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Low Dose Urokinase Preactivated Natural Prourokinase for Thrombolysis in Acute Myocardial Infarction

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By inducing minimal free-fibrinolytic activity with low dose urokinase, the lag phase of prourokinase can be overcome, and the rate of thrombolysis with this substance can be strongly enhanced. The thrombolytic potency of a combination of 250,000 IU of urokinase and 2 doses of prourokinase (4.5 or 6.5 megaunits) was evaluated in an open-label, nonrandomized dose-finding study. Thirty-one patients participated. With 4.5 megaunits of prourokinase (group I, 15 patients) patency was demonstrated angiographically at 60 minutes in 33% while with 6.5 megaunits (group II, 16 patients) 75% patency was achieved ($p < 0.01$). A second angiogram recorded 24 to 36 hours after thrombolysis revealed reocclusion in 60 versus 8% of primarily patent coronary arteries ($p < 0.05$). Hemostatic monitoring in both groups revealed only slight to moderate consumption of fibrinogen (−9 vs −13%), plasminogen (−29 vs −34%) and α_2-antiplasmin (−59 vs −63%), and an increase in D-dimers, the split products of cross-linked fibrin, to a maximum of 1.008 ± 1.211 vs 0.547 ± 0.684 μg/liter. None of these differences was significant. Bleeding complications were more frequently observed in group II (13 vs 37%) (difference not significant), but were mild and related to puncture sites, except in 1 patient with mild oozing from the gum. No major hemorrhage was observed. These results suggest that low dose urokinase preactivation enhances the thrombolytic potency of prourokinase, without affecting its high fibrin specificity. Compared to previous studies using only prourokinase, low dose urokinase preactivation reduces by 50% the prourokinase doses required for effective thrombolysis. Thus, the combination of 250,000 IU of urokinase and 6.5 megaunits of prourokinase infused intravenously over 40 minutes meets the characteristics of an ideal regimen for thrombolysis in patients with acute myocardial infarction.

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The causative role of coronary artery thrombosis in acute myocardial infarction (AMI) has been firmly established.[1,2] As a consequence, the intravenous administration of streptokinase has been introduced to reestablish anterograde blood flow. Meanwhile, thrombolytic therapy has proved to be successful in reperfusing coronary arteries,[3] to salvage jeopardized myocardium[4,5] and to improve in-hospital and long-term mortality rates.[3–7] Despite the favorable results obtained with streptokinase, however, this thrombolytic agent is far from ideal.

This has led to a search for other thrombolytic substances. New, promising drugs such as acylated streptokinase-plasminogen complex and tissue-type plasminogen activater have been developed. Recently, another new thrombolytic agent, prourokinase or single-chain urokinase-type plasminogen activator,[8,9] has become available for clinical investigations. In animal studies[10,11] and in healthy volunteers[12] this substance demonstrates high fibrin specificity. In contrast to other plasminogen activators, the reaction kinetics of prourokinase have a long lag phase, before the substance reaches its final thrombolytic activity.[11] Early clinical results indicated that with low dose urokinase preactivation, this lag phase can be overcome, and thrombolysis with prourokinase can thus be strongly accelerated.[13,14] Hence, we designed this dose-finding study for urokinase preactivated prourokinase in patients with AMI.

METHODS

Patient selection: Patients of either sex who had an AMI with onset of symptoms of <4 hours were considered for study entry. ST-segment elevation of ≥2 mm was required in at least 2 contiguous leads. Patients were excluded from the study for the following reasons:

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(1) chest pain relief by either sublingual nitroglycerin or nifedipine within 10 minutes of administration, (2) age >75 years, (3) a history of recent gastrointestinal bleeding, (4) recent cerebrovascular accident, (5) recent major trauma or surgery, (6) known bleeding tendency or coumadin therapy, (7) pregnancy and (8) all other contraindications for either thrombolytic therapy or cardiac catheterization. A history of remote AMI did not exclude patients from the study. Written informed consent was obtained from all patients before study entry.

Materials: High molecular weight heparin was purchased as Liquemin from Hoffman LaRoche AG; high molecular weight urokinase was supplied by KabiVitrum GmbH. Natural prourokinase (TCL 598; specific activity 135,000 U/mg) was supplied by Sandoz AG.

