Enhancement of thrombolysis with tissue-type plasminogen activator by pretreatment with heparin


ABSTRACT The effect of pretreatment with heparin on lysis of arterial thrombi by tissue-type plasminogen activator (rt-PA) was studied in 19 dogs. Copper coil–induced carotid artery thrombi were weighed, inserted into the femoral arteries, and exposed to a 15 min infusion of rt-PA at 10 μg/kg/min either with (n = 6 thrombi) or without pretreatment with a 200 unit/kg bolus of heparin (n = 6 thrombi). The infusion of rt-PA without pretreatment reduced the thrombus weight by 27.6 ± 7.4%, while infusion of rt-PA with pretreatment reduced it by 79.1 ± 12.3% (p < .0001). To test the hypothesis that heparin enhanced thrombolysis by preventing continued incorporation of new fibrin into the thrombus during thrombolysis we repeated the experiments using pretreatment with 8 U/kg of ancrod, which rapidly depletes fibrinogen. Pretreatment with ancrod (n = 6 thrombi) depleted fibrinogen and enhanced the lytic effect of rt-PA to a similar degree as pretreatment with heparin, resulting in a 67.6 ± 12.3% (NS) decrease in thrombus weight. We conclude that heparin significantly enhances the thrombolytic effect of rt-PA, probably by preventing new fibrin formation and its incorporation into the thrombus during lysis.


IT HAS BEEN SHOWN that thrombi continue to grow by incorporation of new fibrin for up to 72 hr, and that this growth is particularly fast during the first few hours. Thrombus growth that continues during lysis increases the total amount of thrombus to be lysed during thrombolytic treatment and thereby probably delays recanalization of the infarct-related artery and reduces the potential for myocardial salvage in patients with acute myocardial infarction. Heparin has been shown to prevent new fibrin formation and its incorporation into the thrombus.

Accordingly, the purpose of this study was to investigate whether lysis of arterial thrombi after administration of tissue-type plasminogen activator (rt-PA) could be enhanced by pretreatment with heparin.

Methods

Experimental preparation. The study was performed in closed-chest mongrel dogs, 16 to 28 kg in body weight, anesthetized with 30 mg/kg sodium pentobarbital and additional small doses as needed. The dogs were intubated and artificially ventilated with room air with use of a Harvard respirator. An electrocardiographic lead was continuously monitored. Both carotid arteries were exposed and a 25 mm long copper coil of slightly conical shape, 2 mm in outside diameter, was inserted into each artery. The coils were weighed before insertion. One hour after implantation the coils with the thrombi were removed, weighed again to determine the initial thrombus weight, and then inserted into the dissected femoral arteries of the same animal. Shortly before insertion of the coils, the exposed femoral arteries and all their branches had been ligated. After insertion the main femoral artery proximal to the coil and one side branch distal to the coil were released to permit blood flow around the thrombus, which was evidenced in all experiments by the presence of distinctly palpable pulsations in the distal side branch. All the other branches remained ligated during the study to prevent unrecognized embolization of the thrombus disintegrating during lysis.

Protocol of the study. Six series of experiments were performed: (1) the control group received neither rt-PA, heparin, or ancrod, (2) the heparin group received a 200 unit/kg intravenous bolus of heparin only, (3) the ancrod group received an 8 unit/kg intravenous bolus of ancrod only, (4) the rt-PA group received only a 15 min infusion of rt-PA at a rate of 10 μg/kg/min, (5) the rt-PA plus heparin group received a 200 unit/kg bolus of heparin 2 to 4 min before the 15 min infusion of rt-PA, and (6) the rt-PA plus ancrod group received an 8 unit/kg bolus of ancrod 2 to 4 min before the 15 min infusion of rt-PA. The dosage of rt-PA used in our study was shown to be effective in previous canine studies. The dosage of heparin used in the study was found to prevent incorporation of radiolabeled fibrinogen into canine coronary thrombi. The dosage of ancrod was chosen based on information from the study in dogs by Kreisskott and Hofmann.

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Precisely 15 min after the administration of heparin or ancrord in dogs not receiving rt-PA or after the initiation of rt-PA infusion, the proximal trunk of the femoral artery and the open side branch of each dog were clamped and the coil with the residual thrombus was immediately removed and reweighed to determine the weight of the residual thrombus. At the end of the experiment all the branches of the femoral artery were dissected and checked for the presence of embolized thrombus fragments. The percent change in the initial thrombus weight was determined by dividing the difference between the initial weight of the thrombus and its residual weight by its initial weight.

**Drugs.** DNA rt-PA (100,000 U/mg) was kindly supplied by Genentech Inc., South San Francisco, CA. Ancrod (Arvin, 70 U/ml), a purified protein fraction from the venom of the Malaysian pit viser, was supplied by Knoll Pharmaceutical, Whippany, NJ, and heparin (1000 units/ml) was derived from porcine intestines by Elkins-Sinn, Cherry Hill, NY.

