EXPERIMENTAL STUDIES

Thrombolysis With Recombinant Tissue Plasminogen Activator in Atherosclerotic Thrombotic Occlusion

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Human tissue plasminogen activator holds promise for the dissolution of coronary thrombi by intravenous administration and without systemic anticoagulation. Prior animal experiments have been conducted only in vessels without disease. To test the thrombolytic efficacy of recombinant tissue plasminogen activator in the presence of diseased intima, an established model of atherosclerosis was utilized. The aorta of 16 New Zealand white rabbits (2 to 3 kg) was made atherosclerotic by balloon endothelial denudation and concurrent 1% cholesterol feeding for 8 weeks. An aged (24 hour) heterologous (human) clot, labeled with I-125 fibrinogen was injected into the distal aorta and produced thrombotic occlusion. After 1 hour of thrombosis (control period), recombinant tissue plasminogen activator (100,000 IU = 1 mg protein, n = 6) or streptokinase (100,000 IU, n = 5) or saline solution (n = 5) was systemically infused over 30 minutes.

Serial blood samples, obtained to determine fractional change in blood radioactivity over time, showed a fourfold increase of blood radioactivity after tissue plasminogen activator and streptokinase infusion compared with the control period (47,400 ± 3,300 [mean ± standard error] versus 11,800 ± 300 counts/min, p < 0.001). Time to 50% of maximal thrombolysis was 41 ± 14 minutes (± standard deviation) for tissue plasminogen activator versus 63 ± 16 minutes for streptokinase (p < 0.01). In six of six rabbits receiving tissue plasminogen activator and four of five rabbits receiving streptokinase, reestablishment of distal aortic flow was detected via the indwelling catheter within 25 minutes of drug infusion. In the five control rabbits, reperfusion did not occur and there was no change in blood radioactivity over time (up to 2 hours). Gross and histologic studies of the aortas confirmed thrombolysis and diffuse intimal disease at the site of balloon denudation. Thus, in atherosclerotic arteries, intravenous infusion of recombinant tissue plasminogen activator rapidly lysed human thrombus and resulted in reperfusion.
lation to human atherosclerotic, thrombotic occlusion is not readily made. The present study was performed to determine the thrombolytic efficacy of recombinant tissue plasminogen activator and streptokinase in the presence of diseased intima.

Methods

Animal model. We used an established model of atherosclerosis (16–21). Twenty-three New Zealand male white rabbits (2 to 3 kg) were fed an atherogenic (1% cholesterol) diet (19–21) for 10 weeks. After 2 weeks of such feeding, aortic balloon endothelial denudation was performed as follows. Rabbits were anesthetized with ketamine and promazine intramuscularly. A femoral artery was exposed through a 2 cm inguinal incision and a 5F balloon catheter was introduced into the lumen of the artery and advanced to the level of the aortic arch over a distance of 20 cm. The balloon was inflated to 0.5 ml and the catheter was pulled back to within 3 cm of its site of introduction. This procedure was repeated twice. The artery was ligated and the skin wound repaired. Each rabbit received 500,000 units of benzyl penicillin intramuscularly at the end of the denudation procedure.

Acute thrombus preparation. After the rabbits had received the atherogenic diet for a mean time interval of 78 ± 8 days, the thrombolytic experimental procedure was performed. Twenty-four hours before each experiment, the thrombus was prepared as follows. Ten ml of human blood was aspirated into a syringe and 1 hour was allowed for clot formation. All but 2 ml of clot was removed from the syringe and the clot was transferred to a 3 ml syringe. To this thrombus was added 0.1 ml (approximately 500,000 counts/min) of commercially available I-125-labeled human fibrinogen (Amersham) and the clot was refrigerated at 5°C over a 24 hour period. On the day after clot preparation, the rabbits were maintained under deep anesthesia with ketamine and promazine administered intramuscularly. A 21 g butterfly needle was introduced into a marginal ear vein for blood sampling and infusion of the study drug. Sodium iodide (1 ml of a 2% solution) was administered intravenously to block thyroidal uptake of the isotope.

The descending aorta was exposed through a 5 cm midline abdominal incision and reflection of the peritoneal contents. A short 5F end hole catheter was specially designed for the experiment and introduced into the aortic lumen and advanced 2 to 3 cm. Just distal to the catheter insertion point, the aorta was tied to prevent anterograde flow except through the catheter lumen (Fig. 1). Catheter flow was established and the labeled and aged clot was counted immediately before injection. After baseline blood samples were obtained, the clot was injected into the aorta and the catheter was gently flushed with saline solution. Cessation of flow to the distal aortic catheter was observed in each animal as soon as the clot was injected.

