Efficacy of Intravenous Prourokinase and a Combination of Prourokinase and Urokinase in Acute Myocardial Infarction

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Fifty-four patients with Q-wave acute myocardial infarction (AMI) were treated with heparin combined with intravenous single-chain urokinase-type plasminogen activator (prourokinase). To determine the optimal treatment regimen, prourokinase was applied in 3 different ways: group I received a bolus of 7.5 mg and a subsequent infusion of 40.5 mg over 60 minutes. Patency of the infarct artery was observed in 7 patients (50%) at the end of the infusion time. One hour after the end of the infusion the fibrinogen level had decreased to 87 ± 12% of the preinfusion level; the plasminogen and α-2 antiplasmin levels to 61 ± 13% and 59 ± 34%, respectively. In group II prourokinase was administered as a 7.5 mg bolus followed by 66.5 mg over 60 minutes. Eleven patients (55%) had patent infarct-related coronary arteries and fibrinogen, plasminogen and α-2 antiplasmin levels had decreased to 58 ± 29%, 38 ± 18% and 21 ± 14%, respectively. Group III was treated with a bolus of 3.7 mg prourokinase and 250,000 IU urokinase followed by 44.3 mg prourokinase, resulting in a patency rate of 65% (13 patients). Fibrinogen, plasminogen and α-2 antiplasmin levels decreased to 76 ± 15%, 67 ± 15% and 47 ± 29%, respectively. Fibrin-specific thrombolysis can be achieved with glycosylated prourokinase. At higher dosages considerable systemic activation of the fibrinolytic system with little enhancement of the observed therapeutic effect occurred. The combination of prourokinase and urokinase yielded a higher patency rate than either dosage of prourokinase alone, although the difference was not statistically significant in this pilot trial.

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Coronary angiographic studies indicate that most Q-wave acute myocardial infarctions (AMIs) are caused by coronary thrombosis.[1] The use of thrombolytic agents is receiving increasing attention because of recent reports that early coronary reperfusion can preserve left ventricular function[2,3] and reduce both mortality and morbidity.[4,5] Urokinase and streptokinase, plasminogen activators currently in clinical use, have little affinity for fibrin. As a consequence, plasminogen is generally activated systemically and fibrinogen as well as fibrin are degraded, resulting in a severe hemostatic defect.[6] More recently, tissue-type plasminogen activator, which has a fibrin binding site, has been shown to be more selective in promoting fibrinolysis,[7] although fibrinogenolysis and hemorrhage continue to be complications of its use.[8,9] Prourokinase (single-chain urokinase-type plasminogen activator) has been shown to be fibrin specific in vitro and in a variety of animal species.[10–13] In contrast to tissue-type plasminogen activator, relatively few patients have been treated with prourokinase[14–17] and therefore experience with respect to its fibrin specificity and thrombolytic efficacy is limited. Moreover differences seem to exist between glycosylated prourokinase obtained from conditioned cell culture media—as used in this trial—and recombinant, unglycosylated prourokinase obtained by expression of the cDNA encoding human prourokinase in E. coli.[18] This pilot study was undertaken to assess the thrombolytic efficacy and fibrin specificity for different treatment regimens with glycosylated prourokinase.

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TABLE I Therapeutic Regimen

Group	No.	Bolus (mg)	Prourokinase Infusion (mg)	Total Dose (mg)
I	14	7.5	40.5	48
II	20	7.5	66.5	74
III	20	3.7*	44.3	48*

* 250,000 IU urokinase injected immediately before application of the prourokinase bolus.

TABLE II Patient Characteristics

	Group		
	I (n = 14)	II (n = 20)	III (n = 20)
Sex (M:F)	12:2	17:3	20:0
Age (yr)	53 ± 8	61 ± 10	58 ± 9
Height (cm)	173 ± 5	172 ± 6	175 ± 6
Weight (kg)	77 ± 8	75 ± 10	81 ± 12
LAD	6	8	7
Right	4	7	13
LC	4	5	0
Interval (hr)	2.9 ± 1.5	2.7 ± 1.3	2.5 ± 1.0

Interval = interval between onset of pain and initiation of thrombolytic treatment; LAD = left anterior descending coronary artery; LC = left circumflex artery; right = right coronary artery.