**Assessment of fibrinogen level.** Plasma fibrinogen level was assayed for every experimental group except the control group and the group that received heparin only, since in these two groups no change in fibrinogen level was anticipated. In groups that received rt-PA alone or with heparin or ancrord the fibrinogen level was assayed by both the thrombin-clotting9 and the sulphate precipitation method10 and by the thrombin-clotting method only in the group that received ancrord alone. Blood for the determination of fibrinogen level was drawn into test tubes containing aprotinin and 3.8% sodium citrate before and 5, 10, and 15 min after the start of rt-PA infusion. In animals that received only heparin or ancrord blood samples were drawn before and 5, 10, and 15 min after administration of the drug and aprotinin was omitted from the tubes. In animals that received rt-PA with heparin or ancrord, the first blood sample was taken before the administration of heparin or ancrord.

**Assessment of rt-PA level.** Plasma samples for determination of rt-PA levels were taken before and after 5, 10, and 15 min of rt-PA infusion. An assay with an enzyme-linked immunoosorbent assay (ELISA) based on monoclonal antibodies to rt-PA was used.11

**Statistical analysis.** Continuous Gaussian variables are described by their mean ± SD. The one-way analysis of variance was used for comparing the initial thrombus weight and its percent change between subgroups. Differences in the initial thrombus weight were controlled for by an analysis of covariance. Analysis was performed using BMDP biostatistical software and a p value of <.05 was considered to indicate a statistically significant difference.

**Results**

Forty-four thrombi were retrieved from the carotid arteries of 22 dogs. In two dogs the initial weight of the thrombus formed in one coil was minimal and judged too small to allow accurate assessment of change in weight. In three dogs the two coils removed from the carotid arteries could not be reinserted into the femoral arteries. The results are therefore based on 36 thrombi from 19 dogs.

The initial thrombus weight averaged 70 to 80 mg in all except the heparin group, in which it was 44 mg, but the difference was not statistically significant (p = .3). In the control group the weight of the thrombi increased by 40% on the average during the 15 min of the experiment. When administered alone, heparin, ancrord, and rt-PA produced an average 25% to 29% decrease in the initial thrombus weight over the same period. When heparin or ancrord was followed by rt-PA, there was an approximately threefold increase in percent lysis, to 70% to 80%. There was no evidence of peripheral embolization in any experiment. Details of all results are presented in table 1. Figure 1

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect of treatment on weight of thrombus</th>
<th>Initial weight (mg)</th>
<th>Residual weight (mg)</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.8</td>
<td>113.2</td>
<td>+13.4</td>
</tr>
<tr>
<td>Heparin</td>
<td>14.4</td>
<td>11.7</td>
<td>-18.7</td>
</tr>
<tr>
<td>Ancrod</td>
<td>78.0</td>
<td>54.4</td>
<td>-30.3</td>
</tr>
<tr>
<td>rt-PA + heparin</td>
<td>138.9</td>
<td>106.1</td>
<td>-23.6</td>
</tr>
<tr>
<td>rt-PA + ancrord</td>
<td>79.6 ± 24.2</td>
<td>16.7 ± 7.3</td>
<td>-79.1 ± 12.3</td>
</tr>
</tbody>
</table>

Data for every separate experiment and the group mean ± SD are shown. The difference between the initial and residual weight is shown as percent change from the initial weight.
illustrates the percent change in thrombus weight for all groups. The difference between changes in weight was significant even after analysis of covariance to control for the differences in initial weight of thrombus (p < .0001).

The fibrinogen levels obtained by both methods and expressed as percentages of the preinfusion values are listed in table 2. As expected, ancrod depleted fibrinogen virtually completely in all experiments. rt-PA alone or in combination with heparin decreased fibrinogen only moderately. Plasma rt-PA levels were determined in all groups that received rt-PA; the results are presented in table 3. The plasma concentrations achieved were similar in all three groups.

### TABLE 2
Fibrinogen levels (% preinfusion values)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Method</th>
<th>Infusion time (min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancrod</td>
<td>Claus</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>rt-PA</td>
<td>Claus</td>
<td>98 ± 9</td>
<td>98 ± 9</td>
<td>89 ± 22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rampling</td>
<td>78 ± 38</td>
<td>83 ± 23</td>
<td>65 ± 48</td>
<td></td>
</tr>
<tr>
<td>rt-PA + heparin</td>
<td>Claus</td>
<td>114 ± 8</td>
<td>86 ± 33</td>
<td>75 ± 13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rampling</td>
<td>139 ± 26</td>
<td>76 ± 48</td>
<td>73 ± 17</td>
<td></td>
</tr>
<tr>
<td>rt-PA + ancrod</td>
<td>Claus</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rampling</td>
<td>9 ± 7</td>
<td>5 ± 4</td>
<td>2 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD for percent of preinfusion fibrinogen level at 5, 10, and 15 min after the beginning of rt-PA infusion.

