Delayed Treatment With a t-PA Analogue and Streptokinase in a Rabbit Embolic Stroke Model

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Fibrinolytic therapy may be effective in the treatment of ischemic stroke, and clinical trials are under way. We evaluated two fibrinolytic agents, an analogue of tissue plasminogen activator (Fb-Fb-CF, the catalytic fragment of the tissue plasminogen activator molecule with a prolonged serum half-life, n=10) and streptokinase (n=7), in a rabbit model of embolic stroke. Both agents were given 3 hours after stroke onset, a time relevant to the clinical setting. Fb-Fb-CF was significantly better (p<0.04) than saline (n=7) in restoring blood flow to previously occluded intracranial arteries, but streptokinase was ineffective. Neither fibrinolytic agent was associated with a substantial risk for intracerebral hemorrhagic side effects. Our study demonstrates that Fb-Fb-CF can safely and effectively reperfuse rabbit intracranial arteries 3 hours after occlusion, while streptokinase does not. (Stroke 1990;21:602-605)

The potential for treating acute ischemic stroke with fibrinolytic agents to restore cerebral blood flow has generated much interest. Previous animal studies have demonstrated that native, full-molecular tissue plasminogen activator (t-PA) and a t-PA analogue (Fb-Fb-CF) can restore blood flow in occluded cerebral arteries when given 15 or 90 minutes after arterial embolization. Other investigators have observed that t-PA can reduce neurologic damage when given as long as 45 minutes after stroke onset. Hemorrhagic side effects are of major concern, but in these animal models of stroke such side effects have not been a substantial problem.

Streptokinase is a nonfibrin-specific fibrinolytic agent that has been successfully employed in the treatment of acute myocardial infarction (MI). Major hemorrhagic side effects were uncommon (0.1–0.2%) in two large MI treatment trials with streptokinase. Few data are available concerning the safety or efficacy of streptokinase in animal models of embolic stroke. Therefore, we compared the angiographic efficacy and the frequency of intracranial hemorrhagic complications of streptokinase with those of the Fb-Fb-CF in such a model. The two fibrinolytic agents were given 3 hours after arterial embolization, a time delay more relevant to potential clinical usage than those used in previous studies, which employed Fb-Fb-CF 15 and 90 minutes after embolization.

Materials and Methods

The methodology used in our rabbit model of cerebral embolization has been described in detail. Twenty-four New Zealand white rabbits weighing 2–3 kg were anesthetized and intubated. The intracranial arterial circulation was selectively catheterized, and the origin of the proximal internal carotid artery was identified angiographically. The rabbits were then embolized with 0.03 ml of aged (18–22 hours) autologous thrombus. Angiography was repeated within 5 minutes, and embolic occlusion of the distal internal carotid artery or proximal middle cerebral artery was documented.

Before embolization, the rabbits were assigned to treatment with 0.8 mg/kg Fb-Fb-CF given as a bolus over 2 minutes (n=10), saline given as a bolus over 2 minutes (n=7), or 25,000 units/kg of streptokinase infused over 20 minutes (n=7). All treatments began 180 minutes after embolization. Just before the onset of treatment, angiography was performed a third time to document persistent arterial occlusion. Angiography was performed again 30 minutes after treatment was finished and repeated four more times at
TABLE 1. Results of Treatment With Fb-Fb-CF or Streptokinase 180 Minutes After Cerebral Embolization in Rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time to reperfusion (minutes)</th>
<th>Hemorrhagic infarction</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Median</td>
<td>No.</td>
</tr>
<tr>
<td>Fb-Fb-CF</td>
<td>70±37.7</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>104.3±5.7</td>
<td>&gt;110</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;110±0</td>
<td>&gt;110</td>
<td>1</td>
</tr>
</tbody>
</table>

*p<0.04 different from saline by Mann-Whitney U test.
†p<0.05 different from saline by Fisher exact probability test.
‡Brain from one rabbit unavailable for examination.

20-minute intervals, for a total observation period of 110 minutes. All angiograms were reviewed by two investigators to assess reperfusion of the previously occluded cerebral vasculature or persistent occlusion.

The preparation and characteristics of Fb-Fb-CF have been described.4 This analogue consists of the catalytic fragment of the t-PA molecule, including amino acid 262 from the amino terminal of the molecule to the carboxyl terminal, and fragment B of staphylococcal protein A. The protein was expressed in Escherichia coli. The serum half-life of Fb-Fb-CF was previously observed to be 90 minutes.4

The plasma fibrinogen concentration was measured in 10 untreated rabbits and in duplicate in the seven streptokinase-treated rabbits at baseline and 50 and 110 minutes after treatment using the Clauss method and a Stargo (American Bioproducts, Parsippany, New Jersey) fibrinogen kit. The plasma was prepared by centrifugation, and the fibrinogen concentration was estimated by the clotting time in the presence of thrombin and referenced to a standard curve.