Study design: The study was designed as an open-label prospective trial. Upon enrollment, patients immediately received a 5,000 IU heparin bolus followed by a continuous heparin infusion of 1,250 IU/hr, which was not interrupted during thrombolytic therapy (Figure 1).[15] Thrombolysis was started with a 250,000 IU intravenous bolus of high molecular weight urokinase, which was followed by a 40-minute prourokinase infusion of either 4,500,000 U (33 mg) (group I) or 6,500,000 U (48 mg natural prourokinase) (group II). Intravenous nitrates (≥75 mg/day) were given to all patients. Patients were assigned to the treatment groups by time of study entry; the first 15 patients were assigned to group I. All other medications, including antiarrhythmic drugs, sedatives, diuretics and opiate analgetics, were administered as required. To avoid possible interactions between prourokinase and all other medications, prourokinase was administered through a separate intravenous line. For coagulation analysis, blood samples were drawn before thrombolysis, and at 15, 60 and 120 minutes into thrombolytic therapy.

Coronary angiography was performed within 60 minutes after thrombolytic therapy began; the Judkins technique from the right femoral artery was used. If at 60 minutes into thrombolysis the infarct-related coronary artery was still occluded, the investigators were free to decide how to further treat the patient. In patients with a patent infarct-related vessel, the intravenous heparin infusion was continued in a dose resulting in a prolongation of the activated partial thromboplastin time to 60 to 80 seconds. In these patients a second coronary angiogram was recorded the second day after admission.

Assessment of angiograms: Cineangiograms were assessed for perfusion of the infarct-related coronary artery. Perfusion was assessed according to the score used in the Thrombolysis in Myocardial Infarction trial[16]: grade 0 = no perfusion; grade 1 = penetration of the thrombus with minimal perfusion; grade 2 = partial perfusion; grade 3 = complete perfusion. Based on this grading system, total coronary occlusion was considered

