ABSTRACT Coronary thrombolysis was induced by infusion of highly purified human pro-urokinase isolated from a transformed kidney cell line (ACHN) or by infusion of urokinase of urinary origin in anesthetized dogs with 1-hr-old clots in the left anterior descending coronary artery. The clots were induced with a copper coil and thrombolysis was detected by repeat coronary angiography. Intravenous infusion of pro-urokinase at a rate of 10 μg/kg/min for 30 min in two dogs did not induce thrombolysis, which was only obtained after 8 and 15 min of its subsequent intracoronary administration. Intravenous infusion of pro-urokinase at a rate of 20 μg/kg/min for 30 min in four dogs induced coronary thrombolysis within 23 ± 2 min (mean ± SEM). This was not associated with systemic fibrinolytic activation because the α₂-antiplasmin and fibrinogen levels did not decrease. Intravenous infusion of urokinase at a rate of 10 μg/kg/min for 30 min elicited thrombolysis in four of seven dogs within an average of 19 ± 2 min. In the other three dogs thrombolysis was only obtained within 11 ± 3 min of its subsequent intracoronary infusion. Administration of urokinase was associated with systemic fibrinolytic activation as evidenced by a decrease of α₂-antiplasmin to about 10% and of fibrinogen to 43 ± 13% of the preinfusion value. It is concluded that intravenous infusion of pro-urokinase at a sufficiently high rate produces coronary thrombolysis without systemic fibrinolysis in dogs.


CORONARY THROMBOLYSIS has been widely investigated as a means to abort the evolution of acute transmural myocardial infarction. Several attempts have also been made to develop fibrin-specific thrombolytic agents that would be more effective than streptokinase and would induce less systemic fibrinolysis. Thrombolytic agents with anticipated fibrin selectivity now include human tissue-type plasminogen activator (t-PA), acylated plasminogen-streptokinase complex, and pro-urokinase.

Pro-urokinase is a precursor of urokinase, first identified in tissue-culture media and later purified from urine, plasma, conditioned cell culture media, and transformed bacteria. Although the mechanism of action of pro-urokinase remains to be established, recent reports have provided evidence that it can indeed induce more clot-selective thrombolysis in animal preparations than urokinase.

In this study we used a well-characterized preparation for the study of thrombolysis after coronary occlusion in dogs to evaluate the relative fibrinolytic effects of pro-urokinase as compared with urokinase.

Methods

Thirteen dogs weighing approximately 20 kg were anesthetized with 10 to 15 mg/kg sodium pentobarbital and artificially ventilated. Coronary thrombosis was induced by advancing a 3 to 5 mm long copper coil attached to an intracoronary catheter via the carotid artery 3 to 5 cm into the left anterior descending coronary artery distal to the first main diagonal branch, as previously described. An occlusive thrombus formed within 5 to 10 min and was confirmed angiographically in all dogs. All of the animals studied developed electrocardiographic signs typical of ischemia. One hour after induction of the coronary thrombus, pro-urokinase or urokinase was infused via a peripheral vein at a rate of 10 μg/kg/min for 30 min. Whenever complete thrombolysis had not occurred after 30 min of infusion, the agents were administered immediately thereafter at the same rate but via the intracoronary route for an additional 15 min. This was the case in the two dogs treated with pro-urokinase and in three of the seven dogs treated with urokinase. In four dogs given pro-urokinase intravenously at a rate of 20 μg/kg/min for 30 min, complete thrombolysis was observed before the end of the infusion in three dogs and near complete thrombolysis in one. In the latter dog, reocclusion occurred within 10 min after...
the end of the infusion and recanalization was obtained by intracoronary infusion.