Methods

Material: Human prourokinase (scu-PA) was highly purified from the conditioned medium of the transformed kidney cell-line TCL-598. The material was made available by Sandoz AG. The drug was supplied in vials containing 3.75 mg of freeze-dried prourokinase stored at 4°C and dissolved in 10 ml of water immediately before use. The appropriate dose was infused over 1 hour with a constant-rate infusion pump. The purified, glycosylated protein migrated as a single band with an apparent 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. There was no measurable urokinase activity (<1%) in the purified preparation.

Patients: Inclusion criteria consisted of chest pain typical of myocardial infarction lasting for at least 30 minutes, ST-segment elevation of at least 2 mm in at least 3 leads, presentation within 5 hours after the onset of pain, age under 75 years, absence of contraindications for cardiac catheterization or thrombolytic therapy and informed consent.

Treatment: Patients were anticoagulated with 10,000 U of heparin. According to the treatment regimen 3 groups were formed (Table I). The protocol was developed stepwise on the basis of the results obtained in the preceding group. The bolus was applied over 3 minutes, and the subsequent infusion over 60 minutes. Angiographic visualization of the presumed infarct vessel and assessment of patency were performed 60 minutes after the start of treatment. As per the Thrombolysis in Myocardial Infarction trial, grades 0 to III were used to grade patency after treatment; grade II and III were considered successful treatment.

Coagulation parameters: Blood samples were obtained from 44 patients (12, 16 and 16 in groups I, II and III, respectively) prior to application of the bolus and 60, 120 and 240 minutes and 8 (groups I and II) or 24 hours (group III) after the start of treatment. Blood was collected on citrate (final concentration 0.01 M) and immediately centrifuged at 4°C. Fibrinogen levels were measured in plasma immediately thereafter.[19] Plasminogen[20] and α-2 antiplasmin[21] were either determined immediately or from serum quick frozen and stored at −30° for no longer than 2 weeks.

Results

Patients: Patient characteristics are summarized in Table II.

Effectiveness of treatment: Patency of the infarct vessel, defined as rapid or delayed but complete opacification of the coronary artery distal to the occlusion (grade II or III according to the Thrombolysis in Myocardial Infarction trial), was observed in 50% (7 of 14; 95% confidence interval 23 to 77%) of patients in group I treated with 48 mg of prourokinase. In the group treated with 74 mg of prourokinase patency was obtained in 55% (11 of 20; 95% confidence interval 32 to 77%). In group III patients, treated with 250,000 IU urokinase and 48 mg of prourokinase 65% (13 of 20; 95% confidence interval 41 to 85%) of infarct vessels were patent 1 hour after initiation of treatment.

Side effects: In all patients a significant residual diameter stenosis persisted after reperfusion. Seventy-nine, 75 and 95% of patients underwent immediate percutaneous transluminal coronary angioplasty in groups I, II and III, respectively. In group I one patient experienced bleeding necessitating a transfusion. Another patient underwent rapid coronary artery bypass graft surgery because of severe left main stenosis. In group II one 74-year-old female patient died after 30 minutes of unsuccessful treatment for cardiogenic shock without apparent bleeding complications. Autopsy was not permitted. One patient, in whom thrombolytic treatment had been successful, underwent an emergency coronary artery bypass graft because the artery reoccluded shortly after initially successful percutaneous transluminal coronary angioplasty had been performed. The patient died after surgery; bleeding was not a complication. A third patient in whom thrombolysis and percutaneous transluminal coronary angioplasty had been unsuccessful removed the catheter himself 12 hours after the procedures and required transfusions. This patient later died of asystole. In group III no patient required transfusion. One patient died after reinfarction several days after the initial event.

