Cerebral Hemorrhagic Risk of Aspirin or Heparin Therapy With Thrombolytic Treatment in Rabbits

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We studied the incidence of cerebral hemorrhage in an animal model of embolic stroke to determine the safety of aspirin, heparin, and tissue plasminogen activator therapies. We occluded the middle cerebral arteries of rabbits with labeled blood clots and administered either tissue plasminogen activator, heparin, aspirin, tissue plasminogen activator plus aspirin, tissue plasminogen activator plus heparin, or saline at various times after stroke. Compared to saline controls, both the aspirin-only and the tissue plasminogen activator-plus-aspirin groups had a significantly higher incidence of cerebral hemorrhage, whereas the heparin and tissue plasminogen activator combination groups did not. We conclude that aspirin antiplatelet therapy alone may increase the risk of hemorrhagic infarction, whereas heparin or tissue plasminogen activator therapy appears to be relatively safe.

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The recent availability of pharmacologic quantities of the thrombolytic agent tissue plasminogen activator (t-PA) has stimulated renewed clinical interest in thrombolytic therapy for stroke. Tissue plasminogen activator acts on plasminogen predominantly in the presence of fibrin, thus limiting its activity to the accessible surface of the clot. By producing less systemic depletion of fibrinogen and other clotting factors than other thrombolytic agents, t-PA may confer less risk of cerebral hemorrhage.

We previously have used large and small clot experimental embolic stroke models to demonstrate efficacy of t-PA therapy without finding a significant increase in cerebral hemorrhage rate or severity. Animal studies by other investigators using t-PA have produced similar results. However, the risk of cerebral hemorrhage associated with clinical t-PA thrombolysis remains a concern. Data from the Thrombolysis in Myocardial Infarction Trial and other cardiac thrombolysis studies indicate a 0-5% incidence of "spontaneous" cerebral hemorrhage. Initial results from two multicenter acute t-PA stroke trials recently completed also show a 40% (Acute Stroke Study Group Trial-Burroughs Wellcome) rate of cerebral hemorrhagic infarction and a 5% (National Institutes of Health Trial) rate of intracerebral hematoma.

A potential explanation for the difference in cerebral hemorrhage rates between the experimental and clinical studies is the concurrent use of anticoagulant or antiplatelet therapies in the clinical populations. Because the concurrent use of heparin or aspirin may be indicated to prevent vessel reocclusion after thrombolysis, and given that the majority of high-risk patients may be on aspirin before their stroke, an understanding of the risk of their concurrent use with t-PA is critical. To assess the cerebral hemorrhagic potential of t-PA with aspirin or heparin, we undertook the present study using an animal model of embolic occlusion of the middle cerebral artery.

Materials and Methods

The method we used in this study to induce embolic stroke in rabbits has been reported in detail previously. This study was approved by the University of California San Diego Animal Subjects Committee, and the National Institutes of Health guidelines for animal research were followed. In brief, we used male New Zealand White rabbits weighing 2.5-3.0 kg. The animals were anesthetized...
with halothane, and the bifurcation of the common carotid artery was exposed via a lateral neck dissection. After ligation of the common and external carotid arteries, a 20-gauge plastic catheter was placed in the internal carotid artery, oriented anterograde. The catheter was filled with heparinized saline and covered with an injection cap, and the animal was allowed to recover fully from anesthesia.

We mixed whole blood from a donor rabbit with trace quantities of iodine-125–labeled 15-μm diameter plastic microspheres and allowed it to clot at 37°C for 24 hours. The clot was sliced into small cubes and weighed. Cubes weighing 3.8–4.2 mg were selected and suspended in 100 μl Dulbecco’s buffered saline until use 2 hours later. The radioactivity in each cube was measured in a gamma counter.

To induce stroke, we removed the injection cap and used the animal’s blood to flush out the heparinized saline. The catheter was clamped, and the clot was placed into the catheter using forceps. The cap was reattached, the clamp removed, and the clot advanced into the middle cerebral artery by injecting 2.5 ml saline under careful observation to avoid injecting air bubbles. We previously have shown, using serial angiograms, that emboli of this size lodge reliably in the middle cerebral artery. Each animal was examined immediately after embolization, and all had clinical signs of stroke such as hemiparesis, circling, or seizure.