Thrombolysis was generally heralded by the onset of reperfusion arrhythmia and was confirmed in each animal by angiography performed at 10 min intervals. Heparin was administered via intravenous injections of 1000 U at the onset of the infusion of the thrombolytic agents and 15 and 30 min later to prevent reocclusion.14

To measure the turnover rate of pro-urokinase and the extent of systemic activation of the fibrinolytic system, blood samples were collected in citrate before and 2, 5, 10, 20, and 30 min after the onset of intravenous infusion, at the end of intracoronary infusion if one was administered, and at 2, 5, 10, 20, 30, 40, and 50 min after completion of the infusion of thrombolytic agents. Samples were cooled immediately on ice and centrifuged for immediate analysis of fibrinogen and $\alpha_2$-antiplasmin activity, as described previously.14 Circulating levels of pro-urokinase were measured on frozen plasma samples by a classic radioimmunoassay with a rabbit antibody against human urinary urokinase. Titration curves of urokinase and pro-urokinase with this antibody were superimposable.

Pro-urokinase, highly purified from a transformed human kidney cell line (ACHN), was provided by Mochida Chemical Co. The material migrated as a single band (over 90% homogeneity) with $M_r$ 54,000 on sodium dodecyl sulfate gel electrophoresis. The material could be fully activated by plasmin and displayed a specific activity of 95,000 IU/mg protein as measured on fibrin plates. The pro-urokinase preparation used in this study contained approximately 5% of activated molecules as measured by amidolytic assay. This concentration of urokinase is far below the minimum level necessary to measure any fibrinolytic effects in vivo. In addition, we have shown that the activation of plasminogen by pro-urokinase is not dependent on the presence or formation of activated urokinase.15

Urokinase (Winkinase) was a gift from Dr. G. Murano, Bureau of Biologics, Bethesda, MD.

### Results

Pro-urokinase did not induce thrombolysis within 30 min when administered intravenously at a rate of 10 $\mu$g/kg/min in two dogs. Subsequent intracoronary infusion at the same rate produced recanalization after 8 and 15 min, respectively (table 1). Intravenous infusion of pro-urokinase at a rate of 20 $\mu$g/kg/min in four dogs resulted in complete reperfusion in three dogs within 20, 21, and 22 min, respectively, as demonstrated by coronary angiography (figure 1). In one dog recanalization was nearly complete after 30 min and reocclusion occurred within 10 min after the end of the infusion. In this dog recanalization was obtained within 8 min by intracoronary infusion of pro-urokinase at the same rate.

Intravenous infusion of urokinase at a rate of 10 $\mu$g/kg/min in seven dogs produced recanalization in four of them within 19 ± 2 min. In the three other dogs recanalization was obtained within 11 ± 3 min of intracoronary infusion of urokinase at the same rate.

Analysis of the plasma levels of fibrinogen and $\alpha_2$-antiplasmin (figure 2) indicated that intravenous infusion of pro-urokinase at a rate of 20 $\mu$g/kg/min did not cause systemic fibrinolytic activation, whereas infusion of urokinase at half this rate caused extensive $\alpha_2$-antiplasmin consumption and significant fibrinogen breakdown.

### TABLE 1

**Coronary thrombolysis in dogs**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (µg/kg/min)</th>
<th>Number</th>
<th>Infusion</th>
<th>Number</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-urokinase</td>
<td>10</td>
<td>2</td>
<td>IV</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>IV</td>
<td>37 and 45</td>
<td>23 ± 2</td>
</tr>
<tr>
<td></td>
<td>Urokinase</td>
<td>10</td>
<td>7</td>
<td>IV + IC</td>
<td>38 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>19 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IV + IC</td>
<td>3</td>
<td>41 ± 3</td>
</tr>
</tbody>
</table>

IV = intravenous infusion for 30 min; IV + IC = intravenous infusion for 30 min followed by intracoronary infusion for 15 min.

aTime to complete reperfusion in three dogs and near complete reperfusion in 1 dog (mean ± SEM).

bThe reoccluded coronary artery in the dog with near complete reperfusion was recanализed within 8 min of intracoronary administration of pro-urokinase.
FIGURE 2. Changes in fibrinogen and $\alpha_2$-antiplasmin levels in plasma during intravenous infusion of pro-urokinase or urokinase in dogs with acute myocardial infarction. ■, Pro-urokinase (20 $\mu$g/kg/min for 30 min); ●, urokinase (10 $\mu$g/kg/min for 30 min). Results are mean ± SD. The measurements were performed on fresh blood samples.

