Intravenous Thrombolytic Therapy With a Combination of Single-Chain Urokinase-Type Plasminogen Activator and Recombinant Tissue-Type Plasminogen Activator in Acute Myocardial Infarction

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The effects of simultaneous intravenous infusions of 12 mg recombinant tissue-type plasminogen activator (rt-PA) over 30 minutes and 48 mg single-chain urokinase-type plasminogen activator (scuPA) over 40 minutes were studied in 38 patients with acute myocardial infarction. Coronary arterial patency was assessed angiographically 60 minutes and 90 minutes after initiation of treatment. Patency was achieved in 19 of 31 patients (61.3%) (95% confidence limits, 42–78%) at 60 minutes and in 27 of 33 patients (81.8%) (95% confidence limits, 65–93%) at 90 minutes. Nonspecific plasminogen activation was monitored by measuring relevant plasma parameters. At 60 minutes and 120 minutes, the fibrinogen concentration decreased slightly to 82.8±24.3% and 91.2±17.4% of the preinfusion level, and the plasminogen concentration to 66.3±15.2% and 65.3±13.4%, respectively. A greater consumption of α2-antiplasmin was observed, which decreased to 30.7±22.8% and 32.2±21.2% of the preinfusion level at 60 and 120 minutes, respectively. No bleeding necessitating transfusion was observed. Two patients (5.3%) died during hospitalization. The findings suggest that the combined intravenous infusion of rt-PA and scuPA at appropriate doses induces highly effective coronary thrombolysis equal to the best results obtained with either rt-PA or scuPA alone. This efficacy is coupled with high specificity. Thus, the data support the potential use of combinations of rt-PA and scuPA in place of monotherapy. (Circulation 1990;81:907–913)

Coronary angiographic studies indicate that most Q wave acute myocardial infarctions are caused by coronary thrombosis. Intravenous thrombolytic therapy by several plasminogen activators is capable of inducing early coronary reperfusion, thereby preserving left ventricular function and reducing both mortality and morbidity. Streptokinase and urokinase are nonspecific plasminogen activators that induce systemic plasmin generation. Plasminemia causes a hemostatic defect, activation of the complement system with release of anaphylotoxins, and has been implicated in platelet hyperaggregability and rethrombosis. These untoward effects of plasmin can significantly compromise the benefits of thrombolysis. These facts have stimulated different lines of research to develop more specific therapy.

Tissue plasminogen activator (t-PA) and single-chain urokinase-type plasminogen activator (scuPA) induce more selective fibrinolysis. When they are used alone, however, the exceptionally high doses of each activator required to effect coronary thrombolysis cause significant nonspecific plasminogen activation. Recently, it has been shown in vitro and in experimental animals in small pilot trials that recombinant tissue-type plasminogen activator (rt-PA) and scuPA act synergistically in fibrinolysis. As a result, it might be...
possible with certain combinations of rt-PA and scuPA to induce more effective thrombolysis without compromising specificity. By lowering the overall dose requirements, it might be additionally possible to reduce the cost of therapy.

The present study was designed to evaluate this concept. A dosage combination was chosen that was estimated to be just below the threshold at which fibrinogen degradation would be anticipated.

**Methods**

**Material**

Human scuPA was highly purified from the conditioned medium of the transformed kidney cell-line TCL-598. The material was made available by Sandoz AG, Nürnberg, FRG. The drug was supplied in vials containing 3.75 mg freeze-dried scuPA and human serum albumin as a stabilizer. It was stored at 4°C and dissolved in water immediately before use. The purified, glycosylated protein 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 virtually no measurable urokinase activity (<1%) in the purified preparation. Recombinant tissue-type plasminogen activator was purchased from Thomae GmbH, Biberach, FRG. The drug was handled according to the manufacturer's instructions. On sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) it proved to be predominantly single-chain material. Both drugs were infused with constant rate infusion pumps through separate infusion lines.

