Coronary Reperfusion Studies With Pro-Urokinase in Acute Myocardial Infarction: Evidence for Synergism of Low Dose Urokinase

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The beneficial effects of thrombolytic therapy on early and late mortality and cardiac function in acute myocardial infarction have been extensively demonstrated (1–7). Streptokinase and urokinase, the most commonly used thrombolytic agents in clinical practice, have little affinity for fibrin, thus causing a severe hemostatic defect as a result of systemic plasminogen activation. Therefore, recombinant tissue-type plasminogen activator (rt-PA), which has a fibrin-binding site, has been developed and shown to cause reperfusion of occluded coronary arteries in up to 91% of patients (8,9). Systemic fibrinolysis and hemorrhage, however, have also been observed with rt-PA (10). Moreover, its short half-life may predispose patients to reocclusion, resulting in the need for prolonged administration of the agent. Therefore, the search for new thrombolytic agents continues.

Pro-Urokinase, a single chain urokinase-type plasminogen activator, has been demonstrated to exhibit also fibrin specificity both in vitro and in vivo (11–13). Intracoronary administration of pro-urokinase in humans has been shown to reopen occluded coronary arteries in up to 91% of patients (14). Reperfusion rates after the intravenous administration of pro-urokinase, however, have been reported (15–17) only in small groups of patients. Conversely, experimental and preliminary clinical studies (15,18,19) have demonstrated enhanced thrombolytic efficacy of pro-urokinase after the addition of a small amount of urokinase. Therefore, the present study was designed to 1) examine reperfusion rates and fibrin specificity of intravenous pro-urokinase in patients with acute myocardial infarction, and 2) elucidate possible synergistic interactions of pro-urokinase and urokinase in human patients.
Methods

The study was approved by the ethics committee of the University of Freiburg. The trial was conducted at the University Hospital, Freiburg, and the community hospitals of Worms and Villingen-Schwenningen, Federal Republic of Germany.

Patient selection. The study was considered for patients ≤70 years of age who had 1) typical chest pain of ≥30 min duration, and 2) electrocardiographic ST segment elevation of ≥0.1 mV in at least two limb leads or ≥0.2 mV in at least two precordial (V) leads suggestive of myocardial ischemia. Symptoms and signs were not relieved by sublingual nitroglycerin. Thrombolytic therapy had to be started within 6 h of symptom onset after documentation of an occluded (Thrombolysis in Myocardial Infarction trial [TIMI] grade 0 or I) infarct-related artery by means of coronary angiography (20). The following exclusion criteria were applied: ongoing anticoagulant therapy: severe uncontrolled arterial hypertension (systolic pressure >200 mm Hg or diastolic pressure >120 mm Hg); peptic ulceration during the last year; recent history of surgery; a history of coronary artery bypass grafting and no informed consent to participate in the study.

Study protocol. Patients (n = 75) who qualified for the study and who had given informed consent underwent coronary angiography using the Judkins technique. After documentation of an occluded infarct-related artery, 0.2 to 0.3 mg of intracoronary nitroglycerin was administered to exclude coronary artery spasm. On completion of the control angiography, patients were randomized to one of two treatment regimens according to the presence of anterior or inferior myocardial infarction. First, an intravenous bolus injection of 10,000 U of heparin was administered, followed by a continuous infusion of 1,250 U/h of heparin. Patients randomized to receive high dose pro-urokinase therapy (group A, n = 40) received an intravenous bolus injection of 1 million U of pro-urokinase and a subsequent infusion of 8 million U of pro-urokinase over the next hour. In the combined treatment group (group B, n = 35), an intravenous bolus injection of 0.5 million U of pro-urokinase together with 0.2 million U of urokinase was given, followed by an infusion of 4 million U of pro-urokinase over 40 min. If no reperfusion (TIMI grade II or III) could be documented 75 min after the start of the therapy, 0.2 million U of intracoronary urokinase was administered.

Coronary angiography was repeated every 15 min or intermittently when coronary artery reperfusion was suggested by clinical signs. Heparin therapy was continued for a total of 72 to 96 h, with the partial thromboplastin time adjusted to two times the upper limit of normal. No concomitant aspirin therapy was given. Continued anticoagulant therapy was the decision of the responsible physician. Coronary angiography was performed 24 to 36 h after thrombolytic therapy to evaluate coronary reocclusion.