The neuropathologic examination was performed by investigators blinded to the rabbits' treatment. Eighteen rabbits died ≤24 hours after embolization; the remaining six were killed by intracardiac injection of sodium pentothal/lidocaine/curare 2–8 days after embolization. The brain from one streptokinase-treated rabbit was not available for neuropathologic examination because the animal died at a time when the brain could not be harvested. The brains were removed post mortem and fixed by immersion in 10% neutral buffered formalin for at least 2 weeks, sectioned in the coronal plane at 2–3-mm intervals, and examined for the presence of softening or hemorrhage. The sections were numbered sequentially and submitted for standard histologic processing in paraffin. Sections 6 μm thick were cut from each block, stained with hematoxylin and eosin, and evaluated by light microscopy for the presence and location of hemorrhage and infarction. The sections were numbered sequentially and submitted for standard histologic processing in paraffin. Sections 6 μm thick were cut from each block, stained with hematoxylin and eosin, and evaluated by light microscopy for the presence and location of hemorrhage and infarction. Single or multiple hemorrhages in aggregate measuring ≥0.1 cm in diameter were termed macroscopic hemorrhages; hemorrhages measuring <0.1 cm in diameter were termed microscopic hemorrhages. The histologic ages of the infarcts were determined. Recent infarcts (18–24 hours) were characterized by pallor and microvacuolation of the neuropil, acute eosinophilic necrosis of neurons ("red" neurons), and variable infiltration by polymorphonuclear leukocytes. Organizing infarcts (2–8 days) were characterized by coagulation necrosis of the neuropil, with variable infiltration by macrophages and capillary proliferation/hyperplasia.

Data are expressed as mean±SD. Time to reperfusion in the Fb-Fb-CF–treated and streptokinase-treated groups was compared with that in the saline-treated group using the nonparametric Mann-Whitney U test because of the noncontinuous nature of the data. A Fisher exact probability test was performed comparing the frequency of macroscopic hemorrhages and number of animals reperfused in each treatment group.

Results

No control and only one streptokinase-treated rabbit demonstrated reperfusion; six Fb-Fb-CF–treated rabbits did so (Table 1). Time to reperfusion in the Fb-Fb-CF–treated group ranged from 30 to >110 minutes. An example of angiographically documented intracranial arterial reperfusion is presented in Figure 1.

Fibrinogen level in the 10 untreated rabbits was 315±27 mg/dl. That in the streptokinase-treated group was 269±42 mg/dl at baseline, and it declined by 10.8% at 50 minutes and by 14.3% at 110 minutes after treatment (difference not significant).

Neuropathologic examination revealed cerebral infarcts in the territory of the embolized internal carotid artery in all rabbits in both the Fb-Fb-CF– and the streptokinase-treated groups; infarcts were present in six of the seven control rabbits. Nineteen rabbits had recent infarcts, whereas three had organizing infarcts. The histologic ages of the infarcts correlated well with the survival times, except one control rabbit killed 5 days after embolization had histologic evidence of a more recent infarct (1–2 days). Differences in the extent of infarction among treatment groups could not be reliably quantified because of the inherent variability of infarct size.

Macroscopic hemorrhages were observed within infarcts of two Fb-Fb-CF–treated rabbits; none were observed in the streptokinase-treated rabbits. One control rabbit had macroscopic hemorrhage within an infarct. Microscopic hemorrhages within infarcts were present in seven of the 10 Fb-Fb-CF–treated and five of the six streptokinase-treated rabbits as well as in five of the six controls. No rabbit in either treated group had parenchymal hemorrhages in noninfarcted regions of the brain.
FIGURE 1. Left: Lateral cerebral angiograms in rabbit treated with tissue plasminogen activator analogue Fb-Fb-CF. Angiogram taken 5 minutes after embolization with 0.03 ml aged autologous thrombus. Arterial occlusion is indicated by arrow. Right: Repeat angiogram 50 minutes after treatment. Reperfusion is demonstrated by arrows.