**Patients**

Inclusion criteria consisted of chest pain typical of myocardial infarction lasting for more than 30 minutes, diagnostic electrocardiographic ST segment elevation of 2 mm in at least three leads or 1 mm in at least three leads accompanied by ST segment depression in three corresponding leads, presentation within 6 hours after the onset of pain, age less than 75 years, and informed consent.

Exclusion criteria were recent (<3 months) trauma, stroke or major surgery, previous myocardial damage in the infarct territory, cardiopulmonary resuscitation, cardiogenic shock (systolic blood pressure, <80 mm Hg), hypertension (systolic blood pressure, >200 mm Hg; diastolic blood pressure, >110 mm Hg; or both) unresponsive to therapy within 10 minutes, or evidence of recent or active bleeding (e.g., peptic ulcer and hemoptysis). The study protocol had been approved by the institutional ethics committee.

**Treatment**

Patients were anticoagulated with 5,000 units heparin at the start of treatment and 7,500 units just before cardiac catheterization (at 45 minutes). A bolus of 3.7 mg scuPA was applied over 1 minute, and immediately thereafter, two simultaneous infusions were started, that is, 44.3 mg scuPA was infused over 40 minutes, and 12 mg rt-PA was infused over 30 minutes. After completion of the infusions, patients were transferred to the cardiac catheterization laboratory where arterial access was obtained in all patients by the femoral route. Angiographic visualization of the presumed infarct vessel and assessment of patency were performed at 60 minutes and 90 minutes after the start of treatment. Several angiograms in various orthogonal and hemiaxial views were obtained, and all films were analyzed independently by at least two investigators. Angiography of the noninfarct vessels was also performed; ventriculography was not routinely performed. Intracoronary injections other than specified by the study protocol were forbidden, to minimize interference with natural blood flow. As per the Thrombolysis in Myocardial Infarction Trial (TIMI), grades 0 to III were used to grade patency after treatment; grades II and III were considered successful treatment. After 90 minutes, coronary angioplasty was performed at the discretion of the investigator. Balloon dilation of stenoses in the infarct related vessel was accomplished by three to five inflations at 3–8 atm. No intracoronary injections of thrombolytic agents were allowed.

All patients were followed during the time of hospitalization. Intravenous heparin was given at a dose designed to prolong the partial thromboplastin time to threefold for at least 3 days. Immediately after the cardiac catheterization procedure, aspirin (325–500 mg/day) was given. Patients received intravenous β-blockers and nitroglycerin unless contraindicated.

Repeat cardiac catheterization was performed at 21 days to determine late patency of the infarct-related vessel although this was not considered a study end point. Angioplasty of the infarct-related artery was performed during repeat catheterization in cases with high-grade residual stenosis, an exercise thallium-scan positive for ischemia, or both.

**Coagulation Parameters**

Blood samples were obtained from 34 patients before initiation of thrombolytic therapy and 60 and 120 minutes after the start of therapy. Blood was collected on citrate (final concentration, 0.01 M) and immediately centrifuged at 4°C. Serum was quick-frozen and stored at −80°C. Fibrinogen,27 plasminogen,28 and α2-antiplasmin29 levels were determined from frozen samples.

Severe bleeding was defined as either a blood loss resulting in the necessity of transfusions, or a decline in hemoglobin concentration of more than 5 g/dl. All observed bleeding complications had to be recorded in the patient chart.

**Results**

**Patients**

In the 38 patients enrolled, treatment began 2.9 ± 1.1 hours after the onset of symptoms. The mean
age was 56±9 years, and 79% of the patients were male. The infarct-related coronary artery was the left anterior descending artery in 29%, the left circumflex in 21%, and the right coronary artery in 50% of the patients. Patient characteristics are summarized in Table 1.

**Efficacy 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 TIMI Trial), was observed in 19 of 31 patients (61.3%) (95% confidence interval, 42–78%) at 60 minutes and in 27 of 33 patients (81.8%) (95% confidence interval, 65–93%) at 90 minutes.

