Coronary Thrombolysis by Intravenous Infusion of Recombinant Single Chain Urokinase-Type Plasminogen Activator or Recombinant Urokinase in Baboons: Effect on Regional Blood Flow, Infarct Size and Hemostasis

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An occlusive thrombus was produced by thrombin-induced coagulation in the left anterior descending coronary artery of 18 open chest baboons. In six control animals, occlusive thrombosis persisting for 4 hours resulted in a large transmural infarct (66 ± 4% of the perfusion area, mean ± SEM). In six animals, single chain urokinase-type plasminogen activator, obtained by recombinant deoxyribonucleic acid (DNA) technology, was infused intravenously at a rate of 20 µg/kg per min for 60 minutes after approximately 45 minutes of coronary thrombosis. Persistent reperfusion occurred within 21 ± 4 minutes (mean ± SD). The mean duration of occlusion before reperfusion was 72 ± 6 minutes. Recanalization resulted in a reduction of infarct size (42 ± 4%, p < 0.01 versus control animals). Myocardial blood flow in the perfusion area of the left anterior descending coronary artery was 107% of normal 2.5 hours after recanalization. The infusion of recombinant single chain urokinase-type plasminogen activator was not associated with systemic activation of the fibrinolytic system, fibrinogen breakdown or evident bleeding.

In six baboons recombinant low molecular weight urokinase (molecular weight 33,000) was infused intravenously at a rate of 20 µg/kg per min for 60 minutes after approximately 45 minutes of coronary thrombosis. Persistent reperfusion occurred within 14 ± 5 minutes (p < 0.05 versus recombinant single chain urokinase-type plasminogen activator). The mean duration of occlusion was 69 ± 14 minutes. Recanalization resulted in a reduction of infarct size (45 ± 8%, p < 0.05 versus control animals), and myocardial blood flow in the perfusion area of the left anterior descending coronary artery was 97% of normal 2.5 hours after recanalization. Infusion of recombinant low molecular weight urokinase was associated with complete fibrinogen breakdown, a decrease of alpha2-antiplasmin to less than 20% and profuse bleeding from the surgical wounds.

It is concluded that intravenous infusion of recombinant single chain urokinase-type plasminogen activator may recanalize thrombosed coronary vessels without inducing systemic lysis. Recombinant low molecular weight urokinase appears to have a comparable or somewhat higher thrombolytic effect but is associated with extensive systemic fibrinolytic activation.

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breakdown when infused intravenously at a sufficiently high rate (7).

In the present study, we used a primate species (baboon) to establish whether high dose systemic infusion of single chain urokinase-type plasminogen activator, obtained by recombinant DNA technology (10), can induce coronary thrombolysis without the association of systemic fibrinolytic activation, and to evaluate the beneficial effect of reperfusion on the ischemic ventricle. Therefore, coronary angiography was supplemented with measurements of regional myocardial blood flow and determination of infarct size 4 hours after the coronary occlusion.

**Methods**

**Experimental preparation.** Eighteen baboons (*Papio anubis*) of either sex, weighing 7 to 12 kg, were anesthetized with ketamine hydrochloride (Ketalar, Duphar, Amsterdam, The Netherlands), 10 mg/kg body weight and atropine, 0.06 mg/kg intramuscularly, before premedication with diazepam (Valium, Hoffmann-LaRoche, Basel, Switzerland), 5 mg intramuscularly. After endotracheal intubation, the lungs were ventilated with a Bird Mark 7 respirator (Bird Corporation). Anesthesia was maintained with pentobarbital (Nembutal, Abbott Laboratories), 30 mg/kg intravenously. Blood gas analysis was performed repeatedly throughout the experiment using a pH blood gas microanalyzer (166; Corning Glass Works). Catheters were inserted into the descending aorta and the right atrium through the right femoral artery and vein for the measurement of aortic and central venous pressures. A catheter (5F Lehman, USCI) was inserted through the left carotid artery and angiography of the right and left coronary arteries was made. After endotracheal intubation, the lungs were ventilated with a Bird Mark 7 respirator (Bird Corporation). Anesthesia was maintained with pentobarbital (Nembutal, Abbott Laboratories), 30 mg/kg intravenously. Blood gas analysis was performed repeatedly throughout the experiment using a pH blood gas microanalyzer (166; Corning Glass Works). Catheters were inserted into the descending aorta and the right atrium through the right femoral artery and vein for the measurement of aortic and central venous pressures. A catheter (5F Lehman, USCI) was inserted through the left carotid artery and angiography of the right and left coronary arteries was made. Next, a sternotomy was performed and the heart was suspended in a pericardial cradle. A small tube was inserted into the left atrium to measure left atrial pressure and to inject tracer microspheres (NEN Chemicals GmbH, Dreieich, West Germany). For calibrating the tracer microsphere values, arterial blood was withdrawn at a constant speed from the descending aorta using a multispeed transmission pump (Harvard Apparatus Co., Inc.). The left anterior descending coronary artery was dissected free over a small segment to produce a coronary thrombus as described later.