Specificity of treatment: Infusion of prourokinase or the combination of urokinase and prourokinase induced a variable degree of systemic activation of the fibrinolytic system as summarized in Figure 1. Changes in plasma parameters were most pronounced 2 hours after the initiation of therapy. In group I fibrinogen, plasminogen and α-2 antiplasmin had decreased

2 hours after the initiation of treatment to 87 ± 12, 61 ± 13 and 59 ± 34% of pretreatment values. In group II mean values of these parameters dropped to 58 ± 29, 38 ± 18 and 21 ± 14% of pretreatment values. In 2 patients a lytic state with fibrinogen levels of <100 mg/dl was observed. The group treated with the combination of urokinase and prourokinase experienced a decrease to 76 ± 15, 67 ± 15 and 47 ± 29% of pretreatment values.

Appreciable differences between successfully and unsuccessfully treated patients with respect to the changes of plasma parameters were not observed as shown in Figure 2.

Discussion

This pilot study was performed to assess the thrombolytic efficacy, fibrin specificity and safety of different treatment regimens of glycosylated prourokinase in patients with AMI. We decided to administer a minimal dose of 48 mg of the drug on the basis of our own previous experience[16] and that of others.[14,15] This dosage was confirmed to be safe and systemic activation of the fibrinolytic system was mild; however, its efficacy (50% patency) was only moderate. Therefore, the dosage was increased to 74 mg (50% increase), which resulted in a marked systemic fibrinogenolysis and a virtual depletion of α-2 antiplasmin. In 2 patients plasma fibrinogen decreased to <50 mg/dl; however, no bleeding complications occurred. In contrast to the dramatic effect on the hemostatic system, the patency rate with 74 mg prourokinase remained at 55%. The combination of 48 mg of prourokinase and 250,000 IU of urokinase increased the efficacy of treatment to a patency rate of 65% at 1 hour after the initiation of therapy. Further, the changes in plasma parameters were less pronounced compared with the regimen

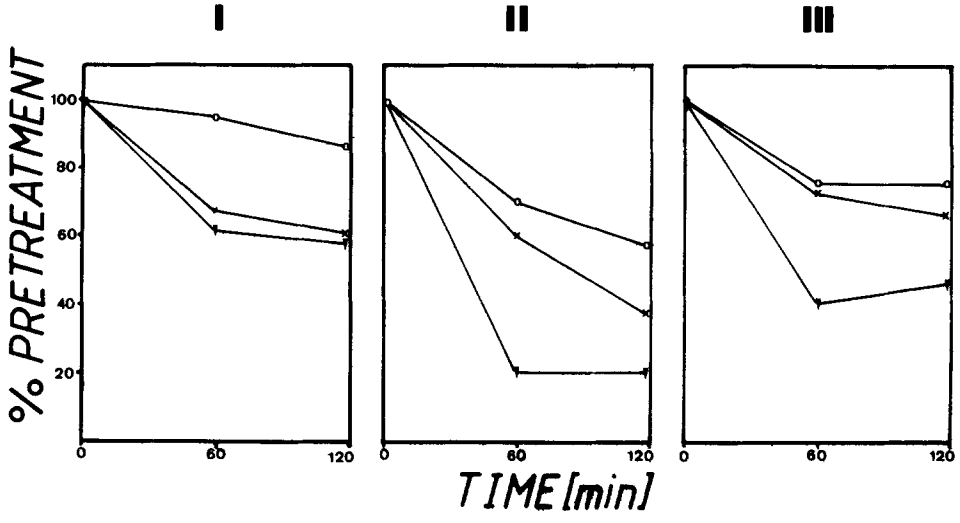

FIGURE 1. Changes of relevant plasma parameters over time with thrombolytic treatment with prourokinase. *Circles* = fibrinogen; *crosses* = plasminogen; *triangles* = α-2 antiplasmin.