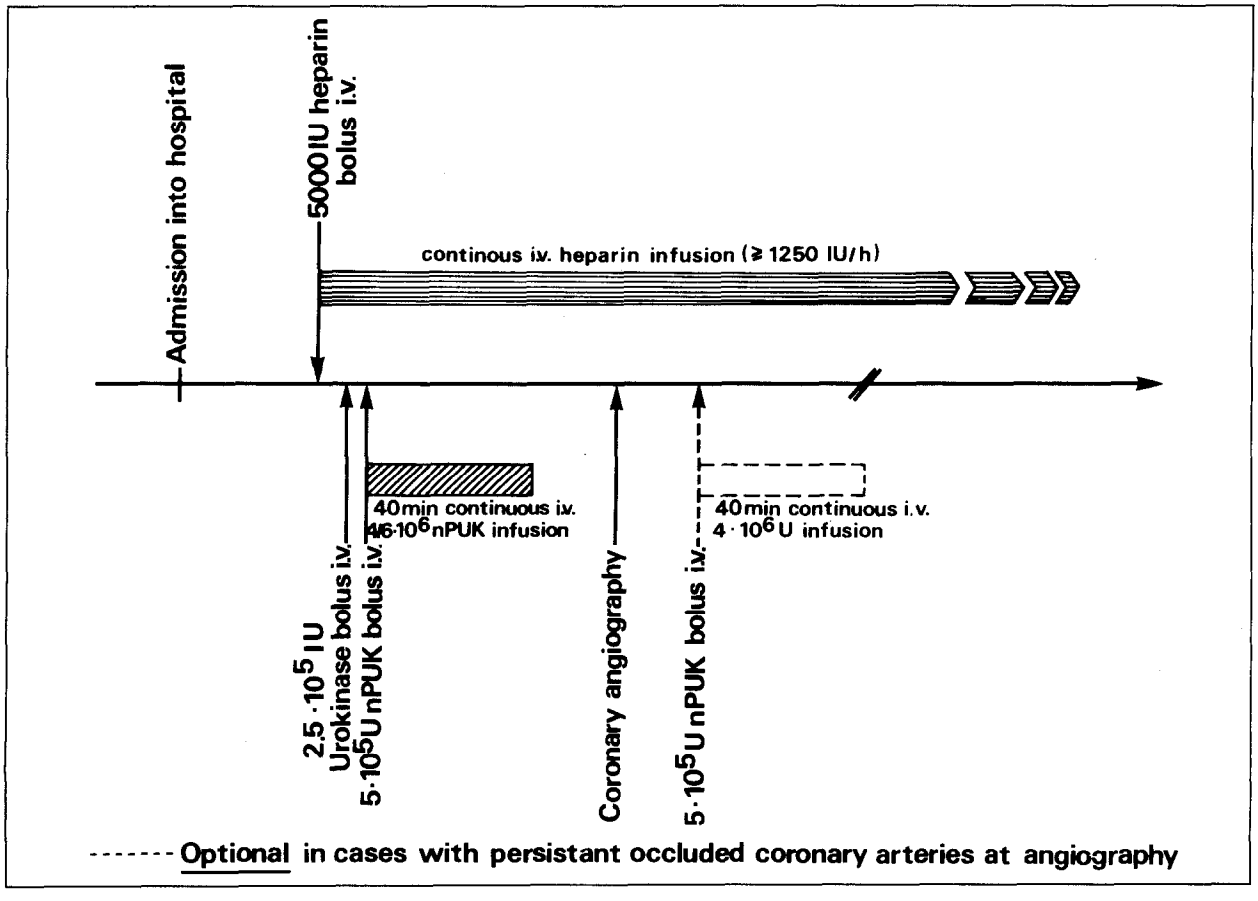

FIGURE 1. Study design. PUK = prourokinase.

TABLE I Patient Data

	Group I	Group II	p Value
Pts (n)	15	16	
Males:Females	12:3	15:1	
Age (yrs)	56 ± 10	58 ± 9	
Body weight (kg)	80 ± 14	82 ± 13	
Height (cm)	171 ± 9	176 ± 5	
Duration of symptoms until start of thrombolysis (hrs)	2.3 ± 1.7	2.4 ± 1.6	
Infarct-related vessel			
LAD:LC:Right	8:0:7	5:2:9	
UK/PUK doses	250,000 U/4.5 · 10^6 U	250,000 U/6.5 · 10^6 U	
Patency achieved (%)	5/15 (33)	12/16 (75)	<0.01
Second PUK infusion	9	2	
Mechanical recanalization	1	1	
Final patency achieved (%)	13/15 (87)	14/16 (88)	NS
Early thrombotic reocclusion/primarily patent vessels (%)	3/5 (60)	1/12 (8)	<0.05
Bleeding complications			
Arterial puncture sites (%)	2* (13)	5 (31)	
Gastrointestinal	0	0	
Urogenital	0	0	
Cerebral	0	0	
Gum (%)	0	1 (6)	
Total no. of bleedings (%)	2 (13)	6 (37)	
Other major complications			
Allergic reaction	0	0	
Acute renal failure (%)	1† (7)	0	
Death (%)	1† (7)	0	
Total no. of other complications	2 (13%)	0	

* One patient with extension into the retroperitoneal space; † artery not reopened by thrombolysis.
LAD = left anterior descending coronary artery; LC = left circumflex artery; PUK = natural prourokinase (TCL 598); UK = urokinase.

to be present when perfusion was grade 0 or 1. Patency of the coronary artery was defined as grade 2 or 3 perfusion.

Coagulation assays: For fibrinogen and D-dimer analysis, blood samples were collected on citrate-containing aprotinin (final concentration, 200 KIU/ml). Citrated blood samples were collected for the determination of plasminogen and α_2-antiplasmin. The samples were centrifuged within 2 hours after collection, and the plasmas were deep frozen at −70°C until they were analyzed.

For fibrinogen determinations, Bangs modification of the Ratnoff-Menzie method was used.[17] For D-dimer measurements the enzyme-linked immunosorbent assay technique described by Stötzer[18] was used. Reagents for D-dimer measurements were purchased as an assay kit (enzyme-linked immunosorbent assay D-dimer) from Boehringer Mannheim GmbH. For plasminogen,[19] and α_2-antiplasmin[19] determinations chromogenic peptide substrate assays were used. The chromogenic peptide substrate H-D-Val-Leu-Lys-pNA used in this assay was purchased as S-2251 from KabiVitrum GmbH.

Statistical analysis: For the comparison of the 2 treatment groups, Fischer's exact test was used. For statistics in the coagulation analysis, Fischer's *t* test was used. P values of <0.05 were considered statistically significant.