To monitor hemodynamic variables, systolic and diastolic aortic pressure, left atrial pressure, lead II electrocardiogram and heart rate were registered on a multichannel recorder (Siemens Corp., Solna, Sweden) throughout the experiment.

**Experimental protocol.** Ten minutes after sternotomy and preparation of the left anterior descending coronary artery, recording of preischemic hemodynamic data was started. Thereafter, a 1 cm long left anterior descending coronary artery segment was ligated at its proximal and distal end using 7-0 Prolene snares, and thrombin (10 μl of 100 National Institutes of Health units/ml) was injected in the isolated vessel segment. The segment was repeatedly crushed with a small forceps. After 15 minutes the Prolene snares were released. This resulted in a clearly visible localized thrombus occluding the left anterior descending coronary artery, which was confirmed by coronary angiography. Twenty minutes after left anterior descending coronary artery occlusion, an injection of tracer microspheres was made for the quantitation of regional myocardial blood flow.

After approximately 45 minutes of coronary thrombosis, recombinant single chain urokinase-type plasminogen activator or recombinant low molecular weight urokinase (20 μg/kg per min) was infused for 60 minutes through the right brachial vein (six baboons in each group). Coronary reperfusion was monitored by visual inspection of the occluded artery and confirmed by control coronary angiography. Immediately after thrombolysis, the animals were heparinized to prevent rethrombosis. During the infusion, blood samples were collected from the left brachial vein into 0.01 M citrate solution to determine fibrinogen, alpha-antiplasmin and single chain urokinase-type plasminogen activator antigen levels as previously described (7). Six other baboons served as a control group. They underwent the same experimental procedure except for the infusion. They were heparinized 60 minutes after left anterior descending coronary artery occlusion.

**Infarct size measurement.** During left anterior descending coronary artery occlusion or reperfusion, or both, all animals received lidocaine (Xylocaine, Göteborg, Sweden), 20 μg/kg intravenously. Two and a half hours after recanalization or 4 hours after acute occlusion of the left anterior descending coronary artery, a second tracer microsphere injection was made in all animals. The animals were then killed with an overdose of pentobarbital and the heart was removed. Infarct size was measured by triphenyltetrazolium staining and expressed as a percent of the perfusion area of the left anterior descending coronary artery, as previously described (11). The area at risk was expressed as a percent of the left ventricular mass.

**Regional myocardial blood flow.** This was measured with radioactive tracer microspheres, as previously described (12,13). The left ventricular slices were unrolled and divided into subepicardial and subendocardial samples (± 1 g tissue blocks). Gamma spectrometry was carried out on all tissue and blood samples (14) with a multichannel analyzer (ND66, Nuclear Data, Inc.) and computer terminal (Nuclear Data, Inc.). Four differently labeled microspheres were used: cerium-141, tin-113, ruthenium-103 and niobium-95.

**Thrombolytic agents.** The recombinant single chain urokinase-type plasminogen activator was obtained by expression of the human cDNA coding for full length pro-urokinase, a peptide chain of 411 amino acids (molar mass...
46,420 daltons), in *Escherichia coli* (10). Recombinant single chain urokinase-type plasminogen activator was purified carefully avoiding conversion to two chain urokinase forms. The isolated protein was unglycosylated and migrated as a main band with molecular weight 47,000 on sodium dodecyl sulfate polyacrylamide gel electrophoresis under native and reducing conditions. It had a specific activity of 175,000 IU/mg as measured on chromogenic substrate (S-2444, Kabi Diagnostica, Stockholm, Sweden) after activation with plasmin and was devoid of urokinase activity and pyrogens.

