulation (p<0.05). The area of ischemic injury was also significantly reduced (p<0.05) by acute fibrinolytic therapy with either tissue plasminogen activator or urokinase. However, only treatment with tissue plasminogen activator significantly reduced (p<0.05) the incidence of infarction. There was no evidence of intracerebral hemorrhage in any rabbit. Early fibrinolytic therapy improved outcome in this model of acute embolic stroke. (Stroke 1990;21:1594-1599)

Both medical and surgical modalities have been proposed as therapeutic options for the reversal and/or amelioration of deficits secondary to focal cerebral ischemia. Emergency bypass and embolectomy procedures have been performed successfully. In most instances, however, the efficacy of these surgical procedures is limited by the time necessary for evaluation and preparation of the patient and execution of the procedure.

Medical manipulations may offer a more readily available means for restoration of blood flow in patients with acute stroke. Fibrinolytic therapy has been reported to be effective in the treatment of patients with acute coronary thrombosis1-7 and has been proposed for the treatment of stroke patients on the basis that most acute strokes result from atherothrombotic or thromboembolic events. Numerous investigators have demonstrated the effectiveness of fibrinolytic therapy in clearing clotted blood from the cerebral arterial system in laboratory models of embolic stroke.8-13 However, the arterial thrombus and thrombotic emboli responsible for clinical stroke differ markedly from the simple blood clots used in these experimental models.14,15 Unlike simple “red” clot, thrombus formed in the arterial system (“white” clot) is composed largely of platelets and fibrin. Thrombus forming on an atherosclerotic plaque, at the origin of the middle cerebral artery, for example, has little resemblance to simple clotted blood.

We have developed a reliable new model of embolic stroke in rabbits using autologous arterial thrombus, which more closely parallels the clinical situation of atherothrombotic and embolic stroke in humans.14 We undertook this protocol in this model to further evaluate the safety and efficacy of fibrinolytic therapy with tissue plasminogen activator (t-PA) and urokinase in the treatment of acute embolic stroke.

Materials and Methods

Details of our rabbit model of embolic stroke have been described.14 Briefly, the day before planned embolization, New Zealand White rabbits weighing 2.5–3.0 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) (KAX cocktail) in a dose of 0.6 ml/kg. The auricular arteries of both ears were cannulated with

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FIGURE 1. Schematic drawing showing method used to create autologous arterial thrombus in rabbit's auricular artery. Inset demonstrates modification of spinal needle used to produce intimal injury. Note that stylet end has been sharpened and angled to assure good contact with intimal lining and that it extends slightly beyond tip of spinal needle. (From Reference 14. Reprinted with permission.)

a modified spinal needle. This modified needle was used to damage a 2-cm segment of the arterial endothelium by “scratching” the vessel lumen. After withdrawing the needle, a ligature was loosely placed proximal to the injured segment of the vessel to diminish blood flow and to enhance thrombus formation (Figure 1). The rabbits were allowed to recover from anesthesia and were returned to their cages.

Twenty-four hours after endothelial injury, the rabbits were returned to the laboratory. They were anesthetized as described above, and the injured segment of the auricular artery was resected. The artery was opened longitudinally under a dissecting microscope, and the thrombus was separated from the vessel wall. The thrombus was placed in Dulbecco's phosphate-buffered saline and sharply divided into 0.5×0.5 mm segments. Three segments were individually aspirated into a 1-ml syringe filled with buffered saline.

The rabbits were placed in the supine position, a tracheostomy was performed, and they were placed on a volume-cycled respirator with a tidal volume of 20 ml/kg and an Fio₂ of 21%. The inspiratory rate was adjusted to maintain Paco₂ between 35 and 45 torr. The rabbits were paralyzed with 0.5 mg/kg tubocurarine followed by an infusion of 0.75 mg/hr. 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 was maintained by adjusting a heating blanket.

The left common, internal, and external carotid arteries were exposed and isolated using standard microsurgical techniques. An 18-gauge Teflon catheter was introduced retrogradely via the external carotid artery to the origin of the internal carotid artery and secured with a 5-0 silk ligature. The common carotid artery was occluded with a Yasargil microvascular temporary aneurysm clip just proximal to the bifurcation. The arterial thrombi were immediately injected through the catheter into the internal carotid artery, and the clip on the common carotid artery was removed (Figure 2). Temporary occlusion of the common carotid artery eliminated the possibility of retrograde passage of the emboli into the systemic circulation.

