**Effect of Tissue Plasminogen Activator on Intimal Platelet Accumulation in Cerebral Arteries After Subarachnoid Hemorrhage in Cats**

Yoshihiro Yamamoto, MD; Ben R. Clower, PhD; Joseph L. Haining, PhD; and Robert R. Smith, MD

Recombinant tissue plasminogen activator may be effective in preventing cerebral vasospasm after subarachnoid hemorrhage by resolving subarachnoid clots. We previously demonstrated that blood placed on the adventitial surface of cerebral arteries enhances intimal platelet accumulation, positively correlating with endothelial damage and other pathologic changes in vessel walls. In this study, we investigated the ability of a single bolus injection of tissue plasminogen activator to prevent platelet accumulation in cerebral vessels after subarachnoid hemorrhage. Subarachnoid hemorrhage was produced in cats by the transorbital intracisternal injection of 2.5 ml autologous arterial blood around the proximal part of the right middle cerebral artery. In 10 animals, 25 μg tissue plasminogen activator was injected at intervals of 10 (five cats) and 60 minutes (five cats) after subarachnoid hemorrhage. Intracisternal physiological saline (0.5 ml) was injected in six cats 10 minutes after subarachnoid hemorrhage. Platelets labeled with indium-111 were injected intravenously just before subarachnoid hemorrhage, and their radioactivity was measured in cerebral arteries at death. The results indicated that, after subarachnoid hemorrhage, early injection of tissue plasminogen activator inhibited intimal platelet accumulation, but later injection did not, although the extent of subarachnoid clot was reduced at both plasminogen injection times. (Stroke 1991;22:780-784)

Although the pathophysiology of cerebral vasospasm after subarachnoid hemorrhage (SAH) has not yet been clarified, a positive linear correlation seems to exist between the extent of SAH and the severity of delayed cerebral vasospasm. To prevent or arrest vasospasm, attempts have been made to remove or neutralize subarachnoid clots, either by early surgical exploration or by the use of various intracisternal irrigation techniques.

In addition to sustained contraction of medial smooth muscle cells, SAH produces pathologic changes within the cerebral artery, a phenomenon that also correlates positively with the severity of SAH. Endothelial injury and intimal platelet accumulation, the first tissue alterations after SAH, occur in the intimal layer of the cerebral vessel in the early phase of SAH, but which event is primary is not known. Both events have been suggested to have a pivotal role in the contractile and pathologic status of the cerebral vessel in SAH.

Recently, a new fibrinolytic agent, recombinant tissue plasminogen activator (tPA or rtPA), has been used both experimentally and clinically to dissolve subarachnoid clots. This study examines the effect of a single intracisternal bolus injection of tPA on intimal platelet accumulation in cerebral arteries after experimental SAH.

**Materials and Methods**

Sixteen mongrel cats (2.5–3.5 kg) were divided randomly into three groups and underwent induced SAH. All animals in each group were subjected to the subarachnoid injection of autologous arterial blood. These studies are in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Institutional Animal Care and Use Committee.
FIGURE 1. Ventral surfaces of brains from cats in group 1 show massive subarachnoid clots around right middle cerebral arteries and in the basal cisterns.

The method for producing SAH has been described previously and is based on the transorbital injection of 2.5 ml whole arterial blood into the cisternal space in the region of the proximal main trunk of the right middle cerebral artery (MCA). Each animal was anesthetized with a combination of ketamine hydrochloride (22 mg/kg i.m.) and xylazine (5.5 mg/kg i.m.). The head was immobilized in a stereotactic instrument, and the right eye was removed. After orbital exenteration, a small (4-mm) craniectomy was performed just superior to the right optic canal, exposing a short segment of the main trunk of the right MCA. After vessel exposure, a polyethylene tube (total length, 50 mm; i.d., 0.28 mm; o.d., 0.61 mm; PE-10, Clay Adams, Parsippany, N.J.) was inserted into the subarachnoid space for 3 mm, adjacent to the proximal part of the right MCA, without causing bleeding. The orifice of the tube was covered with a fragment of extraocular muscle, which was irrigated repeatedly with sterile saline. A drop of sterile methyl-2-cyanoacrylate was applied to the surface of the muscle fragment to occlude the orifice. After the animal’s head was tilted right side down, 2.5 ml autologous arterial blood was injected through the tube on the adventitial surface of the right MCA. Blood left in the tube was immediately irrigated with saline to avoid coagulation. This tube later was used for tPA or saline injections.