We assigned animals to one of six groups, with protocols designed to approximate clinical therapy: 1) Aspirin only: Animals were given 20 mg/kg (70% bioavailability) aspirin by intravenous infusion into an ear vein as described by Kelton et al 18 hours before embolization. The dose was chosen to approximate clinical therapy and was based on prior rabbit aspirin studies. 21 22 In a pilot study (n=6), this dosage tripled the animal’s bleeding time at 18 hours (baseline mean, 4±0.5 minutes; 18 hours mean, 12±1 minutes, mean±SEM) as measured by a 1-cm ear incision using a modified template technique. 22 2) Aspirin plus t-PA: Aspirin treatment was identical to the aspirin-only group. In addition, animals received 10 mg/kg intravenous t-PA 90 minutes after embolization (t-PA, 300,000 IU/mg; a gift from Burroughs Wellcome Co., Research Triangle Park, N.C.). A 20% t-PA bolus was given, with the remainder infused at a constant rate over 30 minutes. 3) t-PA only: Animals received 10 mg/kg intravenous t-PA 90 minutes after embolization by the same infusion schedule. 4) Heparin only: Animals received a 2,000 unit intravenous heparin bolus 5 minutes after embolization. This produced anticoagulation for at least 6 hours (partial thromboplastin time (PTT)>100 seconds). 5) Heparin plus t-PA: Heparin treatment was identical to the previous group. In addition, animals received 5 mg/kg intravenous t-PA starting 90 minutes after embolization using the same infusion protocol. 6) Controls: Controls were salinetreated ischemic animals. Animals received 1 ml/kg intravenous saline at 90 minutes, using a 20% bolus, with the remainder infused over 30 minutes.

Twenty-four hours after stroke, an observer blinded to treatment group assessed the neurologic function of each animal. All surviving animals were killed using 0.5 ml intravenous Terminal (Anpro Pharmaceutical, Arcadia, Calif.). The brains were removed and carefully examined (blind to treatment group) for the presence of visible clot or surface hemorrhage, and the large arteries were stripped from the brain. The brains were placed in formalin and, after 7 days’ fixation, each brain was sliced into coronal 5.0-mm thick blocks. The two faces (total 10 faces per brain) were examined for evidence of visible hemorrhage. The size of each hemorrhage was quantified by the number of faces in which it was observed (0–10). A larger volume of hemorrhage will be apparent on more faces. We have found this to be a sensitive and reproducible method of quantifying cerebral hemorrhage. 5 6 20 This technique identifies visible cerebral hemorrhage but does not differentiate between intracerebral hematomas and hemorrhagic infarction.

The retained radioactivity was counted in each of the blocks of brain and in the cerebral vessels. If a visible clot was present, it was included with the vessels. The total recovered radioactivity was defined as the sum recovered in the brain blocks and vessels. We rejected any animal in which the total recovery was less than 10% of the original clot activity. These animals probably were infarcted by a different mechanism, such as air emboli or catheter tip emboli. We defined thrombolysis as the presence of less than 20% of the recovered radioactivity remaining in the vessels. This definition produced an excellent agreement with presence or absence of visible clot. Occasionally, we observed a spurious visible clot that contained less than 20% activity. Because this was thought to represent inadvertent injection of an unlabeled catheter tip embolus or postmortem thrombosis, these animals were not scored as having a significant residual clot.

Results

The effect of treatment on hemorrhage incidence is shown in the “Hemorrhage” column of Table 1. There was a significant overall difference between groups (p=0.02) by χ² test. In the saline-treated animals, we observed hemorrhage in 29% and spontaneous thrombolysis in 29% of the animals. Tissue plasminogen activator or heparin combinations did not significantly increase the hemorrhage rate compared to controls. However, both groups that received aspirin had a significant increase in hemorrhage rates compared to controls (p=0.03 for t-PA plus aspirin, and p=0.002 for aspirin) by Fischer’s exact test. There was no significant difference in hemorrhage incidence between the two aspirin groups.

The effect of treatment on hemorrhage size is shown in the “Faces” column of Table 1. Because this measurement uses an ordinal scale, the median
number of faces is given for each group. There was not a significant overall difference in hemorrhage size between groups by Kruskal-Wallis one-way analysis of variance. The frequency of hemorrhage sizes for each group is shown in Table 2. An example of a small hemorrhagic infarction is shown in Figure 1.

