Incidence of Cerebral Hemorrhage After Antifibrinolytic Treatment for Embolic Stroke in Rabbits

P.D. Lyden, MD, K.P. Madden, MD, PhD, W.M. Clark, MD, MA, K.C. Sasse, BS, and J.A. Zivin, MD, PhD

We studied thrombolysis in an animal model of embolic stroke to determine the safety of tissue plasminogen activator and streptokinase. We occluded the middle cerebral arteries of 137 rabbits with radiolabeled blood clots and administered tissue plasminogen activator (n=49), streptokinase (n=40), or saline (n=48) at various times after embolization. We assessed the rate of thrombolysis and cerebral hemorrhage 24 hours later. Both drugs were very effective in producing thrombolysis. Compared with saline, streptokinase caused a significant increase in the rate of cerebral hemorrhage (p<0.05), but tissue plasminogen activator did not. We conclude that thrombolytic therapy for acute stroke should be safer with tissue plasminogen activator than with streptokinase. (Stroke 1990;21:1589-1593)

In previous reports, we showed that thrombolysis with tissue plasminogen activator (t-PA) reduced neurologic damage after experimental embolic stroke.1-3 At the doses used, t-PA did not increase the size or incidence of postischemic cerebral hemorrhage when given 5 minutes or 4, 8, or 24 hours after stroke. Despite these encouraging experimental findings, the clinical use of thrombolytic agents has been linked to an increased risk of brain hemorrhage.4-9 For example, streptokinase (SK) and urokinase used in clinical trials for stroke treatment have been associated with brain hemorrhage.4-8 Furthermore, during recent trials of t-PA thrombolysis for acute myocardial infarction, some investigators have expressed concern that there may be an increased risk of brain hemorrhage in patients treated with higher doses of t-PA.8,9 Thus, it is vital to understand the risk of brain hemorrhage associated with systemic thrombolytic treatment. We adapted the animal model that we had used previously by using radiolabeled emboli instead of angiography to document thrombolysis. This modification allowed us to avoid the complications associated with serial angiography in animals.10-12

Materials and Methods

We used 186 male New Zealand White rabbits weighing 2.5–3.0 kg. The surgical method for implanting a catheter in the extracranial carotid artery is described in detail elsewhere.2,13 The catheter was filled with heparinized saline and capped with a rubber injection port. We prepared the emboli by mixing 3.0 ml whole blood from a donor rabbit with trace quantities of iodine-125-labeled 15-μm-diameter plastic microspheres. After 24 hours' incubation at 37° C, we sliced the clot into small cubes weighing 3.7–4.2 mg, each containing approximately 250 microspheres. Each cube was suspended in 100 μl Dulbecco's phosphate-buffered saline containing 1.0 g/l bovine serum albumin and stored at room temperature until use 2–3 hours later. The radioactivity in each cube was measured by placing the test tube holding the suspended cube in a gamma counter. Immediately before embolization, we removed the heparinized saline in the catheter by mixing 3.0 ml whole blood from a donor rabbit with a rubber injection port. We prepared the emboli by mixing 3.0 ml whole blood from a donor rabbit with trace quantities of iodine-125-labeled 15-μm-diameter plastic microspheres. After 24 hours' incubation at 37° C, we sliced the clot into small cubes weighing 3.7–4.2 mg, each containing approximately 250 microspheres. Each cube was suspended in 100 μl Dulbecco's phosphate-buffered saline containing 1.0 g/l bovine serum albumin and stored at room temperature until use 2–3 hours later. The radioactivity in each cube was measured by placing the test tube holding the suspended cube in a gamma counter. Immediately before embolization, we removed the heparinized saline in the catheter by using a syringe and 22-gauge needle inserted through the injection port. Then the catheter was clamped, and the port was removed. Using microforceps, we took a cube from its test tube and placed it in the open end of the catheter. The injection port was replaced, and the clamp was removed. A syringe and 22-gauge needle was inserted through the port, and 2.5 ml saline was injected to advance the embolus to the middle cerebral artery (MCA). The entire procedure required <60 seconds, and no blood clotting occurred in the...
catheter during clamping. Using serial angiography, we have previously shown that emboli of this size reliably lodge in the MCA.\(^3\)