The pharmacokinetics of pro-urokinase after its intravenous infusion at a rate of 20 $\mu$g/kg/min for 30 min, as measured by radioimmunoassay, are summarized in figure 3. A gradual increase of the plasma level was observed to reach a plateau level of 3.5 $\mu$g/ml. After the end of the infusion, the pro-urokinase antigen disappeared from plasma with an initial $t_\frac{1}{2}$ of 7 min, but the disappearance curve was not monotone.

In contrast to the measured plasma levels of pro-urokinase for which dilution curves were uniformly linear, those for samples containing urokinase were nonlinear when serially diluted. This observation was unexpected in view of the identical titers of the antibody to each agent as measured in buffer systems and must reflect interference with the measurement of urokinase by plasma factors such as antiproteinases.

FIGURE 3. Pro-urokinase associated antigen levels in plasma during and after intravenous infusion of pro-urokinase at a rate of 20 $\mu$g/kg/min for 30 min. Results are mean ± SD. The disappearance rate after cessation of the infusion occurs with a $t_\frac{1}{2}$ of 7 min.
which react with urokinase but not with pro-urokinase.3

Discussion

The results of this study indicate that pro-urokinase, when administered intravenously at a sufficiently high rate (20 μg/kg/min), can cause dissolution of occlusive coronary clots in dogs without systemic fibrinolytic activation. Urokinase, although causing coronary thrombolysis with at least equal potency, does so only in the presence of significant systemic fibrinolysis as reflected by decreased levels of fibrinogen and α2-antiplasmin. This confirms and extends previous observations that natural3,11 and recombinant1 pro-urokinase cause thrombolysis with increased fibrin specificity relative to their two-chain counterparts. Our observation that urokinase effects thrombolysis at relatively similar doses as pro-urokinase is contrary to those of previous studies.3,11,13 The reason is not readily apparent, although all of these previous reports used preparations of venous thrombosis, while we have made the first comparison between pro-urokinase and urokinase in arterial thrombosis.

Although t-PA was not used directly in this study, our preparation of coronary thrombolysis has been applied previously to the study of fibrinolysis induced by recombinant t-PA. Coronary reperfusion was achieved regularly within 13.7 ± 1.9 min without systemic fibrinolysis by an infusion of 10 μg/kg/min t-PA with control values from urokinase comparable to those obtained in this study. Although clearly not a controlled comparison, it would nonetheless appear that the specific thrombolytic activity of t-PA is at least twofold higher than that of pro-urokinase. Similar results were noted in a controlled comparison between the two in rabbits with jugular vein thrombosis,13 although the rabbit is a relatively urokinase-resistant species and might constitute a less-than-optimal animal preparation for such a comparison.

Pro-urokinase is rapidly removed from the circulation, with a postinfusion t1/2 in plasma of 7 min. This clearance rate is comparable to that of t-PA in dogs, which has a t1/2 of 5 min.14 The clearance rate of pro-urokinase is also comparable with that of urokinase in man (t1/2 16 min),15 considering the inverse relationship between body weight and turnover. The similar turnover rate of recombinant pro-urokinase and active urokinase was also established in rabbits.17 Therefore the maintenance of a therapeutic level of pro-urokinase in plasma will most likely, as is the case for t-PA and for urokinase, require continuous infusion.

Because of the large interspecies variability of the response to thrombolytic agents, the results obtained in dogs cannot as such be extrapolated to man. Using a system composed of a radioactive plasma clot immersed in plasma,16 we have previously confirmed this large interspecies variability but have observed comparable relative thrombolytic effects of t-PA, pro-urokinase, and urokinase within the same species. On the basis of the finding that the fibrinolytic system of man is more responsive to activation by t-PA and urokinase than that of most other mammalian species, it may be anticipated that the human fibrinolytic system may be more responsive to pro-urokinase than that of the dog.

In conclusion, this study confirms the results of previous reports that natural and recombinant pro-urokinase are more clot-selective thrombolytic agents than active urokinase in animals with venous thrombosis. We have now extended this observation to an experimental preparation of arterial thrombosis, which may be relevant for acute myocardial infarction. Whether pro-urokinase might constitute an alternative to t-PA for coronary thrombolysis in man remains to be further investigated.

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