At 60 minutes, 35.5% of the patients (11/31) had TIMI grade III reperfusion, whereas 25.8% (8/31) had TIMI grade II reperfusion, and 38.7% (12/31) had a completely occluded infarct artery (TIMI grade 0). At 90 minutes, 57.6% of the patients (19/33) had TIMI grade III reperfusion, and 24.2% (8/33), 3.0% (1/33), and 15.2% (5/33) showed TIMI grades II, I, and 0 reperfusion, respectively, as shown in Figure 1.

In two patients, assessment was only possible after 90 minutes because the catheterization laboratory was not available at 60 minutes. Five patients had to be excluded from the angiographic part of the study for the following reasons: In three patients, a coronary angiogram could not be obtained at either 60 or 90 minutes (nor within 30 minutes, thereafter) because both catheterization facilities were occupied. In one patient, a percutaneous transluminal coronary angioplasty (PTCA) procedure was performed at 45 minutes because the clinical situation became unstable, and the infarct vessel was still occluded. In one patient, the infarct artery could not be determined unequivocally, the right coronary artery was completely occluded but well collateralized and could not be reopened by PTCA (presumably an old occlusion). The circumflex artery showed a high-grade stenosis with TIMI grade III blood flow at 60 minutes, however, electrocardiographic changes persisted (judged as left circumflex artery in Table 1 and as “not assessable” for Figure 1).

**Specificity of Treatment**

Intravenous thrombolytic therapy with the combination of rt-PA and scuPA induced little systemic activation of the fibrinolytic system as summarized in Figure 2. At 60 and 120 minutes, the fibrinogen level had declined to 82.8±24.3% (n=34) and 91.2±17.4% (n=32) of pretreatment values, respectively. The initial decline probably also reflects dilution by the various infusions and possibly some in vitro degradation. Notably, at 60 minutes, only four of 34 patients (11.8%) had a transient fibrinogen reduction to less than 100 mg/dl, among them was one patient (2.9%) with less than 50 mg/dl. At 120 minutes, the fibrinogen level was well above 100 mg/dl in all patients. The mean plasminogen concentration in the patients was 88.2±18.2% (n=34) of a normal reference plasma at the beginning of therapy. At 60 minutes, plasminogen levels had declined to 66.3±15.2% (n=31) of pretreatment levels, and they remained stable at 120 minutes at 65.3±13.4 (n=31) of the initial plasminogen level. α2-Antiplasmin, the most sensitive measure of plasmin generation, was 98.8±16.4% (n=33) of a normal reference plasma at the outset of therapy. At 60 and 120 minutes, α2-antiplasmin levels had declined to 30.7±22.8% (n=33) and 32.2±21.2% (n=31) of pretreatment values, respectively. This indicates that, although some free plasmin was generated, the neutralizing capacity of the antiplasmin system was not exhausted. Therefore, the total dose of plasminogen activators used seems to be just below the threshold of specificity, as was estimated at the start of this trial.

**Side Effects**

No bleeding complications necessitating transfusion occurred. There was no report of overt bleeding or excessive hematoma at the puncture site. Hemoglobin concentration 1 day after thrombolytic treatment was 87.9±8.1% of the pretreatment value (15.0 vs. 13.2 g/dl). Three patients had a decline in hemoglobin concentration of more than 3 g/dl but no bleeding was detected. No patient experienced a decrease in hemoglobin concentration of more than 5 g/dl. No other side effects of the combination therapy

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**TABLE 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>November 1, 1987–December 31, 1988</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>79 (30/38)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56.1±9.0 (range, 33–69)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.3±8.3 (range, 155–191)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.6±13.0 (range, 45–115)</td>
</tr>
<tr>
<td>LAD (%)</td>
<td>29 (11/38)</td>
</tr>
<tr>
<td>LCx (%)</td>
<td>21 (8/38)</td>
</tr>
<tr>
<td>RCA (%)</td>
<td>50 (19/38)</td>
</tr>
<tr>
<td>Interval (hr)</td>
<td>2.9±1.1 (range, 1.0–6.0)</td>
</tr>
</tbody>
</table>

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

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**Figure 1.** Bar graph showing patency of infarct-related coronary arteries (in percentages).
were detected clinically (i.e., no hypotension and no allergic reaction) or by routine laboratory analysis.