Treatment was initiated 30 minutes after embolization. Rabbits were assigned randomly to one of three treatment groups: t-PA solution (1 mg/ml), initial bolus of 1 mg followed by infusion of 1 mg/kg/hr for 2 hours; urokinase solution (25,000 units/ml), initial bolus of 25,000 units followed by infusion of 25,000 units/hr.
units/kg/hr for 2 hours; or control solution of heparinized saline (1 unit/ml), initial bolus of 1 unit followed by infusion of 1 unit/kg/hr. Laboratory personnel were blinded to the animals’ treatment group. All solutions were delivered at identical rates using a Harvard infusion pump (South Natick, Mass.). The embolization catheter was kept patent before and after treatment with a continuous infusion of heparin solution (1 unit/ml) at 5 ml/hr. Connection to an arterial strain gauge allowed monitoring of arterial blood pressure via the embolization catheter.

Six hours after embolization, the rabbits were killed with an intracardiac injection of saturated KCl. The brains were immediately removed and inspected under a dissecting microscope for the presence of emboli and thrombosis. The location of emboli was recorded on schematic drawings of the rabbit cerebral arterial supply (Figure 3). The brains were then briefly cooled in ice-cold saline and cut into five coronal sections approximately 0.5 cm thick. The sections were examined for evidence of hemorrhage and then stained with a 2% solution of triphenyltetrazolium chloride (TTC) in a 37°C warm water bath for 30 minutes. The stained sections were immersed in 10% phosphate-buffered formalin and examined after 1 week for measurement of infarct size. Transparent plastic sheets were placed over each section, and the total area of the brain slice and the area of infarction (as outlined by TTC staining) were traced on the overlay. The tracings were digitized, and the percentage area of whole brain infarction was calculated for each rabbit as (sum of infarcted areas/sum of brain slice areas) × 100%.

All evaluations and statistical comparisons were performed without knowledge of the animals’ treatment group. Statistical significance was determined by χ² test or Fisher’s exact test for attribute data. Continuous data were analyzed with one-way analysis of variance (ANOVA) using the Newman-Keuls test for post-hoc comparisons.

**Results**

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

One-way ANOVA showed significant differences (p<0.05) in the number of emboli detected between the t-PA and control groups and the urokinase and control groups (Table 1). In the t-PA group emboli were found in 13 of 21 animals (62%, with an average of 0.9 embolus/rabbit. In the urokinase group emboli were found in 13 of 20 animals (65%), with an average of 1.0 embolus/rabbit. In the control group, emboli were found in 20 of 20 (100%) animals, with an average of 2.0 emboli/rabbit.

Fibrinolytic therapy also significantly affected the distribution of emboli found in the proximal and distal portions of the vascular tree (χ² analysis, p=0.008). In the proximal vasculature (internal carotid artery and M₁ segment of the middle cerebral artery) of the control group 22 emboli were found, compared with only three emboli in the t-PA group and seven emboli in the urokinase group (Table 1).

One-way ANOVA of infarct size showed significant differences (p<0.05) between the t-PA and control groups and the urokinase and control groups. The percentage area of whole brain infarction averaged 5.1% in the t-PA group, 6.1% in the urokinase group, and 12.9% in the control group.

Whereas treatment with either t-PA or urokinase significantly reduced the average size of the infarcts, only t-PA significantly reduced the incidence of infarction (χ² analysis, p=0.013). In the t-PA group nine of 21 rabbits (43%) had infarction, compared
TABLE 1. Results Comparing Fibrinolytic Therapy With t-PA and Urokinase to Controls in Treatment of Acute Embolic Stroke in Rabbit Model

<table>
<thead>
<tr>
<th></th>
<th>t-PA (n=21)</th>
<th>Urokinase (n=20)</th>
<th>Control (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With emboli (No.)</td>
<td>13</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Embolization incidence (%)</td>
<td>62</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>Emboli per rabbit (mean)</td>
<td>0.9</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Proximal emboli (ICA-M₃)</td>
<td>3</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Distal emboli (M₄-M₆)</td>
<td>16</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>With infarction (No.)</td>
<td>9</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Infarction incidence (%)</td>
<td>43</td>
<td>75</td>
<td>85</td>
</tr>
</tbody>
</table>
% area of whole brain infarction
| Affected rabbits Mean±SD | 11.9±10.9 | 8.1±7.4         | 15.1±12.4     |
| Range                 | 2.0–33.2   | 0.4–25.0        | 1.0–34.9      |
| Entire group Mean±SD  | 5.1±9.1    | 6.1±7.29        | 12.9±12.7     |
| Range                 | 0–33.2     | 0–25.0          | 0–34.9        |
| Bleeding from surgical wound margins incidence (%) | 52         | 5                | 0             |
| Hemorrhagic infarction incidence (%) | 0          | 0                | 0             |