Figure 1. Schematic of thrombolytic experiment. The rabbit’s marginal ear vein was used for both blood sampling of I-125 radioactivity and administration of the thrombolytic agent. The distal aorta was cannulated and ligated distal to the catheter insertion, and the aged human thrombus was injected into the aorta. The aortic catheter was monitored for reestablishment of antegrade flow.

Thrombolytic experiment. Blood samples of I-125 radioactivity were obtained every 15 minutes for 1 hour before infusion of the study drug. At the end of the hour control period, 1 mg (= 100,000 IU) of recombinant tissue plasminogen activator (Genentech) or 100,000 IU streptokinase (Hoechst-Roussel) or saline solution was intravenously administered by a Harvard pump over 30 minutes. For 2 hours from the start of the infusion, serial blood samples were again drawn every 15 minutes. Flow to the distal aortic indwelling catheter was monitored. After the last blood sample, the rabbit was given 2,000 U of heparin before sacrifice with air embolism. After sacrifice, the aorta was removed intact and cross sections of the vessel through the diseased portion of the aorta were obtained.

Sixteen animals were successfully studied; six with recombinant tissue plasminogen activator, five with streptokinase and five with saline solution. Of 23 rabbits successfully deendothelialized, 7 were excluded as follows: 4 died from an overdose of anesthetic before study drug infusion, 1 died from aortic rupture and 2 rabbits did not develop aortic clot adherence so that cessation of the flow did not occur.

Statistical analysis. Paired t tests were used to test for significant differences in radioactivity release between baseline and drug infusion periods. Analysis of variance for repeated measurements and analysis of covariance were used to test the hypothesis that there was a significant difference in rate of thrombolyis (as a reflection of fractional change in blood radioactivity). The Student’s t test was used to reject the null hypothesis that there was no difference in time to half maximal thrombolysis between the two study drugs. Unless otherwise noted, the values reported are mean ± standard error of the mean.
Figure 2. Blood I-125 radioactivity in counts/min over time is shown for tissue plasminogen activator (TPA), streptokinase (SK) and control rabbits (saline). There was no detectable increase in blood radioactivity during the 1 hour period of thrombotic occlusion, before administration of the thrombolytic agent or for 2.5 hours after infusion in the saline-treated rabbits. Both tissue plasminogen activator and streptokinase led to a fourfold increase in blood radioactivity, but the rate of thrombolysis (reflected by release of I-125 into blood) was faster for tissue plasminogen activator than for streptokinase ($p = 0.03, F = 24.3$ by analysis of covariance and $p < 0.01$ by ANOVA with repeated measures). Values are mean ± standard deviation.

Results

Thrombolysis. During the 1 hour control period (before study drug infusion), there was negligible variation in the repeated measurements of blood radioactivity for each animal group (Fig. 2). In the recombinant tissue plasminogen activator group, I-125 radioactivity ranged from $10,500 ± 1,000$ to $11,500 ± 500$ counts/min; in the streptokinase group from $11,600 ± 400$ to $12,300 ± 250$ counts/min and in the control group from $10,400 ± 350$ to $11,600 ± 700$ counts/min. Counts obtained during and 90 minutes after study drug infusion showed no detectable increase in the saline solution control group (minimum $11,200 ± 500$ versus maximum $12,200 ± 500$, $p = not significant$). However, recombinant tissue plasminogen activator led to a peak fourfold increase in I-125 blood radioactivity compared with saline ($47,400 ± 3,300$ versus $11,800 ± 300$ counts/min, $p < 0.001$). Similarly, streptokinase resulted in a 4.2 times increase in blood radioactivity ($49,600 ± 1,600$ versus $11,800 ± 300$ counts/min, $p < 0.001$). The peak counts of recombinant tissue plasminogen activator compared with streptokinase were not significantly different.

The data for the 2 hour period, beginning with the onset of drug infusion, are displayed in Figure 3. Calculating the fractional change in blood radioactivity tends to minimize the effects of initial differences in the prepared clot radioactivity, which varied 21% among the 16 experiments (minimum $420,000$ counts/min; maximum $530,000$ counts/min).

Figure 3. The fractional increase in blood radioactivity over time corrects for the initial differences in the prepared clot radioactivity, which varied 21% among the 16 experiments. Both tissue plasminogen activator (TPA) and streptokinase (SK) led to a fourfold increase of I-125 blood radioactivity compared with the control experiments. The peak in I-125 blood radioactivity between tissue plasminogen activator and streptokinase is not significantly different. Values are mean ± standard deviation.
Table 1. Results in Three Experimental Groups

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Thrombolysis Time to 1/2 Max (minutes)</th>
<th>Reperfusion</th>
<th>Bleeding</th>
<th>Residual Thrombus</th>
</tr>
</thead>
<tbody>
<tr>
<td>rTPA (n = 6)</td>
<td>41 ± 14*</td>
<td>6 of 6</td>
<td>0 of 6</td>
<td>5 of 6</td>
</tr>
<tr>
<td>SK (n = 5)</td>
<td>63 ± 16*</td>
<td>4 of 5</td>
<td>3 of 5</td>
<td>5 of 5</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>—</td>
<td>0 of 5</td>
<td>0 of 5</td>
<td>5 of 5</td>
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*p < 0.01, unpaired t test; rTPA = recombinant tissue plasminogen activator; SK = streptokinase; thrombolysis time to 1/2 max = minutes of half-maximal 1-125 blood radioactivity.