Each rabbit was examined immediately after embolization and again shortly before treatment. All had clinical signs of stroke such as hemiparesis, circling, or seizure. Approximately half of the rabbits were randomly assigned to receive saline or 3, 5, or 10 mg/kg t-PA. A gift from Burroughs Wellcome Company (Research Triangle Park, N.C.), the t-PA had a specific activity of 300,000 IU/mg in a clot lysis assay. The drug was dissolved in saline, and 1 ml/kg was administered intravenously beginning 90 minutes after embolization. A bolus containing 20% of the dose was followed by a constant infusion of the remaining 80% of the dose over 30 minutes. The other rabbits were randomly assigned to receive saline or 30,000 units/kg SK (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.) 5, 90, or 300 minutes after embolization. Each group received 1 ml/kg solution intravenously over 30-60 minutes using an initial bolus containing 20% of the dose.

All rabbits were sacrificed with CO\(_2\) 24 hours after treatment. The brains were removed from each animal and examined carefully for the presence of a visible clot in the superficial cerebral vessels (MCA and anterior cerebral artery [ACA]). After 7 days' fixation in 10% phosphate-buffered formalin, each brain was sliced into coronal 5.0-mm-thick blocks. The faces of each block were examined for grossly apparent hemorrhage. We estimated the size of the hemorrhage by counting the number of blocks that contained visible hemorrhage. A larger hemorrhage will be visible in more blocks. In each treatment group, we calculated the percentage of animals with hemorrhage in none, one, two, or three blocks.

To measure thrombolysis, we stripped all epicerebral arteries (MCA and ACA) from the brain using microforceps and placed them in a gamma counter to measure radioactivity. If a visible clot was present, it was included with the vessels. Likewise, we measured the radioactivity in each block from every brain. The total recovered radioactivity was defined as the sum of the radioactivity in the vessels, clot, and brain blocks. We rejected any rabbit in which the total recovered radioactivity was <50% of that originally injected, reasoning that in such cases most of the embolus never reached the brain. We defined thrombolysis as the localization in the brain blocks of >80% of the total recovered radioactivity (i.e., <20% of the radioactivity remained in the vessels).

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We chose this definition based on the observation that if a drug successfully lysed the embolus (no clot visible in the epicerebral arteries), we never found more than approximately 20% of the total recovered radioactivity in the vessel.

Results in each drug-treated group were compared with results in the combined saline-treated control groups, after checking that there were no differences in the rates of hemorrhage among the control groups. Statistical significance was determined with the \(x^2\) test using the Bonferroni correction for multiple comparisons\(^4,15\) or analysis of variance as appropriate.

Results

We rejected 49 rabbits because of low total recovered radioactivity. These animals were distributed equally among all the groups. In these rejected rabbits we found all of the residual radioactivity in the extracranial internal carotid artery, suggesting that the embolus had lodged in the extracerebral circulation without ever reaching the brain. No radioactivity was found in the lungs of four rabbits examined. In some of these rejected rabbits we also found a visible clot in an epicerebral vessel such as the MCA. These spurious clots contained no microspheres, indicating that we had inadvertently injected an unlabeled embolus that had formed on the catheter tip.

In the remaining 137 rabbits we found excellent agreement between the direct visual inspection for clot and quantitative measurements of thrombolysis. In the 66 brains with no clot visible in any epicerebral vessel, the mean ±SD radioactivity in these vessels was 11±14% of the total recovered radioactivity. In the 71 brains with a clot clearly visible in an epicerebral vessel, we found 62±24% of the total recovered radioactivity in the vessels and clot. Based on this analysis, we defined thrombolysis as the localization in the brain blocks of ≥80% of the total recovered radioactivity.