The recombinant low molecular weight urokinase was obtained from the same recombinant *Escherichia coli* strain as recombinant single chain urokinase-type plasminogen activator (10) after conversion of the primary expression product during purification. The isolated protein proved to be unglycosylated but otherwise structurally identical and functionally equivalent to low molecular weight urokinase from human urine (15). Recombinant low molecular weight urokinase (molar mass 30,852 daltons) had a specific activity of 360,000 IU/mg as measured on chromogenic substrate and was devoid of pyrogens.

**Results**

**Coronary thrombolysis.** Coronary thrombolysis was achieved in all 12 baboons receiving an infusion of either recombinant single chain urokinase-type plasminogen activator (n = 6) or recombinant low molecular weight urokinase (n = 6). The time to reperfusion was 21 ± 4 minutes (mean ± SD) for recombinant single chain urokinase-type plasminogen activator and 14 ± 5 minutes for recombinant low molecular weight urokinase (p < 0.05). The mean duration of coronary occlusion before reperfusion was 72 ± 6 minutes for recombinant single chain urokinase-type plasminogen activator and 69 ± 14 minutes for recombinant low molecular weight urokinase. Acute reocclusion of the left anterior descending coronary artery was not observed. Figure 1 shows angiographic evidence of thrombolysis in a typical experiment. In the control group, complete occlusion of the left anterior descending coronary artery persisted throughout the experiment in all six animals.

**Regional myocardial blood flow.** Acute thrombosis of the left anterior descending coronary artery resulted in a severe reduction of myocardial blood flow in its perfusion area (Fig. 2). This relatively low collateral flow (14, 20 and 16% of flow to the nonoccluded area in the three groups, respectively) persisted in the control group with permanent coronary artery occlusion (Fig. 2). In both the recombinant single chain urokinase-type plasminogen activator and recombinant low molecular weight urokinase groups, lysis of the coronary thrombus resulted in a reactive hyperemia in the left anterior descending coronary artery area as evidenced by a persisting visible blush. Two and a half hours after thrombolysis, myocardial blood flow was homogeneously distributed in the left anterior descending and circumflex artery areas (Table 1, Fig. 2).

**Effects of reperfusion on infarct size.** Occlusion of the left anterior descending coronary artery for 4 hours resulted in large transmural infarcts in all six control baboons. The mean percent of the perfusion area of the occluded artery showing infarction was 66 ± 4% (mean ± SEM). In the six baboons undergoing reperfusion with recombinant single chain urokinase-type plasminogen activator, the percent of

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**Figure 1.** Coronary arteriograms before and after intravenous administration of recombinant single chain urokinase-type plasminogen activator in a baboon with thrombotic occlusion of the left anterior descending coronary artery. **A,** After thrombotic occlusion (asterisk). **B,** After reperfusion.
Figure 2. Regional myocardial blood flow (MBF) distribution after left anterior descending (LAD) coronary artery occlusion (OCCL) and at the end of the experiment. The results represent the mean ± SEM of the left anterior descending coronary artery-myocardial blood flow values, expressed as percent of the circumflex coronary artery (CX)-myocardial blood flow values. A = control group; B = recombinant low molecular weight urokinase; C = recombinant single chain urokinase-type plasminogen activator.

Infarcted tissue was significantly lower than that in the control animals: 42 ± 4% (p < 0.001) (Fig. 3). In the six baboons undergoing reperfusion with recombinant low molecular weight urokinase the percent of infarcted tissue was also significantly lower than that in the control animals: 45 ± 8% (p < 0.05).

In the control group, none of the infarcts were hemorrhagic. After about 70 minutes of coronary thrombosis, reperfusion induced macroscopically visible hemorrhage in 10 of the 12 animals. Characteristically, the hemorrhage was located in the midwall and never extended outside the infarct into viable myocardium.

The volume of the perfusion area of the occluded artery was not different between the recombinant single chain urokinase-type plasminogen activator group (17 ± 3%) or the recombinant low molecular weight urokinase group (22 ± 4%) and the control group (23 ± 3%) (p > 0.05).

**Hemodynamic changes.** Both in the animals with permanent left anterior descending coronary artery occlusion and in animals with reperfusion, the main hemodynamic variables (heart rate, aortic pressure and left atrial pressure) remained essentially unchanged throughout the experiment (Table 2). In the animals with reperfusion, the incidence of arrhythmias (ventricular ectopic beats or runs of ventricular tachycardia) was, however, higher than in the group with permanent coronary artery occlusion. Throughout the experiments, blood pH, partial oxygen tension, partial carbon dioxide tension, bicarbonate and total carbon dioxide levels were kept within normal limits.