Comparison of recombinant tissue plasminogen activator with streptokinase rate of thrombolysis, reflected by the rate of increase in blood radioactivity over time, was done by analysis of variance for repeated measures and analysis of covariance, both yielding the same conclusion: thrombolysis mediated by tissue plasminogen activator was more rapid (by covariance p = 0.03, by repeated measures p < 0.01). Another way to demonstrate the contrast with respect to time is to select a point and use the t test. For the time to 50% maximal thrombolysis, recombinant tissue plasminogen activator was significantly shorter: 41 ± 14 minutes (± standard deviation) versus 63 ± 16 minutes (p < 0.01).

Reperfusion and bleeding complications (Table 1). Reperfusion was determined by reestablishment of antegrade aortic flow to the indwelling distal catheter. This occurred in all six rabbits treated with recombinant tissue plasminogen activator and in four of five rabbits treated with streptokinase but did not occur in any control animal. In the 10 thrombolytic experiments in which reperfusion was noted, this occurred within 25 minutes but in not less than 15 minutes. No aorta, once reperfused, was noted to reocclude during the remaining 90 minutes of blood sampling period.

Bleeding during the experiment was not apparent in any rabbit receiving recombinant tissue plasminogen activator or saline solution. However, two rabbits treated with streptokinase had excessive wound and retroperitoneal bleeding during the last 30 minutes of the experiment and one rabbit had intraperitoneal bleeding profusely at 15 minutes after the streptokinase was infused, leading to exsanguination and early termination of the experiment.

Pathologic findings (Fig. 4 to 7). Postmortem gross and histologic studies of each aorta were performed. An occlusive, extensive thrombus was identified in each control animal (Fig. 4 and 5). Small fragments of clot were demonstrated in all but one rabbit receiving recombinant tissue plasminogen activator and in all rabbits given streptokinase, without appreciable difference in amount of material between these two groups.

Atherosclerosis, grossly resembling human fatty streaks and fibrous plaques, was present in each distal aorta. Diffuse, advanced, circumferential intimal involvement with yellowish discoloration and raised plaque was noted grossly throughout the descending thoracic and abdominal aortas, most intense in the 6 to 8 cm proximal to iliac bifurcation (Fig. 4).

Histologic studies revealed luminal filling with clot material in the control rabbits in contrast to little or no thrombus in the recombinant tissue plasminogen activator or streptokinase groups (Fig. 5). There was extensive circumferential involvement of the atherosclerotic plaque noted in all distal aortic sections of each animal; little or no uninvolved intimal surface was present. When a clot was present histologically, it appeared to adhere to intimal plaque (Fig. 6).

The atherosclerotic plaque was at least three to six times the thickness of the underlying arterial wall (Fig. 7). Within the typical plaque, extensive atheronecrosis was manifested by cellular dropout and foam cells (Fig. 7). In several specimens (two rabbits in each group), dissection of the media with fragmentation of the internal elastic membrane was observed. Particularly noteworthy was the involvement of the media by foam cells and the presence of calcified ma-
Figure 5. Low power (×4, reduced by 15%) distal aortic histologic cross section stained with hematoxylin-eosin. A. Occlusive thrombus within circumferential atherosclerotic intima in a control rabbit. B. No residual thrombus was present in the aorta of this rabbit treated with tissue plasminogen activator. The similar extent of circumferential atherosclerotic plaque in both aortas is demonstrated.

Discussion

Recombinant human plasminogen activator. This study shows that intravenously infused recombinant human plasminogen activator was highly effective in achieving thrombolysis in atherosclerotic arteries. Although the extent of thrombus dissolution (as reflected by peak fractional blood radioactivity) and reperfusion results for recombinant tissue plasminogen activator and streptokinase were not different, the rate of thrombolysis with plasminogen activator was more rapid. Prior studies with tissue plasminogen activator have documented its clot selectivity. Compared with streptokinase and urokinase, which have no specific affinity for fibrin, tissue plasminogen activator binds with very high affinity (\(K_m = 0.14 \text{ mM}\)) to the fibrin-plasminogen complex. This property probably explains the enhanced rapidity of plasminogen activator-mediated thrombolysis that was found in our study. Further support of the local lysis concept is rendered by the lack of depletion of circulating fibrinogen, plasminogen or alpha-2-antiplasmin in all prior studies of tissue plasminogen activator.