RESULTS

Patients: Of the 31 patients enrolled in this dose-finding study, 15 were assigned to 4,500,000 U (group I) and 16 received 6,500,000 U of prourokinase (group

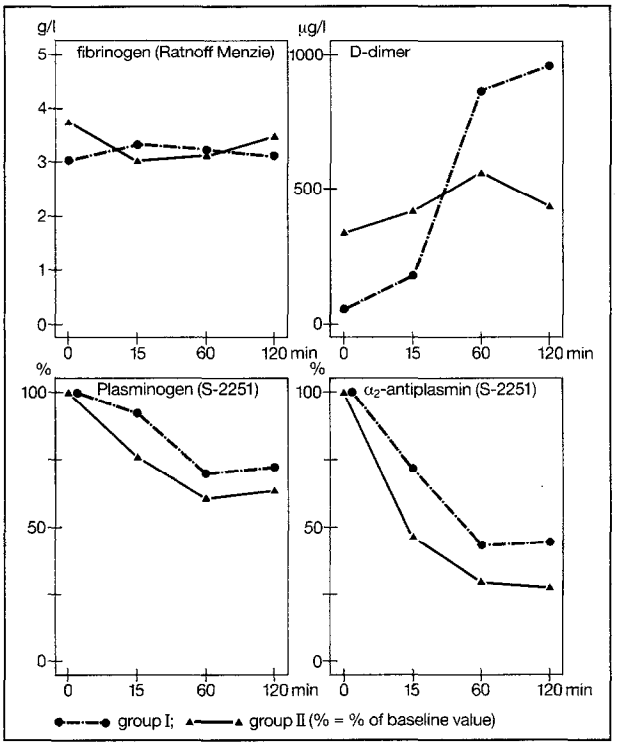

FIGURE 2. Hemostatic parameters before, and 15, 60 and 120 minutes into thrombolysis for acute myocardial infarction with natural prourokinase. *Closed circles* = 250,000 U of urokinase plus 4.5 megaunits of prourokinase (group I), *closed triangles* = 250,000 U of urokinase plus 6.5 megaunits of prourokinase (group II). Plasminogen and α_2-antiplasmin are given in percent of baseline value.

II). At the time of study entry no differences between both treatment groups were observed with respect to demographic or clinical characteristics (Table I).

Coronary angiographic findings: Using the previously mentioned criteria, angiography 60 minutes after onset of thrombolysis revealed a patent infarct-related coronary artery in only 5 of 15 patients in group I (33%). In contrast, in group II patients patency at 60 minutes was achieved in 12 of 16 patients (75%) ($p < 0.01$). A second intravenous prourokinase infusion was given to 11 patients in whom primary patency was not achieved (9 in group I; 2 in group II), resulting in secondary reopening in 9 patients (7 group I; 2 group II). Mechanical recanalization of the vessel was successfully performed in 2 patients and 1 patient was left with the infarct-related vessel occluded.

Coronary angiography repeated the second day after thrombolysis—in all 17 patients in whom patency of the infarct vessel was achieved by the first prourokinase infusion—revealed reocclusion in 4. Thus, the reocclusion rate was 23%. It is noteworthy that there was a tendency toward a higher reocclusion rate in the lower dose group (3 of 5 primary patent vessels) than in the higher dose group (1 of 12 primary patent vessels) ($p < 0.05$). Because of the small number of patients, however, these results should be regarded with caution (Table I).

Adverse effects: Bleeding complications were observed in 8 patients, with a tendency toward a higher prevalence in group II (2 of 15 vs 6 of 16; Table I). The difference was not statistically significant. Bleeding was most frequently located at arterial puncture sites after cardiac catheterization (7 of 8 bleeding complications, Table I). In 1 patient, mild oozing from the gingiva was observed 36 hours after prourokinase infusion. Bleeding complications were generally mild, and none of the patients required blood transfusion.

Major complications other than hemorrhage were observed in 2 patients, (Table I) both receiving the lower prourokinase dose. The first angiogram recorded 60 minutes into thrombolysis in both of these patients revealed that the infarct-related vessel was still occluded. One of these patients went into cardiogenic shock and died 2 days later. The other patient underwent emergency catheter angioplasty. He developed acute renal failure, which was probably related to the high doses of contrast medium infused during acute intervention. This patient recovered without renal dysfunction (Table I).