FIGURE 3. Schematic drawing of rabbit's intracranial circulation illustrating division of middle cerebral artery (MCA) into six segments for localization of emboli. Results of post-mortem inspections were recorded on these forms for each animal. PCom A, posterior communicating artery; ICA, internal carotid artery.

with 15 of 20 (75%) in the urokinase group and 17 of 20 (85%) in the control group.

There was no evidence of intracerebral hemorrhagic complications in any rabbit (Table 1). Systemic bleeding occurred more frequently with t-PA. In the t-PA group 11 rabbits (52%) exhibited minor bleeding from the surgical wound margins (easily controlled with bipolar cautery), compared with one rabbit (5%) in the urokinase group and no rabbits in the control group. There was no correlation in the t-PA group among bleeding from the surgical wound margins, size of infarction, or number of emboli found at postmortem examination (Fisher’s exact test).

Discussion

In this randomized, blinded, controlled trial, fibrinolytic therapy with both t-PA and urokinase was found to be safe and effective in the treatment of acute embolic stroke. Our study differs from earlier laboratory reports in that autologous arterial thrombus was used as the embolic material rather than simple clotted blood.14

The dosages of t-PA and urokinase used in this study are similar to those reported in previous laboratory and clinical protocols.8-12,17-20 Intra-arterial administration was chosen to maximize the fibrinolytic effect of urokinase,17,19,21-24 and the t-PA and control solutions were administered by the same route to maintain the blinded protocol.

Based on our results (Table 1), t-PA and urokinase appear equally effective in dissolving clots and reducing the number of emboli by approximately 50%. In both the t-PA and urokinase groups, emboli were not only less frequent but were identified most commonly in the distal branches of the middle cerebral artery. This distribution of emboli is consistent with fragmentation and distal migration prior to complete lysis.

More importantly, fibrinolytic therapy with both agents significantly reduced the size of infarction (Table 1). Although both t-PA and urokinase reduced the average size of the infarct, only t-PA significantly reduced the incidence of infarction. In the t-PA group 43% of the rabbits had pathologic evidence of a cerebral infarct, compared with 75% in the urokinase group and 85% in the control group. While infarcts occurred more frequently in the urokinase group than in the t-PA group, their average size tended to be smaller in the urokinase group. When
analysis of infarct size was confined to the subgroup of rabbits with evidence of infarction, the average percentage area of whole brain infarction was 11.9% for the t-PA group, 8.1% for the urokinase group, and 15.1% for the control group. Reviewing the data for additional clues to explain these results, we found that five rabbits in the urokinase group (25%) had small cortical infarcts with no evidence of residual emboli, while only one rabbit in the t-PA group had this combination of findings.

Focal cerebral ischemia is a dynamic process with outcome dependent on both the depth and duration of ischemia. Thus, while it cleared emboli from the cerebral circulation, urokinase appears to have been less efficient than t-PA in clearing emboli before the onset of ischemic injury. The higher incidence of bleeding from surgical wound margins in the t-PA group also suggests that t-PA had greater fibrinolytic activity than urokinase. It is possible that the use of a higher dosage of urokinase would have effected more rapid clearing of emboli.

The risk of intracerebral hemorrhage with fibrinolytic therapy is a major concern in patients with acute stroke. Our results suggest that the risk is small, at least with acute therapy. Other authors have reported similar laboratory results when therapy is initiated ≤2 hours after embolism. One group has reported no significant increase in the risk of hemorrhage in a rabbit model of embolic stroke when fibrinolytic therapy was delayed as long as 8–24 hours after embolism. Additional studies are needed to confirm the safety of fibrinolytic therapy at these delayed intervals.

Results in our model demonstrate that acute fibrinolytic therapy can improve outcome after embolic stroke. Intra-arterial treatment with t-PA and urokinase significantly decreased the number of emboli and the average size of infarction. However, only t-PA significantly reduced the incidence of infarction.

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References


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