Figure 6. Histologic cross section of the aorta of a control rabbit shows thrombus adherent to the atherosclerotic intima (hematoxylin-eosin ×10, reduced by 49%). Residual clot fragments, adherent to the intima, were identified in all thrombolytic experiments with streptokinase and in all but one with tissue plasminogen activator.

Thrombolysis. The time of onset of clot lysis in the current study was noted by time to reperfusion and fractional change of blood radioactivity. The time to half maximal thrombolysis was 50% longer in the streptokinase-treated rabbits. However, the order of magnitude of our results is in contrast with that of Bergmann et al. (15), who found that in a dog coronary artery thrombotic model there was a 10-fold increase in time to onset of lysis of canine thrombus by intravenously administered streptokinase compared with melanoma-derived tissue plasminogen activator. The differences in the rate of thrombolysis observed between the two studies may be the result of dissimilar animal models. But in addition, the role of the atherosclerotic intima in adhering to clot may account, at least in part, for impeding tissue plasminogen activator-mediated thrombolysis.

In the current study, a disparity is noted between the short half-life of tissue plasminogen activator (2.4 minutes) (24) and the ongoing thrombolysis detected through blood radioactivity. Despite only a 30 minute duration of infusion, continued thrombolysis mediated by plasminogen activator...
was evident for over 60 minutes. This finding is consonant with those of Matsuo, Korninger and Collen and their colleagues (8–10). One possible explanation for a disproportionately long time of thrombolysis mediated by tissue plasminogen activator, compared with its short half-life, may be changes in plasminogen activator binding once reperfusion is achieved. Once antegrade flow is reestablished, considerably more surface area of clot is exposed to plasminogen activator and, thus, much lower levels of this enzyme may be associated with its continued ability to digest fibrin. Alternatively, local activation may persist for a long period of time after delivery of the agent to the clot surface. One other explanation invokes endogenous effects, perhaps native plasminogen activator once partial dissolution is accomplished. Thus, although it might be expected that intravenous tissue plasminogen activator could allow minute to minute titration of fibrinolytic activity, biologic activity (assessed by thrombolysis) may continue for some period of time and at least up to 1 hour in the current study.

**Role of atherosclerosis.** The underlying atherosclerotic lesion is a key factor in the period immediately after successful thrombolysis. Most patients who undergo successful coronary recanalization are left with a critical stenosis (25), often requiring definitive intervention to prevent reocclusion. In the first six patients with acute myocardial infarction who received melanoma-derived plasminogen activator, two developed reinfarction and three required coronary artery bypass surgery (22). Although the atherosclerotic plaque generated in the present study was circumferential and typically three to six times the thickness of the aortic wall, the lesions were not greater than 50% stenotic and reocclusion was not observed. Three factors appear to establish a predisposition for reocclusion: 1) underlying critical atherosclerosis, 2) the short half-life of tissue plasminogen activator, and 3) residual thrombus, observed in all but one of the experiments in the current study. Should reocclusion be a frequent clinical occurrence, the role of recombinant tissue plasminogen activator in the future may indeed be to "bide time" before either percutaneous transluminal angioplasty or coronary artery grafting is performed.

**In the present study we did not quantify actual thrombolysis.** Prior studies (8–10) have used an I-125 isotope recovery balance to calculate percent thrombolysis. However, these calculations involve a quantitative assumption of extravascular distribution and urine radioactivity. In studies that have utilized such an analysis, monitoring of reperfusion was not possible and gross or histologic studies were not performed. The pathologic and reflow data in the present study were well correlated with the fractional change in blood radioactivity obtained for each experiment. The finding of residual thrombus, albeit in small amounts, in the recombinant tissue plasminogen activator experiments may be a common finding when diseased atherosclerotic vessels are subject to thrombolysis. The uniform dose of intravenous recombinant human tissue plasminogen activator used in this study (0.375 mg/kg for 30 minutes) is comparable with an effective therapeutic dose that has been used in patients to achieve coronary thrombolysis (12) and is equimolar, but not necessarily equipotent, to the streptokinase dose that was utilized. It remains possible, however, that a greater dosage or a longer infusion period may lead to more complete thrombolysis.

**Implications.** Recombinant tissue plasminogen activator has many properties that approach an ideal coronary thrombolytic agent. The present study confirms its ability to lyse human thrombus in experimentally induced atherosclerotic intima and points to possible advantages over streptokinase with respect to time required and bleeding complications. Studies are currently underway to test this promising agent in patients with acute myocardial infarction.

**References**


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