The effect of treatment on the rates of hemorrhage and thrombolysis is shown in Table 1. Figure 1 illustrates the effect of treatment on hemorrhage size. In the combined saline-treated group we observed hemorrhage in 25% and spontaneous thrombolysis in 35% of the rabbits. High rates of thrombolysis were achieved with both t-PA and SK. Compared with saline, t-PA did not cause an increase in the rate of hemorrhage at any dose and did not cause an increase in the size of the hemorrhage. Compared with saline, SK significantly increased the incidence (\(p<0.05\)) and size (\(p<0.001\) by analysis of variance) of cerebral hemorrhages. There was a trend toward a higher incidence of hemorrhage with an increased interval between embolization and the initiation of SK therapy.

Within a treatment, rate of hemorrhage did not differ in rabbits with and without thrombolysis (Table 2). That is, thrombolysis was not a factor that correlated with the development of hemorrhage.

Discussion

There are two methods for documenting thrombolysis: angiography and direct visual inspection. Some authors have used serial angiography before and after treatment to determine whether the occluded vessel has opened.\(^2,3,12,16\) We found that repeated injections of contrast dye are quite toxic and that many animals with brain infarction die ≤12 hours after serial angiography. This problem may explain
TABLE 1. Rates of Hemorrhage and Thrombolysis in Rabbits After Treatment With t-PA, SK, or Saline for Embolic Stroke

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Time (min)</th>
<th>n</th>
<th>Hemorrhage</th>
<th>Thrombolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>*</td>
<td>48</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>t-PA</td>
<td>3 mg/kg</td>
<td>90</td>
<td>16</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>t-PA</td>
<td>5 mg/kg</td>
<td>90</td>
<td>22</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>t-PA</td>
<td>10 mg/kg</td>
<td>90</td>
<td>11</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>SK</td>
<td>30,000 units/kg</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>SK</td>
<td>30,000 units/kg</td>
<td>90</td>
<td>17</td>
<td>11</td>
<td>65†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
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</tr>
</thead>
<tbody>
<tr>
<td>SK</td>
<td>30,000 units/kg</td>
<td>300</td>
<td>12</td>
<td>10</td>
<td>83†</td>
</tr>
</tbody>
</table>

*Saline-treated control rabbits were treated 5, 90, or 300 minutes after embolization.
†p<0.05 different from saline by $\chi^2$ test.

TABLE 1. Rates of Hemorrhage and Thrombolysis in Rabbits After Treatment With t-PA, SK, or Saline for Embolic Stroke

- t-PA, tissue plasminogen activator; SK, streptokinase; Time, time after embolization that treatment was initiated.
- Results of t-PA treatment at 5 minutes and 4, 8, and 24 hours are contained in References 2 and 3.

The high mortality rates that other investigators have observed. To avoid angiography, we examined the brain directly 24 hours after treatment. However, direct inspection at a single time does not reveal when thrombolysis occurred, and one cannot know if the clot seen at 24 hours is the injected embolus or a spurious embolus inadvertently injected from the tip of the indwelling catheter. Most problematic of all, if no clot is visible in the brain at 24 hours, without angiography one cannot know that the embolus was ever in the brain in the first place. That is, one might incorrectly deduce that thrombolysis had occurred because no clot was seen in the brain, when in fact the injected embolus had lodged in the neck. To avoid these problems we prepared emboli with iodine-125-labeled 15-$\mu$m microspheres. Such microspheres are widely used to study blood flow of any organ because they lodge in arterioles and capillaries. We verified that the microspheres did not "wash through" the brain by examining the lungs in four rabbits; no radioactivity was found. The labeling technique allowed us to avoid angiography. We re-
The hemorrhage size is quite variable, so a huge sparsely with areas of normal tissue. Therefore, areas of petechial hemorrhage are interspersed with areas of normal tissue. 20 Therefore, more exact measurement of hemorrhage size would give arbitrary results. Figure 1 shows that the semiquantitative method we devised allowed detection of an increase in hemorrhage size in the SK-treated groups. The dose of SK that we used is comparable to the dose effective for human coronary thrombolysis.