**Changes in hemostatic variables and bleeding.** During infusion of recombinant single chain urokinase-type plasminogen activator, a steady state plasma level of 6 ± 2 μg/ml was maintained. After cessation of the infusion, the plasminogen activator antigen disappeared from plasma with an initial half-life of 5 minutes but the disappearance curve was not monotone (Fig. 4). Infusion of recombinant single chain urokinase-type plasminogen activator was not associated with systemic fibrinolytic activation because after the infusion, the fibrinogen and the alpha-antiplasmin levels had not significantly changed as compared with those in the control group (Fig. 5). Bleeding from the puncture site in the coronary artery was observed in two animals, but profuse bleeding from surgical wounds was not observed.

Infusion of recombinant low molecular weight urokinase caused extensive systemic fibrinolytic activation with total depletion of both fibrinogen and alpha2-antiplasmin. This was reflected by profuse bleeding from surgical wounds in five of the six baboons infused with this agent.

**Table 1. Regional Myocardial Blood Flow (ml/min per 100 g)**

<table>
<thead>
<tr>
<th></th>
<th>Circumflex Coronary Artery Area</th>
<th>Left Anterior Descending Coronary Artery Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Endo</td>
</tr>
<tr>
<td><strong>30 minute LAD thrombosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group (n = 6)</td>
<td>175 ± 118</td>
<td>194 ± 105</td>
</tr>
<tr>
<td>rscu-PA group (n = 6)</td>
<td>172 ± 70</td>
<td>193 ± 100</td>
</tr>
<tr>
<td>rL-UK group (n = 6)</td>
<td>134 ± 46</td>
<td>143 ± 38</td>
</tr>
<tr>
<td><strong>240 minute LAD thrombosis</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control group</td>
<td>188 ± 118</td>
<td>226 ± 197</td>
</tr>
<tr>
<td>150 minute reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rscu-PA group</td>
<td>109 ± 43</td>
<td>125 ± 60</td>
</tr>
<tr>
<td>rL-UK group</td>
<td>94 ± 27</td>
<td>108 ± 28</td>
</tr>
</tbody>
</table>

*p < 0.001 versus circumflex value. Epi = subepicardial layer; Endo = subendocardial layer; rL-UK = recombinant low molecular weight urokinase; rscu-PA = recombinant single chain urokinase-type plasminogen activator. The data represent mean values ± SD.
Figure 3. Effect of reperfusion on infarct size. The values represent mean ± SEM of the mass of infarcted tissue, expressed as percent of the mass of the perfusion bed of the occluded artery. A = control group; B = recombinant single chain urokinase-type plasminogen activator (rscu-PA); C = recombinant low molecular weight urokinase-type plasminogen activator (rL-UK).

Discussion

Coronary thrombolysis. This study demonstrates that intravenous administration of recombinant single chain urokinase-type plasminogen activator can effectively produce coronary thrombolysis in primates, which confirms and extends our findings with human single chain urokinase-type plasminogen activator and coronary thrombolysis in dogs (7). The time to achieve thrombolysis (21 ± 4 minutes) in this study is comparable with that achieved in dogs with natural single chain urokinase-type plasminogen activator (23 ± 2 minutes). Interestingly, a significantly shorter mean time to reperfusion (14 ± 5 minutes) was observed with