Hemostatic variables: During the infusion of prourokinase, fibrinogen levels in group I remained essentially unchanged, while in group II patients they decreased slightly, the minimum averaging 79% of the baseline value (Figure 2). These decreases in fibrinogen levels were not statistically significant. Surprisingly, in the lower prourokinase dose group, D-dimers—the specific degradation products of cross-linked fibrin—increased more markedly, from 56 ± 24 to 1.008 ± 1.211 μg/liter

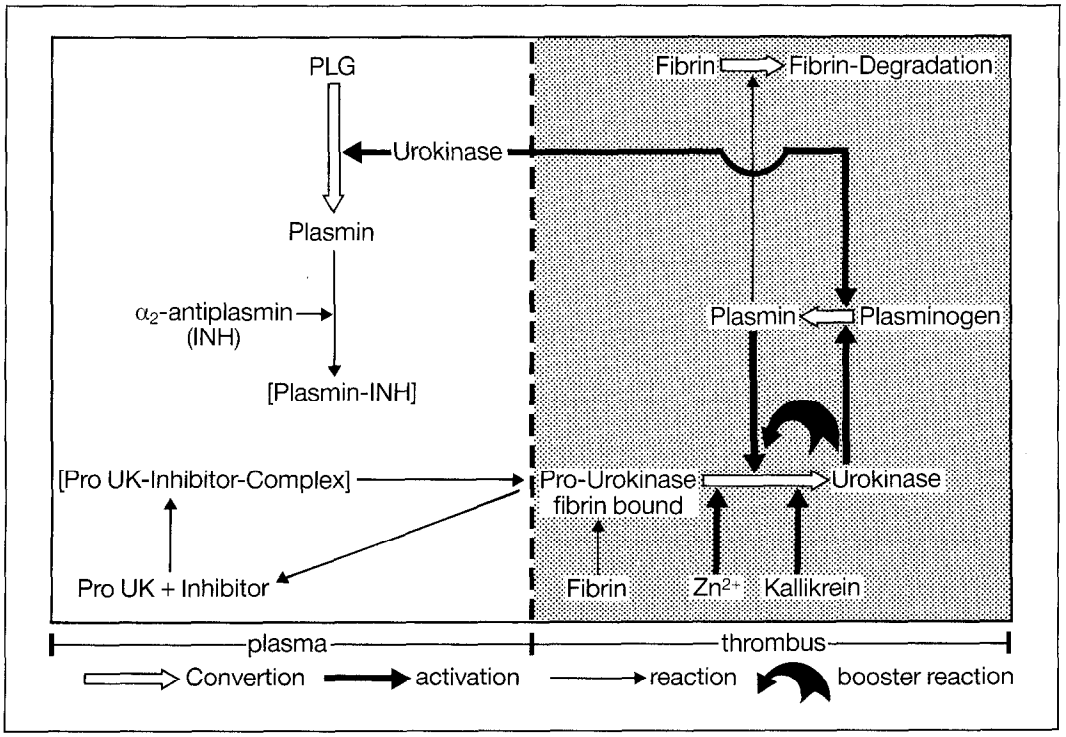

FIGURE 3. Circulating prourokinase is reversibly bound to a specific prourokinase (pro-UK) inhibitor. On contact with clotted fibrin, free pro-UK is liberated from this enzyme-inhibitor complex. Free pro-UK, with a low intrinsic activity, then converts plasminogen to plasmin, which in a second reaction converts pro-UK to urokinase, a plasminogen activator with a far higher catalytic rate constant. As soon as urokinase is formed, thrombolysis, in a feedback reaction is accelerated by itself, resulting in rapid thrombolysis. As urokinase is only formed at the clot surface, thrombolysis with pro-UK is fibrin specific.

than in the higher prourokinase dose group (347 ± 646 to 547 ± 684 μg/liter. The difference between both groups was not significant (Figure 2).

In both groups a significant decrease in plasminogen levels was observed (Figure 2). Plasminogen levels in the lower dose group decreased −30.5 ± 39.6% from baseline ($p < 0.02$), while in the higher dose group, they decreased −38.4 ± 26.1% from baseline ($p < 0.002$). Consumption of α_2-antiplasmin in group I with values of −57.6 ± 35.0% ($p < 0.001$) and −70.9 ± 12.5% in group II ($p < 0.001$) was even more marked. When both groups were compared with respect to plasminogen and α_2-antiplasmin consumption, however, the differences were not significant (Figure 2).