We did not study t-PA and SK in a parallel design because we have previously reported that 1, 3, or 5 mg/kg t-PA does not promote cerebral hemorrhage when given 5 minutes or 4, 8, or 24 hours after embolization. Three t-PA-treated groups were included in this study to allow direct comparisons with the SK-treated groups. Previous studies have shown that even using supratherapeutic doses, delayed t-PA thrombolysis does not increase the rate of hemorrhage. 23 Given 5, 90, or 300 minutes after embolization, SK at a dose similar to that used in patients for coronary thrombolysis significantly increased the rate of hemorrhage. We found a trend toward an increased rate of hemorrhage with a longer delay from embolization to the onset of SK treatment. Although not statistically significant, this trend suggests that delayed therapy may pose a greater risk than acute thrombolysis. 10 Slivka and Pulvinelli 21 found that 10,000 or 30,000 units/kg SK did not increase hemorrhage size when given 24 hours after ligation of the MCA in rabbits. However, three of four rabbits treated with t-PA 24 hours after ligation did suffer cerebral hemorrhage. These authors' permanent ligation method did not allow for thrombolysis and reperfusion and may have traumatized the arteries, so it is difficult to interpret these findings.

In our model the greater potential for hemorrhage with SK than with t-PA may be related to several factors. SK is less specific for fibrin-bound plasminogen than t-PA. 22 SK will cleave plasminogen as well as fibrinogen and other clotting factors in the blood, resulting in a systemic thrombolytic and fibrinolytic state. 22-24 Cleavage of circulating fibrinogen produces fibrinogen degradation products, which are potent anticoagulants. The fibrinolytic effects of SK can be measured in the circulation much longer than those of t-PA. 24 Although previous clinical observations have suggested that coagulopathy related to heparin or SK treatment may promote cerebral hemorrhage, 25-27 we have shown that neither heparin nor t-PA-induced coagulopathy promoted cerebral hemorrhage in this animal model. 2,3,13 Further studies will be necessary to fully understand why thrombolysis with SK promotes more hemorrhage than that with t-PA.

There was a 25% incidence of hemorrhage in the combined saline-treated group. This parallels the rate of spontaneous hemorrhagic conversion in untreated human embolic infarction. 19,20,28,29 The pathophysiologic mechanism of cerebral hemorrhage after embolic stroke is not clear. Fisher and Adams 19 suggested that after an occluding embolus fragments and migrates distally, blood flow is restored at full pressure to an ischemia-weakened vessel. However, other investigators have noted that emboli are more
likely than intra-arterial thrombi to cause hemorrhage only because embolic infarcts tend to be larger.\textsuperscript{20,28} The correlation between large infarcts and hemorrhage has been suggested in clinical and experimental studies.\textsuperscript{20,29–32} Another mechanism of hemorrhage may be that blood enters an ischemic zone via collateral vessels.\textsuperscript{21,25} This idea would explain our finding in this and previous studies\textsuperscript{2,3} that hemorrhage is not associated with thrombolysis per se. As shown in Table 2, hemorrhage occurred whether the occluding embolus was lysed or not. In a recent study, hemorrhagic infarction developed in the brains of patients after cardioembolic stroke without reopening of the occluded vessels.\textsuperscript{33} The occurrence of hemorrhage appeared to be related to transient hypertension.\textsuperscript{33}

The results of this study should be viewed with care as the animals used were young and free of hypertension or coexisting vascular disease. However, t-PA is likely to be effective in reducing neurologic damage after embolic stroke\textsuperscript{1–3} and probably is relatively safe.

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