To determine the amount of platelet accumulation in the cerebral arteries, platelets labeled with indium-111 were prepared according to the technique of Hawker et al., except that commercially available In-oxine was used as the labeling agent. The labeled platelets were injected intravenously immediately before SAH was induced.

In group 1 (six cats), 0.5 ml sterile physiological saline was injected into the subarachnoid space 10 minutes after induced SAH. In groups 2 (five cats) and 3 (five cats), 0.5 ml tPA (Genentech Inc., South San Francisco, Calif.) (50 μg/ml) was injected into the subarachnoid space 10 and 60 minutes after SAH, respectively. Animals in each group were killed 4 hours after SAH.

<table>
<thead>
<tr>
<th>RMCA, right middle cerebral artery; LMCA, left middle cerebral artery.</th>
<th>Radioactivity (cpm)</th>
<th>Ratio</th>
<th>RMCA/LMCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Cat no.</td>
<td>Radioactivity (cpm)</td>
<td>RMCA</td>
</tr>
<tr>
<td>1</td>
<td>B53</td>
<td>331</td>
<td>201</td>
</tr>
<tr>
<td>1</td>
<td>B45</td>
<td>760</td>
<td>269</td>
</tr>
<tr>
<td>1</td>
<td>B74</td>
<td>153</td>
<td>38</td>
</tr>
<tr>
<td>1</td>
<td>A63</td>
<td>209</td>
<td>135</td>
</tr>
<tr>
<td>1</td>
<td>P15</td>
<td>227</td>
<td>104</td>
</tr>
<tr>
<td>1</td>
<td>Q32</td>
<td>273</td>
<td>206</td>
</tr>
<tr>
<td>2</td>
<td>B46</td>
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</tr>
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<td>B66</td>
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<td>248</td>
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<td>3</td>
<td>B83</td>
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</tr>
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<td>3</td>
<td>B87</td>
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<td>264</td>
</tr>
<tr>
<td>3</td>
<td>B72</td>
<td>455</td>
<td>361</td>
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</table>

<table>
<thead>
<tr>
<th>Table 2. Duncan’s Multiple Comparison of Transformed Data</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of (ratio)</td>
<td>0.788</td>
<td>1.029*</td>
<td>0.902</td>
</tr>
<tr>
<td>Standard deviation of (ratio)</td>
<td>0.106</td>
<td>0.057</td>
<td>0.105</td>
</tr>
</tbody>
</table>

*Groups 1 and 3 are the same; group 2 is different from both (α=0.05 level).
At death, each cat was given 100 mg i.m. ketamine, and its femoral vein was exposed and infused with 150 mg sodium pentobarbital. After the brain was excised, its base was photographed to record the extent of SAH. Measured segments of equal length (15 mm from the origin) of both the right and left MCA were dissected from their vascular beds and placed in 12×75 mm tubes containing 2.5% phosphate buffered glutaraldehyde solution. Their radioactivity in counts per minute was measured using an automatic gamma counter (model 1185, Searle Analytic, Inc., Des Plaines, Ill.). After background correction, the radioactivity of the right MCA segment was divided by that of the left MCA segment to produce a radioactivity ratio. This ratio is an important figure inasmuch as the left MCA is an internal control. As long as the ratio is significantly greater than 1, preferential accumulation in the right MCA is indicated regardless of the amount of radioactivity. A standard analysis of variance and a randomization test were performed to determine significant differences among groups.