Discussion

This study demonstrates that the t-PA analogue Fb-Fb-CF can successfully reperfuse occluded intracranial arteries when given 3 hours after an embolic event. Reperfusion was not associated with an increased risk for hemorrhagic side effects, an important potential problem with any fibrinolytic agent that may be used for stroke therapy. This observation extends previous studies with Fb-Fb-CF that demonstrated the angiographic efficacy and safety of this agent when employed 15 or 90 minutes after embolic occlusion.4 A 3-hour interval to treatment is probably more realistic for patient accrual in the clinical setting. The model employed does not allow for the assessment of a fibrinolytic agent's effect on clinical outcome or on the extent of ischemic damage because there is a large degree of inherent variability in the extent of infarction within the embolized arterial tree. Fibrinolytic therapy with t-PA has been demonstrated to improve clinical outcome in a similar rabbit model of embolic stroke when given up to 45 minutes after embolization.5 It is uncertain whether reperfusion several hours after the onset of stroke will ameliorate ischemic deficits, but very few patients will be candidates for fibrinolytic therapy if the time window for such intervention is minutes and not hours. Experimental evidence suggests that at least a portion of ischemic tissue may be viable and therefore amenable to treatment hours after an ischemic insult.10,11 Additionally, fibrinolytic therapy may be combined with therapeutic measures designed to salvage metabolically impaired tissue by correcting or improving the basic metabolic or cellular consequences of ischemia.12,13 Such combined therapy may allow for substantial delays before initiating successful fibrinolytic therapy.

This t-PA analogue has been shown to have a longer serum half-life (90 minutes) than native, full-molecular t-PA (5–6 minutes).4 Fibrinogen consumption was also quite modest with Fb-Fb-CF when analyzed previously.4 Full-molecular t-PA offers relative fibrin specificity compared with exogenous fibrinolytic drugs such as streptokinase and urokinase, but t-PA is still plagued by frequent major hemorrhagic side effects and a propensity toward reocclusion of coronary arteries that are successfully reperfused.14,15 Both problems suggest that, although it can effectively treat conditions associated with acute arterial thromboembolism such as acute MI and pulmonary embolism, t-PA is not an ideal fibrinolytic agent. The bleeding side effects associated with t-PA may be reduced by employing fibrinolytic agents with enhanced fibrin-specificity that effectively induce fibrinolysis at lower doses.16 A prolonged serum half-life allows for bolus therapy and the ability to maintain a therapeutic plasma level without continuous infusion. Intravenous anticoagulants, agents that are commonly used in conjunction with t-PA to prevent arterial reocclusion, may then not be necessary. Anticoagulant therapy appears to enhance the risk for intracranial hemorrhagic side effects, and avoiding such therapy may be important.17 Frag-
ments or analogues of t-PA (such as Fb-Fb-CF) allow for the design of fibrinolytic drugs with specific characteristics that may enhance fibrinolytic effectiveness and reduce potential hemorrhagic risk. A t-PA analogue with a reduced propensity for hemorrhagic side effects and more rapid and sustained fibrinolytic action may be better suited for treating large-vessel thromboembolic stroke than native t-PA.

We used streptokinase in our rabbit model of cerebral embolization because this drug has been shown to be effective in the treatment of acute MI with a very low risk for intracranial hemorrhagic side effects. The therapeutic efficacies of streptokinase and t-PA for the treatment of MI appear at this time to be equivalent, with similar risks for major hemorrhagic complications. In our model, streptokinase at doses comparable to those used in human MI trials was ineffective in promoting reperfusion. Hemorrhagic complications were minimal, and fibrinogen consumption was modest. Centeno et al have previously observed that intra-arterial streptokinase therapy for common carotid artery occlusion in rabbits was ineffective. These two studies imply that rabbit models of stroke may be inappropriate in assessing the effectiveness of streptokinase. Perhaps treatment with higher doses of streptokinase or the use of a stroke model in another species may be necessary. A recent study by Clark et al in another rabbit model of embolic stroke suggests that streptokinase may have substantial intracranial hemorrhagic complications when given 6 hours after stroke onset.

In previous trials in patients with acute stroke, exogenous fibrinolytic agents were associated with frequent hemorrhagic side effects, but several recent nonrandomized studies with these agents given intra-arterially have demonstrated reperfusion with infrequent hemorrhagic side effects. Although exogenous fibrinolytic agents given intravenously appear to be efficacious in the treatment of acute MI, their potential application in the treatment of acute stroke remains uncertain and controversial. Additional laboratory investigations in other models of stroke in different species will be necessary before clinical trials are contemplated on the basis of effective and safe animal data. We believe that fibrin-specific fibrinolytic therapy with t-PA or a t-PA analogue offers greater promise than that with exogenous fibrinolytic agents for clinical trials in thromboembolic stroke.

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References


Key Words • animal models • plasminogen activator, tissue-type • streptokinase • rabbits
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