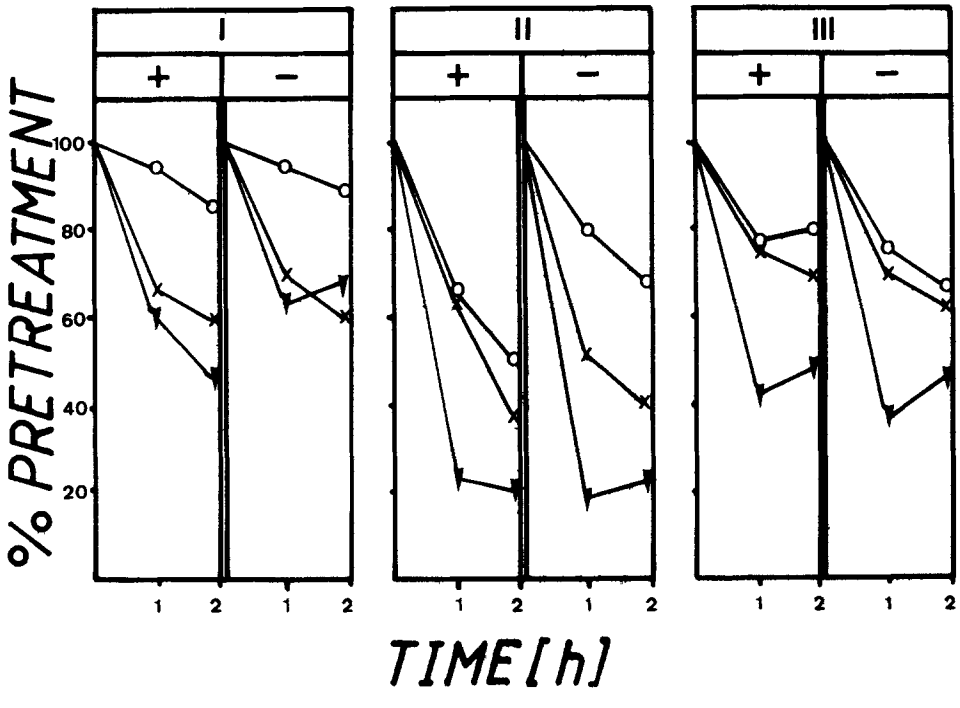

FIGURE 2. Comparison of changes in relevant plasma parameters in successfully versus unsuccessfully treated patients. *Circles* = fibrinogen; *crosses* = plasminogen; *triangles* = α-2 antiplasmin; + = successfully treated; − = unsuccessfully treated.

with 74 mg of prourokinase. No appreciable difference in the systemic activation of the fibrinolytic system was observed between successfully and unsuccessfully treated patients; this suggests that systemic fibrinogenolysis is not a prerequisite for successful thrombolysis. This finding is in accordance with published data about coronary thrombolysis with urokinase.[22] Whether or not fibrinogenolysis is an important parameter to predict reocclusion after initially successful recanalization cannot be evaluated as a considerable number of the patients in our study underwent immediate percutaneous transluminal coronary angioplasty for high grade residual stenosis. The patency rates in this study are lower than those reported for recombinant prourokinase, whereas the changes in plasma parameters are roughly comparable.[15] In comparing the dosages for glycosylated and recombinant prourokinase on a molar basis, a factor of 1.2 has to be taken into account. Thus, 48 mg of glycosylated prourokinase is equimolar to 40 mg of recombinant prourokinase. Further, repeated visualization of the occluded vessel before the endpoint of the study was omitted in our study to avoid artificial perfusion of the infarct vessel proximal to the occlusion. Finally, there may be differences in the enzymatic properties of natural and recombinant prourokinase not only in vitro,[18] but also in vivo, that could be accountable for the differences observed between glycosylated and recombinant prourokinase.

The combination of plasminogen activators and a possible synergism of their action has been the subject of controversial investigations in vitro.[23-28] One pilot study in patients has been performed[29] and 1 preliminary report[17] has come to our attention. At present it is unclear which combinations will prove useful and practical in patients with AMI.

The results of the present study suggest that coronary thrombolysis can be achieved by glycosylated prourokinase in patients with AMI. With 48 mg of prourokinase clot specificity is well maintained, while part of this specificity is lost at higher dosage. The combination of urokinase and prourokinase achieved the highest patency rates while systemic activation of the fibrinolytic system remained mild. This combination and a combination of prourokinase and tissue plasminogen activator deserve further study and are currently being compared in a prospective, randomized trial at our institution.

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