Study agent. Human pro-urokinase was highly purified from the conditioned medium of the transformed kidney cell line TCL-598 (Sandoz AG). The drug was supplied in vials containing 0.5 million U of freeze-dried pro-urokinase stored at 4°C and dissolved in water immediately before use. The purified glycosylated protein migrated as a single band with a molecular weight of approximately 55,000 on sodium dodecyl sulfate gel electrophoresis under reducing conditions. It had a latent specific activity of about 130,000 IU/mg as measured with the chromogenic substrate S-2444 (Kabi Diagnostica) after activation with plasmin. Measurable urokinase activity was <1% in the purified preparation. The appropriate dose of pro-urokinase was infused with a constant rate infusion pump.

Coagulation studies. Citrated blood samples were collected with and without 500 U/ml of aprotinin to prevent in vitro fibrinolysis. Analysis of fibrinogen (normal range 150 to 450 mg/dl) and alpha 2-antiplasmin was performed. Alpha 2-antiplasmin levels were measured utilizing chromogenic substrate (S-2251, Kabi-Vitrum).

Statistics. Values are expressed as mean values ± SD. Statistical comparisons of hematologic results before and after pro-urokinase administration were performed by means of Student's t test. Fisher's exact test and the Wilcoxon test were used where appropriate. Comparisons between both treatment regimens with respect to time to reperfusion were carried out by the log-rank test. A p value ≤0.05 was considered significant.

Results

Entry characteristics of study groups (Table I). Forty patients (group A) received high dose pro-urokinase therapy; 35 patients (group B) were treated according to the combined regimen. There were no significant differences in baseline characteristics of the two groups. The duration between the onset of symptoms and the start of thrombolytic therapy averaged 199 min in group A and 190 min in group B. Control angiography showed TIMI grade 0 perfusion of the infarct-

Table 1. Clinical Characteristics of the Two Study Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A (n = 40)</th>
<th>Group B (n = 35)</th>
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</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>31/9</td>
<td>31/4</td>
</tr>
<tr>
<td>Age (range) (yr)</td>
<td>57 (38–70)</td>
<td>57 (37–70)</td>
</tr>
<tr>
<td>Anterior MI</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Inferior MI</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Infarct-related artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMI 0</td>
<td>38</td>
<td>34</td>
</tr>
<tr>
<td>TIMI I</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Duration of symptoms (min)</td>
<td>199 ± 83</td>
<td>190 ± 89</td>
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Group A = high dose pro-urokinase; Group B = low dose pro-urokinase plus low dose urokinase; MI = myocardial infarction; TIMI = Thrombolysis in Myocardial Infarction trial grade.
related artery in 72 patients and TIMI grade I in the remaining 3 patients.

Reperfusion results. "Anytime reperfusion" of TIMI grade II or III was observed within 75 min in 29 (73%) of the 40 patients in group A compared with 23 (66%) of the 35 patients in group B (p = NS). At 15, 30 and 45 min after the start of thrombolytic therapy, there was a trend toward a higher reperfusion rate in group B; however, this difference did not reach statistical significance at any time. A parallel comparison of reperfusion rates for the two treatment regimens for each 15 min time interval (Fig. 1) indicated that in group A, 22 (85%) of 26 patients who were treated within 4 h of symptom onset showed TIMI grade II or III reperfusion compared with 8 (57%) of 14 treated within 4 to 6 h (p = 0.04; Fisher's exact test). The comparable numbers in group B were 21 (75%) of 28 patients with therapy started within 4 h compared with 2 (29%) of 7 patients treated later (p = 0.03).

Intracoronary interventions. In 10 group A patients, 0.2 million U of intracoronary urokinase was administered, with late reperfusion occurring in 3 patients. This resulted in a total reperfusion rate of 80% (32 of 40 patients). In group B, intracoronary intervention was also performed in 11 patients, and reperfusion was observed in 6 additional patients, resulting in a total reperfusion rate of 83% (29 of 35 patients) (p = NS).