### TABLE 3
rt-PA levels (ng/ml)

<table>
<thead>
<tr>
<th></th>
<th>Infusion time (min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>rt-PA</td>
<td>714 ± 522</td>
<td>1255 ± 728</td>
<td>1480 ± 898</td>
<td></td>
</tr>
<tr>
<td>rt-PA + heparin</td>
<td>855 ± 90</td>
<td>1397 ± 142</td>
<td>1820 ± 523</td>
<td></td>
</tr>
<tr>
<td>rt-PA + ancrod</td>
<td>834 ± 324</td>
<td>1161 ± 191</td>
<td>1010 ± 208</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD for rt-PA level (in ng/ml) 5, 10, and 15 min after the beginning of rt-PA infusion.

### Discussion

The results of this study confirm that pretreatment with heparin can enhance the thrombolytic efficacy of rt-PA. This finding is consistent with our earlier observation that pretreatment with heparin markedly accelerated the lysis of occlusive coronary artery thrombi by streptokinase or urokinase in dogs. Our findings are in agreement with experimental and clinical observations that administration of heparin allowed a significant reduction of the dosage of streptokinase or urokinase necessary for the treatment of pulmonary emboli. The fact that pretreatment with heparin enhances the effectiveness not only of rt-PA but also of streptokinase and urokinase suggests that the mechanism of enhancement is not specific for rt-PA. In light of our experiments and reports from other laboratories, it is likely that heparin enhances thrombolysis by...
blocking formation of new fibrin and its incorporation into the thrombus during thrombolytic therapy. The actual decrease in the weight of the thrombi after administration of heparin can be explained by the potent endogenous thrombolysis that occurs in dogs\textsuperscript{15} when it is unopposed by continued thrombus growth.

The concept that heparin enhances thrombolysis by preventing formation of new fibrin is further supported by the fact that pretreatment with ancród produced a quantitatively similar enhancement of the thrombolytic effect of rt-PA as did pretreatment with heparin. Ancréd prevented fibrin formation and enhanced the thrombolytic effect of rt-PA by the virtually complete depletion of fibrinogen. Enhancement of thrombolysis by ancréd was also observed in patients treated with streptokinase for deep-vein thrombosis\textsuperscript{16} and in those treated with urokinase for pulmonary emboli.\textsuperscript{17} Heparin\textsuperscript{18} and ancréd\textsuperscript{19} are known to have no direct fibrinolytic activity, but were shown to stimulate the release of tissue plasminogen activator.\textsuperscript{20, 21} The amounts released are negligible when rt-PA is administered in therapeutic doses.\textsuperscript{22}

The favorable effect of depletion of fibrinogen on the rate of thrombolysis was also observed in our experimental studies with urokinase\textsuperscript{23} and in clinical studies by Rothbard et al.\textsuperscript{24} and Burket et al.\textsuperscript{25} These authors reported higher rates of coronary artery recanalization in patients in whom the depletion of fibrinogen by intracoronary streptokinase was more extensive. Furthermore, in Burket's study intracoronary urokinase depleted fibrinogen less than did streptokinase and also produced a lower rate of recanalization. In these two studies the patients did not receive heparin. In contrast, in studies of intracoronary streptokinase by Cowley and White and their colleagues,\textsuperscript{26, 27} the differences in the degree of fibrinogen depletion had no effect on the rate of recanalization. In these two studies patients were pretreated with heparin, suggesting that depletion of fibrinogen does not further enhance thrombolysis.

The apparent discrepancy between the efficacy of rt-PA administered without heparin in this study and that reported in previous studies\textsuperscript{6, 7} can be explained by the fact that these studies administered heparin before rt-PA for anticoagulation.

Potential limitations of the study. The method of placing copper coils in the carotid arteries and subsequently transferring them with the developed thrombi into the femoral arteries was designed to allow quantification of lysis. It is possible that the transfer of the coil with the thrombus from the carotid artery into the femoral artery stimulated an accelerated growth of the thrombus, as observed in our control experiments. Although this may have affected the quantity of the observed changes, it seems unlikely that it affected the direction of changes and thus our conclusions.

Clinical implications. Consistent with some clinical and experimental observations, the present study strongly suggests that the thrombolytic efficacy of rt-PA can be enhanced by pretreatment with heparin or ancréd. Since the effect of heparin is readily reversible and fibrinogen depletion is considered undesirable, the use of heparin seems preferable.

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References
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