The effect of treatment on mortality is shown in the "Death" column of Table 1. There is a trend toward increased mortality in the groups with the highest hemorrhage rates (aspirin groups), although this was not significantly different than controls. However, across all groups, we did find a significantly increased mortality in the animals with cerebral hemorrhage (34 of 47 dead; 72%) versus those without hemorrhage (18 of 43 dead; 42%) (p=0.01) by Fischer's exact test.

We saw significantly higher rates of thrombolysis in animals that received t-PA, regardless of group (see "Thrombolysis" column of Table 1; p<0.05 by Fischer's exact test). Across all groups, we found a significantly decreased rate of cerebral hemorrhage in animals with thrombolysis (19 of 48 with hemorrhage; 40%) versus those without thrombolysis (28 of 42 with hemorrhage; 67%) (p=0.01) by Fischer's exact test.

We rejected 37 animals because of less than 10% radioactivity recovery for the brain and vessels. These animals were distributed equally among all treatment groups. In these animals, we found greater than 90% of residual activity in the extracranial internal carotid, confirming that the clot never reached the brain.

**Discussion**

In this study, we used an experimental embolic stroke model to assess the cerebral hemorrhagic risks of aspirin, heparin, and t-PA therapies. This model involves the use of labeled blood clots to confirm infarct etiology and thrombolysis. This method previously has been found to have an excellent agreement with cerebral angiogram and avoids the problems inherent with repeated angiograms. The small number of spheres released from each clot (approximately 200) is too low to cause any ischemic effect in the brain, for it is necessary to inject several thousand to cause significant brain ischemia.

The hemorrhage and thrombolysis rates in this control group are similar to those seen in our previous studies: approximately 25% hemorrhage and approximately 35% spontaneous thrombolysis at 24 hours. The hemorrhage and thrombolysis rates in this t-PA–only group are also very similar to our previous findings: 3 mg/kg t-PA produced 31% cerebral hemorrhage, and 5 mg/kg t-PA produced 14% cerebral hemorrhage using identical infusional protocols. These hemorrhage rates are also similar to the incidence of spontaneous hemorrhagic conversion in untreated human cerebral infarction within the first week after stroke. This study also confirms our previous findings that thrombolysis per se does not increase the incidence of cerebral hemorrhage. Early thrombolysis appears to be protective against cerebral hemorrhage in this model.

In our study, cerebral hemorrhage incidence in the heparin-only and heparin–plus–t-PA groups was not significantly different from controls, despite the markedly prolonged PTT for at least 6 hours. Although there is a nonsignificant trend toward increased hemorrhage incidence, power analysis indicates that approximately 180 animals per group would be required to achieve significance, assuming this difference is real. These hemorrhage incidence results are similar to our previous study, which also did not detect an increased incidence of hemorrhage using various heparin doses in this model (an approximate 37% hemorrhage rate). Caution is needed when generalizing the results from young, previously

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**TABLE 1. Relationship of Treatment to Incidence of Cerebral Hemorrhage, Hemorrhage Size, Mortality, and Thrombolysis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Hemorrhage</th>
<th>Faces</th>
<th>Death</th>
<th>Thrombolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>17</td>
<td>5 (29%)</td>
<td>4</td>
<td>11 (65%)</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>t-PA</td>
<td>11</td>
<td>4 (36%)</td>
<td>2</td>
<td>5 (45%)</td>
<td>11 (100%)§</td>
</tr>
<tr>
<td>Heparin/t-PA</td>
<td>13</td>
<td>6 (46%)</td>
<td>3.5</td>
<td>6 (46%)</td>
<td>9 (69%)§</td>
</tr>
<tr>
<td>Heparin</td>
<td>15</td>
<td>7 (47%)</td>
<td>2</td>
<td>5 (33%)</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>14</td>
<td>12 (86%)§</td>
<td>4</td>
<td>12 (86%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Aspirin/t-PA</td>
<td>20</td>
<td>13 (65%)§</td>
<td>4</td>
<td>13 (65%)</td>
<td>13 (65%)§</td>
</tr>
</tbody>
</table>

t-PA, tissue plasminogen activator.