Reocclusion and side effects. Early reocclusion (within 75 min after the start of therapy) of the infarct-related artery was observed in one patient in group A treated with high dose pro-urokinase. Reocclusion within 24 to 36 h was assessed by later repeat coronary angiography in 49 patients whose infarct-related artery had shown TIMI grade II or III reperfusion at 75 min after the start of therapy. Three group A patients and one group B patient were documented to have a reoccluded vessel. Thus, a total of five patients showed early (n = 1) or late (n = 4) reocclusion (reocclusion rate 10%). Three group A patients and one group B patient died within the first 24 h because of cardiogenic shock or intracerebral ventricular fibrillation. In three of the four patients, no reperfusion of the infarct artery had been achieved; the fourth patient did show reocclusion of the infarct-related artery and died thereafter. Bleeding from the arterial puncture site was observed in four patients (three in group A, one in group B) and resulted in the need for blood transfusion in one of the four patients. Two of the three group A patients had received intracoronary urokinase in addition to pro-urokinase. Intracerebral hemorrhage, allergic reactions or a significant decrease in arterial blood pressure during thrombolysis were not observed.

Coagulation studies (Table 2). Of the 75 patients, 63 had blood samples evaluated for fibrinogen determinations at control and 1, 2 and 3 h after the start of thrombolytic therapy. Samples from the 22 patients who received intracoronary urokinase were excluded from the analysis. Thus, fibrinogen changes are reported for 21 group A and 20 group B patients. In patients in group A, fibrinogen decreased from a mean of 269 ± 62 mg/dl to a minimum of 228 mg/dl after 2 h (15% decrease; p < 0.01); in three of these patients, fibrinogen concentration decreased briefly to <100 mg/dl. In patients in group B, a mean decrease in fibrinogen of 13% occurred (from 294 ± 77 to 256 ± 86 mg/dl; p < 0.01); no patient showed a decrease in fibrinogen concentration to <100 mg/dl. The decrease in fibrinogen was not different between the groups. In patients in group A, alpha 2-antiplasmin declined to 93%, 86%, 71% and 46% of the pretreatment value at 5, 10, 30 and 60 min, respectively, after the start of fibrinolytic therapy. In patients in group B, the respective values were 75%, 65%, 62% and 51% (Fig. 2). At 5 and 10 min, this difference was statistically significant (Wilcoxon test).

Discussion

Thrombolytic efficacy. In this angiographically controlled study, a reperfusion rate of 73% was achieved after the administration of 9 million U of pro-urokinase. Reperfusion rates after administration of this new thrombolytic agent have been reported in only a few small studies (15-17). In a study (16) utilizing a total dose of 80 mg of recombinant pro-urokinase, 10 of 11 patients showed reperfusion 90 min after the start of therapy. Most recently, a reperfusion rate of 67% was reported (17) in a cohort of 12 patients receiving 9 million U of glycosylated pro-urokinase, which is equivalent to approximately 65 mg of the recombinant form of pro-

Table 2. Fibrinogen (mg/dl) Degradation During Thrombolytic Therapy

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Group A</td>
<td>269 ± 62</td>
<td>234 ± 93</td>
<td>228 ± 97</td>
<td>211 ± 86</td>
</tr>
<tr>
<td>Group B</td>
<td>294 ± 77</td>
<td>261 ± 90</td>
<td>264 ± 83</td>
<td>256 ± 86</td>
</tr>
</tbody>
</table>

p < 0.05 compared with control in both groups. Definitions as in Table 1.
urokinase. In a recent multicenter trial (21), a coronary artery patency rate of 71.8% was observed after the administration of 80 mg of recombinant pro-urokinase. When pro-urokinase was administered within the first 4 h of symptom onset, thrombolytic efficacy appeared to be significantly higher, resulting in a reperfusion rate of 80%. Thus, pro-urokinase shares the time dependence of its thrombolytic efficacy with streptokinase (22), urokinase (23) and anisoylated plasminogen streptokinase activator complex (APSAC) (24). Only rt-PA seems to be equally effective when given within the first 3 h or between 4 and 6 h after the onset of infarction (22).