Results

Photographs of the base of the brain showed that the saline-injected animals in group 1 (Figure 1) demonstrated larger areas of SAH than those in the other experimental groups (Figures 2 and 3). The SAH of group 1 was primarily localized in the right lateral fissure, with moderate amounts of blood in the basilar and interpeduncular cisterns, and to a lesser degree, in the left lateral fissure. Although the size of SAH in brains of animals in group 2 (tPA injection, 10 minutes) and group 3 (tPA injection, 60 minutes) were comparable, both showed less than that observed in group 1.

Table 1 summarizes the net radioactivity of MCAs and ratios in each group. Statistically, the variances of the groups were not homogeneous. Therefore, we applied a variance-stabilizing transformation to the data before analysis of variance to improve the power of the tests. Namely, we created a new variable, the reciprocal of the cube root of the original ratio. One-way analysis of variance and Duncan's multiple comparisons of the transformed data showed that groups 1 and 3 were not statistically different but that they were both different from group 2 (Table 2).

Table 3 summarizes the results of the nonparametric randomization test. We analyzed the original ratios without transformation. The results showed that groups 1 and 3 were not different, but they were both significantly different from group 2 (p<0.017).

In summary, the injection of tPA 10 minutes after SAH could inhibit intimal platelet accumulation, but injection 60 minutes after SAH could not, although the extent of adventitial clot was significantly reduced in both groups.

Discussion

A new fibrinolytic agent, tPA or rtPA, has several advantages compared to regular fibrinolytic agents such as urokinase. It has stronger affinity to fibrin-bound plasmin and produces "clot-selective" fibrinolysis without causing systemic coagulopathy. These features make tPA the ideal fibrinolytic substance to dissolve subarachnoid clots in patients who had aneurysm surgery during the acute stage of SAH to prevent delayed cerebral vasospasm. Using a "double-hemorrhage" dog model, Seifert and colleagues reported that an intracisternal bolus inje-
tion of 25 \( \mu g \) tPA is highly efficacious in preventing angiographic as well as pathomorphologic vasospasm on day 8 after SAH. Using a primate model, Findlay and coworkers demonstrated that 0.75 mg tPA is effective in preventing angiographic vasospasm on day 7 after SAH, as long as it is applied within 72 hours of SAH. A cooperative clinical trial is now under way. Some cases that showed adverse events, that is, posttreatment hematoma, also have been reported (personal communication, T. Brott, Department of Neurology, University of Cincinnati).

Previous studies in our laboratory showed that platelet accumulation in the cerebral arteries can be observed as early as 2 hours after experimental SAH. Platelets accumulated consistently 4 hours after SAH, and the extent of accumulation appeared to correlate with the presence of red blood cells on the adventitial surface. Electron microscopic studies of these vessels demonstrated the pathologic changes of the intimal side of the vessel wall: endothelial corrugation, detachment, crater formation, and accumulating blood elements. Endothelial cells affect the contractility of medial smooth muscle cells by means of the endothelium-derived relaxation factor and endothelin; consequently, the pathologic changes of the endothelium appear to be an important factor in producing delayed cerebral vasospasm. Platelets can both reflect the severity of endothelial damage and accelerate vasculopathic change by secreting growth factors, serotonin, and prostaglandins. Therefore, we expected tPA to prevent endothelial damage by reducing the contact of the vessel wall with the subarachnoid clot.

The results of this study showed, however, that intimal platelet accumulation could not be prevented when tPA was injected 60 minutes after SAH although tPA can significantly dissolve subarachnoid clots within 4 hours. It is thought that platelets start accumulating on the endothelial surface within 60 minutes after SAH and that the accumulation cannot be reversed within 3 hours, even when the adventitial clot is dissolved by tPA.

Further studies determining both the amount and the time course of platelet accumulation after tPA injection are necessary. In clinical cases, however, tPA may not be able to prevent vascular damage when it is injected days after initial SAH.

Acknowledgments

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References


KEY WORDS • blood platelets • plasminogen activator, tissue type • subarachnoid hemorrhage • cats
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