*In animals with a hemorrhage, median number of faces that had visible hemorrhage.
†Percent of animals dead at 24 hours.
‡Thrombolysis is measured as defined in text.
§p<0.05 compared to saline.

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**TABLE 2. Frequency of Hemorrhage Sizes for Each Experimental Group**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>0 (0%)</td>
<td>5</td>
</tr>
<tr>
<td>t-PA</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>4</td>
</tr>
<tr>
<td>Heparin/t-PA</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>6</td>
</tr>
<tr>
<td>Heparin</td>
<td>5 (71%)</td>
<td>0 (0%)</td>
<td>2 (29%)</td>
<td>7</td>
</tr>
<tr>
<td>Aspirin</td>
<td>4 (33%)</td>
<td>4 (33%)</td>
<td>4 (33%)</td>
<td>12</td>
</tr>
<tr>
<td>Aspirin/t-PA</td>
<td>2 (15%)</td>
<td>9 (70%)</td>
<td>2 (15%)</td>
<td>13</td>
</tr>
</tbody>
</table>

t-PA, tissue plasminogen activator.

*Small hemorrhages were classified as visible on one or two faces only; medium, on three or four faces; and large, on five faces or more.
healthy, ischemic animals to the clinical situation. However, the combined results from our two heparin experimental embolic stroke studies, representing 69 heparin-treated animals, did not show an increased cerebral hemorrhage rate or size with heparin anticoagulation, even when used concurrently with t-PA thrombolysis. This potentially is an important finding, given that heparin may be indicated immediately after t-PA thrombolysis to prevent rethrombosis.

In contrast to the heparin results, aspirin therapy did significantly increase the rate of cerebral hemorrhage after experimental embolic stroke in our study. This increased rate is seen in both the t-PA-plus-aspirin and aspirin-only groups. Although there was no significant difference in incidence between these two groups, the high rates in both groups may have produced a ceiling effect leading to a Type II error. This study also suggests that these are "clinically relevant" hemorrhages because animals with hemorrhages had a significantly higher mortality. The only other agent to produce such an increased rate of hemorrhage in this model was high-dose intravenous streptokinase (30,000 IU/kg). The possibility that these results represent a species-specific paradoxical thrombogenic effect of aspirin must be considered. However, prior studies have reported a thrombogenic effect with aspirin in rabbits only with doses five times higher than those used in our study. In addition, we found no evidence of increased thrombosis on our postmortem examinations. To our knowledge, our study is the first experimental stroke study to assess the cerebral hemorrhagic risk of aspirin. Although some clinical trials have found a slightly higher rate of cerebral hemorrhage in aspirin-treated groups, these findings are indirect and uncontrolled.

The mechanism by which aspirin or combined aspirin plus t-PA may increase the rate of hemorrhagic infarction may be related to their antiplatelet effects. Tissue plasminogen activator substrates have been found to cause a delayed, prolonged inhibition of platelet aggregation. In an experimental study, Vaughan et al found that combined aspirin and t-PA administration resulted in both a markedly prolonged bleeding time and systemic bleeding complications. Gimple et al found a marked prolongation of bleeding time after t-PA in their patients who had used aspirin before t-PA treatment, despite normal pre-t-PA treatment bleeding times. This increased bleeding time correlated with the risk of systemic bleeding, suggesting a potentiation of the t-PA-induced antiplatelet effect with concurrent aspirin pretreatment. It is possible that a similar increase in the risk of cerebral hemorrhagic infarction would also occur. Because equally increased hemorrhage rates occurred in both the aspirin-only and aspirin-plus-t-PA groups, our study supports the theory that platelet dysfunction and not a fibrinolytic state increases the risk of cerebral hemorrhage. Because aspirin inhibits platelet cyclooxygenase function for up to 8 days, combined antiplatelet effects of t-PA and aspirin may occur even with relatively remote aspirin treatment.

We are unaware of any clinical studies on the hemorrhage risk of premorbid or early aspirin therapy in a patient with stroke. Until clinical studies addressing this issue are conducted, we suggest caution in the early...
use of aspirin after stroke and in the management of a patient taking aspirin at stroke onset.

References


Key Words • cerebral hemorrhage • thrombolytic therapy • aspirin • rabbits
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