Synergistic interactions. Another important finding of the present study was that the combined treatment with pro-urokinase and low dose urokinase was equally effective as high dose pro-urokinase treatment. Substantial evidence from experimental studies (25-28) indicates that different thrombolytic agents may have additive or even synergistic effects with regard to their thrombolytic potential. Pannell and Gurewich (25) reported that pro-urokinase is converted to its active form (high molecular weight two chain urokinase) by plasmin that is generated on the fibrin surface. In vitro studies (26) demonstrated that this process may be accelerated by a small bolus injection of urokinase, which exposes more fibrin binding sites for plasminogen on the thrombus. We demonstrated (15) coronary reperfusion in 9 of 11 patients utilizing 6.5 million U of glycosylated pro-urokinase together with a bolus injection of 0.2 million U of urokinase, which resulted in only minimal fibrinogen degradation. In subsequent studies (19,29), patency rates of 65% to 75% were reported when similar combined pro-urokinase-urokinase treatment regimens were used. In the present study, the combined treatment using only half of the high pro-urokinase dosage (4.5 million U) was as effective as high dose therapy. One has to assume that both substances exert synergistic effects with regard to coronary reperfusion because the dosage of 0.2 million U of urokinase has no demonstrable independent thrombolytic effect (18). Instead, dosages of 2 to 3 million U of urokinase are necessary to achieve patency rates of 60% to 65% (23,30). Thus, the combination of 4.5 million U of pro-urokinase and 0.2 million U of urokinase results in an algebraic fraction of 0.6 of equally effective dosages. This potentiation of clot lysis obeyed Berenbaum’s mathematic definition of synergy (31), that is, the same thrombolytic effect can be achieved by pro-urokinase plus low dose urokinase at algebraic fractional combinations <1. Synergism of these two thrombolytic substances is further suggested by the fact that alpha 2-antiplasmin depletion was significantly more rapid in the combined treatment group. Finally, in nine patients with failed intravenous pro-urokinase administration, the addition of 0.2 million U of intracoronary urokinase achieved coronary reperfusion within 1 to 3 min, indicating rapid activation of pro-urokinase at the thrombus. Thus, the present study appears to demonstrate an additive and synergistic effect of pro-urokinase and low dose urokinase.

Fibrin specificity of pro-urokinase. Fibrinogen levels decreased by only 15% after pro-urokinase administration irrespective of whether a small bolus injection of urokinase was also administered. This value is comparable with that obtained by Loscalzo et al. (17). The more pronounced decrease in fibrinogen observed in another study (21) was due to the higher dosage of pro-urokinase used. As with other thrombolytic agents, clot selectivity vanishes with increasing pro-urokinase dosages. The minimal change in fibrinogen observed in this and other trials (15-17) places pro-urokinase in one class with rt-PA concerning fibrin specificity. It clearly distinguishes pro-urokinase from non-selective thrombolytic agents such as streptokinase, urokinase or APSAC.

Coronary reocclusion. Early reocclusion after thrombolysis with or without combined coronary angioplasty remains a major problem with old thrombolytic agents like streptokinase (32-34) as well as with newer substances such as rt-PA (8,35). This has resulted in recommendations of prolonged rt-PA administration over 3 to 8 h to avoid reocclusion (35). In a recent trial (21) utilizing recombinant pro-urokinase, a low (5%) incidence of early reocclusion of the infarct-related artery was observed. In the present study, reocclusion occurred in 10% of patients, with no significant difference between the two treatment regimens tested. This compares favorably with other thrombolytic substances (8,22,24) despite the fact that in the present study, no concomitant aspirin therapy was given. It has been recently demonstrated (36) that heparin may potentiate the thrombolytic efficacy of natural pro-urokinase in human patients. Thus, the concomitant administration of heparin during and 72 to 96 h after thrombolysis with pro-urokinase has to be considered to be of major importance with regard to both a high reperfusion
rate and a low incidence of early reoclusion of infarct-related arteries.

**Side effects of pro-urokinase.** Despite the fact that heparin was administered together with pro-urokinase, a remarkably low incidence of bleeding complications (6%) was observed in the present study. Hemorrhage necessitating blood transfusion occurred in only one patient. The Pro-Urokinase in Myocardial Infarction (PRIMI) study group (21) reported bleeding complications in 14.1% of their pro-urokinase-treated patients compared with 24.6% of their streptokinase-treated patients. In the study of Loscalzo et al. (17), pro-urokinase caused a major hemorrhagic complication in only 1 of 40 patients. In addition, other side effects such as allergic reactions or a decrease in arterial blood pressure—complications well known to occur with other thrombolytic agents (2,4)—were not observed in the present study or other trials (15,16) using pro-urokinase. Thus, pro-urokinase seems to be a new thrombolytic agent with a remarkably favorable side effect profile.

**Conclusions.** The present data indicate that pro-urokinase is a very effective new thrombolytic agent that achieves coronary reperfusion in 65% to 70% of patients. There is substantial evidence for synergism when pro-urokinase is administered together with a small bolus injection of urokinase. Side effects, particularly bleeding complications, are rare because of a high degree of fibrin specificity.

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**References**