DISCUSSION

Mechanism of action of prourokinase: Prourokinase is a new physiologic plasminogen activator that was first isolated in 1982.[8,20] In vitro[8,21] and in vivo studies[10,11] of this serine protease[9] revealed a high thrombolytic potency associated with a high clot selectivity. The mechanism of action includes a lag time before the substance reaches its final activity.[11]

The underlying mechanism of action is still under debate.[22,23] According to 1 theory[22] (Figure 3), circulating prourokinase is reversibly bound by a specific prourokinase inhibitor. On contact with clotted fibrin, free prourokinase is liberated from this enzyme-inhibitor complex. Free prourokinase, with a low intrinsic activity, then converts plasminogen to plasmin, which, in a second reaction can convert prourokinase to urokinase, a plasminogen activator with a far higher catalytic rate constant.[22] As soon as urokinase is formed, the reaction is accelerated by itself. Pannell and Gurewich,[23] however, attribute the clot selectivity of prourokinase to its binding to and predominant activation of Lys-plasminogen (Figure 4). While the bulk of circulating plasminogen is in its Glu-form, plasminogen in its Lys-form—mediated by the exposure of lysine binding sites—is specifically bound to fibrin surfaces.[24] Fibrin-bound plasminogen, therefore, is predominantly in its Lys-form. Once prourokinase has activated Lys-plasminogen, Lys-plasmin will induce modification of the fibrin molecule, which exposes additional plasminogen binding sites.[25] Thus, the reaction progressively accelerates. Both mechanisms are not necessarily exclusive.

Preactivation of prourokinase with urokinase: The initial lag phase observed with prourokinase would be in accordance with both suggested mechanisms. From a theoretical standpoint, by inducing a small amount of plasmin activity at fibrin surfaces, fibrinolysis should be enhanced, no matter which of the 2 mechanisms is actually involved. By the addition of low doses of urokinase given in advance, either conversion of prourokinase to