Table 2. Hemodynamic Data

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Heart Rate (beats/min)</th>
<th>LAP (mm Hg)</th>
<th>AoP-s (mm Hg)</th>
<th>AoP-d (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
<td>Control group (n = 6)</td>
<td>154 ± 19</td>
<td>5 ± 2</td>
<td>109 ± 18</td>
</tr>
<tr>
<td></td>
<td>rscu-PA group (n = 6)</td>
<td>157 ± 20</td>
<td>3 ± 2</td>
<td>112 ± 19</td>
</tr>
<tr>
<td></td>
<td>rL-UK group (n = 6)</td>
<td>152 ± 21</td>
<td>5 ± 1</td>
<td>120 ± 14</td>
</tr>
<tr>
<td>30 minute LAD thrombosis</td>
<td>Control group</td>
<td>164 ± 19</td>
<td>8 ± 5</td>
<td>112 ± 11</td>
</tr>
<tr>
<td></td>
<td>rscu-PA group</td>
<td>159 ± 17</td>
<td>4 ± 2</td>
<td>109 ± 13</td>
</tr>
<tr>
<td></td>
<td>rL-UK group</td>
<td>153 ± 22</td>
<td>5 ± 2</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>240 minute LAD thrombosis</td>
<td>Control group</td>
<td>153 ± 24</td>
<td>7 ± 3</td>
<td>103 ± 21</td>
</tr>
<tr>
<td></td>
<td>rscu-PA group</td>
<td>157 ± 29</td>
<td>2 ± 1</td>
<td>106 ± 17</td>
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<tr>
<td></td>
<td>rL-UK group</td>
<td>156 ± 25</td>
<td>2 ± 1</td>
<td>103 ± 10</td>
</tr>
</tbody>
</table>

AoP-d = diastolic aortic pressure; AoP-s = systolic aortic pressure; LAP = left atrial pressure; other abbreviations as in Table 1. The data represent mean values ± SD.
recombinant low molecular weight urokinase (p < 0.05). This is probably explained by the twofold higher specific activity (360,000 versus 175,000 IU/mg) of the low molecular weight urokinase relative to that of the single chain urokinase-type plasminogen activator. Thus, with equal infusion rates on a weight basis, twice the total activity was administered to the animals receiving low molecular weight urokinase, resulting in shorter reperfusion times.

**Effects of reperfusion on infarct size.** The impact of coronary reperfusion on the reduction of infarct size was only moderate, not an unexpected observation. This is consistent with a previous report (11) demonstrating the severe time constraints on coronary occlusion in baboons.

**Regional myocardial blood flow.** Despite the lack of a more significant impact on myocardial infarct size, a surprising absence of the no-reflow phenomenon (that is, the severe impairment of tissue perfusion in the recanalized zone [16,17]) was found. Recanalization was associated with perfect distribution of myocardial blood flow in the recanalized zone. This is distinct from results previously obtained in the same model with recombinant tissue-type plasminogen activator (11), where post-thrombotic flow was impaired. One possible explanation for this discrepancy is the longer postreperfusion infusion time of the thrombolytic agents utilized in the present study. This may have resulted in more complete lysis of microvascular thrombi.

**Pharmacokinetics of recombinant single chain urokinase-type plasminogen activator.** The pharmacokinetic behavior of this plasminogen activator was comparable with that observed with other preparations of single chain urokinase-type plasminogen activator in other species. A somewhat higher steady state level (6 µg/ml) was achieved than with a similarly effective administration of natural single chain urokinase-type plasminogen activator in dogs (3 µg/ml) (7). However, the plasma half-lives were similarly short, 5 and 7 minutes, respectively. The only other measurement of the clearance rate for recombinant single chain urokinase-type plasminogen activator was obtained after a single intravenous injection in rabbits where a half-life of 3.5 minutes resulted (18). In the present study as well as in other studies with thrombolytic infusions of single chain urokinase-type plasminogen activator, disappearance rate curves from plasma were not monotone. These short half-lives will probably make the use of continuous drug infusions necessary for effective thrombolytic therapy.

**Therapeutic implications.** An important aspect of this study is the demonstration of the fibrin-specific thrombolytic potential of recombinant single chain urokinase-type plasminogen activator. With peak plasma levels of 6 µg/ml maintained for 45 minutes, no decrease in either alpha_2-antiplasmin or fibrinogen levels occurred, which is indicative of a high degree of fibrin specificity. The resulting maintenance of normal systemic fibrinolytic function parallel with a site-specific thrombolytic effect is an attractive therapeutic quality with which risks of hemorrhage may be reduced. In this study the apparent lack of bleeding seen with the animals receiving recombinant single chain urokinase-type plasminogen activator contrasted sharply with the hemorrhaging at incisional and vessel puncture sites of the recombinant low molecular weight urokinase-treated baboons. Still, one must use caution in the extrapolation of relative fibrin specificity of thrombolytic agents from animal models to humans, because interspecies variability in sensitivity of the fibrinolytic system to activation is marked. Nonetheless, recombinant single chain urokinase-type plasminogen activator offers promise as an intravenous agent for effective and specific thrombolytic therapy of acute myocardial infarction in humans.
References