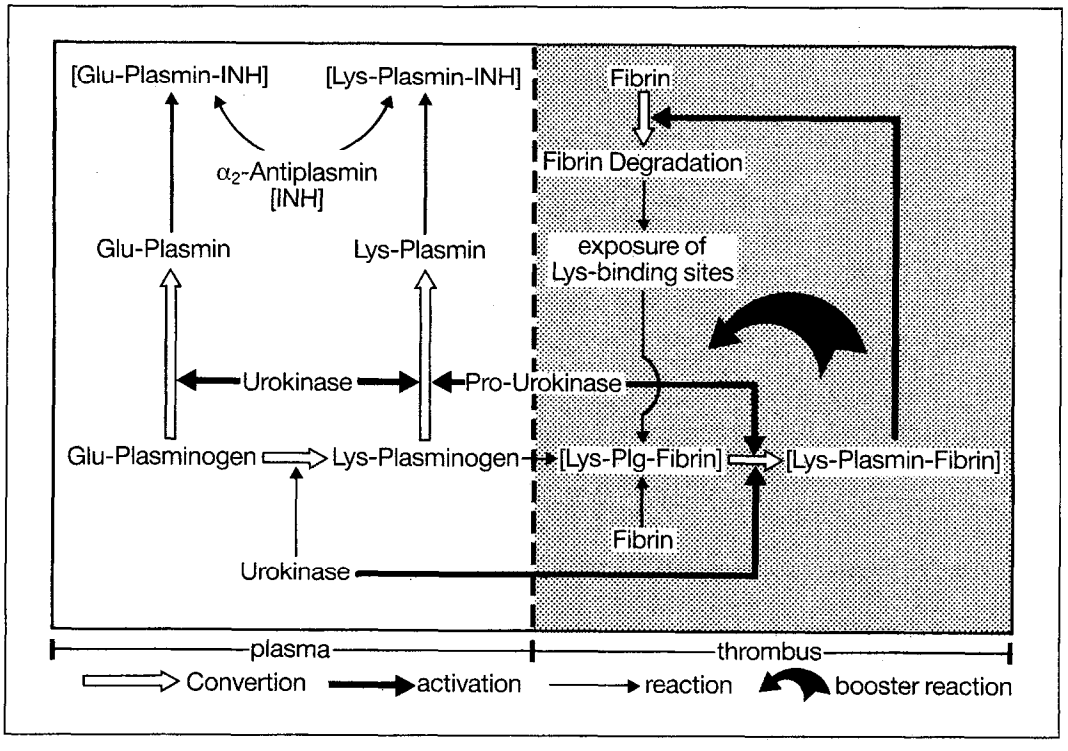

FIGURE 4. Plasminogen exists in 2 molecular forms: Glu-plasminogen and Lys-plasminogen. Lys-plasminogen is formed from Glu-plasminogen by limited proteolysis, induced by urokinase or pro-urokinase, for example. While the bulk of circulating plasminogen is in its Glu-form, Lys-plasminogen rapidly binds to Lys-binding sites on the fibrin surface. Fibrin-bound plasminogen is therefore predominantly in its Lys-form. Once Lys-plasminogen is activated to Lys-plasmin, fibrinolysis will induce modifications in the fibrin molecule that expose additional Lys-binding sites, and additional Lys-plasminogen can thus be bound. As pro-urokinase predominantly activates Lys-plasminogen, thrombolysis with pro-urokinase is fibrin specific and progressively accelerates.

urokinase at the clot surface[22] or the exposure of additional Lys-binding sites on the thrombus[23,25] can be achieved. The positive effect of a preactivation with low dose urokinase has recently been confirmed in clinical trials.[13,14]

With a low patency rate of only 40% using up to 11 megaunits of prourokinase (81.5 mg) without urokinase preactivation,[13] we regarded this thrombolytic regimen as ineffective. From a theoretical standpoint, however, combination of prourokinase with low doses of urokinase seemed promising. In this dose-finding study, we studied the combination of prourokinase with a 250,000 IU urokinase preactivation. Of the 2 prourokinase doses tested, only the higher dose (6,500,000 U) achieved adequate patency rates (75%). In contrast, with 4,500,000 U of prourokinase, patency rates were as low as 33%, which is about what can be expected from spontaneous reperfusion in a population not undergoing thrombolytic therapy.[26] Furthermore, the lower prourokinase dose was associated with a reocclusion rate as high as 60%, while in the high dose group the reocclusion rate was only 8%. Comparing these results with our previous findings[13] and other studies, applying up to 14 megaunits of prourokinase (80 mg recombinant prourokinase corresponding to 103.7 mg of natural prourokinase) without urokinase preactivation yields patency rates up to 70%.[13,14,27,28] Thus, preactivation with low dose urokinase actually seems to reduce the prourokinase dose required to reach therapeutic efficacy.

Hemostatic variables: Even after preactivation with low dose urokinase, thrombolysis with prourokinase is highly fibrin specific. In this study only minor changes of the fibrinogen levels were observed. It has been assumed that systemic fibrinogen depletion would contribute essentially to the success rate of thrombolysis.[29,30] In this study, with 250,000 IU urokinase and 6,500,000 U prourokinase, patency rates of 75% could be achieved without causing severe systemic fibrinogen breakdown.

The high residual fibrinogen levels observed in group II after thrombolysis do not seem to influence reocclusion rates adversely. In view of the high reocclusion rate in the low dose prourokinase group, one can assume that thrombotic reocclusion is most likely due to incomplete thrombolysis, thrombotic material remaining at the site of the ruptured coronary artery stenosis, which may serve as a nidus for further thrombus apposition rather than for a second thrombotic event.

Alpha$_2$-antiplasmin, the fast inhibitor system of plasmin, inactivates circulating plasmin activities almost immediately.[24] Therefore, as long as the pool of α_2-antiplasmin is not completely depleted, circulating free-fibrinolytic activity is prevented, and breakdown of plasma fibrinogen is precluded. In our study, neither dose tested resulted in complete consumption of α_2-antiplasmin. This is in accordance with our observation that fibrinogen levels remain almost unaltered. Physiologically, the plasma concentration of plasminogen is about double that of α_2-antiplasmin.[24] Stronger activation of the systemic fibrinolytic system than that induced with the combination of 250,000 U urokinase and 6.5 megaunits of prourokinase used in this study would therefore most likely lead to systemic fibrinogen breakdown.

Adverse effects: Bleeding complications in this study were observed in 25.8% of the patients and were mainly related to arterial puncture sites due to cardiac catheterization. Blood loss due to the bleeding complications was only mild or moderate, and none of the patients required blood transfusion. Complications other than hemorrhage were observed in 2 patients, both directly related to treatment failure. With regard to the bleeding complications observed, the combination of low dose urokinase preactivation and prourokinase infusion seems reasonably safe. The low number of patients treated in this study, however, precludes definite judgment of the hemorrhagic risk.

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