Clinical In-Hospital Follow-up

In all patients, a significant residual diameter stenosis persisted after reperfusion. Of the 27 successfully treated patients, 20 underwent an immediate (>90 minutes after onset of thrombolytic therapy) PTCA procedure. Sixteen of these interventions were successful in reducing the grade of stenosis, and four were not. One patient, in whom thrombolysis had been initially successful but who suffered reoclusion of the infarct artery 1 hour after a nonsuccessful PTCA procedure, died in cardiogenic shock. In five of the six patients who did not respond to thrombolytic treatment, PTCA was attempted. The procedure was successful in four cases. A second patient died of infarction 17 days after admission. The acute angiogram had been judged “not accessible,” because the right coronary artery was occluded and the left circumflex artery showed high-grade stenosis.

Because repeat angiography was not performed in all patients and because there was no uniform protocol for postthrombolysis PTCA, no firm conclusions could be drawn with respect to the reclosure rate after thrombolytic treatment alone.

Discussion

As originally documented by DeWood et al.,1 almost 90% of patients with acute myocardial infarction have an occlusive coronary thrombus. Optimally, efficacious coronary thrombolytic therapy should provide a corresponding patency, if not recanalization, rate. Judged by this standard, results with intravenous streptokinase, urokinase, acylated plasminogen streptokinase complex (APSAC), rt-PA, and scuPA fall short of this objective.18,26,30,31 Moreover, with respect to specificity, the current thrombolytic regimens are associated with a substantial consumption of plasminogen and with significant degradation of fibrinogen. Although these changes are greater with streptokinase, APSAC, and urokinase, monotherapy with rt-PA and scuPA are also almost nonspecific at the high doses required for efficacy. In a recent randomized trial, 80 mg recombinant unglycosylated scuPA produced patency in 71.2% of patients, at 90 minutes, associated with extensive fibrinogenolysis (mean of 79 mg/dl at 60 minutes).18 It is important to realize that, calculated from the specific activities of the two agents, 80 mg recombinant scuPA (specific activity, 175,000 IU)32 corresponds to approximately 100 mg glycosylated scuPA (specific activity, 130,000 IU).17,33,34 Thus, only about one half of the standard amount of scuPA was used in this trial in combination with rt-PA. As a consequence, the observed changes in plasma parameters were mild and comparable with those observed using similar amounts of glycosylated scuPA alone17,33 or in combination with urokinase.17,34 rt-PA used as a single agent in doses of 100 mg seems to be of a similar efficacy as scuPA, inducing early recanalization in 65–75% of infarct vessels26,35–39 and with fibrinogen decreasing to about 60% of pretreatment levels. At higher doses (150 mg), fibrinogen depletion to about 40% of pretreatment values has been reported26 along with an increased incidence of intracranial hemorrhages.40 An increased rate and a reduced duration of the infusion of 100 mg rt-PA has been reported to result in a patency rate of 86% at 90 minutes; no data about specificity were given in a preliminary report.41 Thus, it seems possible that changes in the application modalities can improve the efficacy of rt-PA.

The patency rate of 81.8% at 90 minutes, obtained in this study, equals the best results obtained with scuPA or rt-PA alone, as well as the patency rates reported for a combination of rt-PA and urokinase (UK) (73%)42 or scuPA and UK, for which patency rates of 65% and 75% were reported.17,34 Moreover,
this efficacy was accompanied by far greater specificity as reflected by a negligible decrease in fibrinogen (10% at 120 minutes). At fibrin-specific doses, neither t-PA nor scuPA are very effective. These findings support the potential use of combinations rt-PA and scuPA in place of monotherapy or other combinations.

The present findings reflect the experience obtained in patients recruited sequentially at one institution. Although this is a pilot study, it represents the largest clinical trial of combinations of rt-PA and scuPA, to date. The findings are consistent with those of two pilot studies involving three and nine patients, respectively.24, 25 Moreover, the results can be explained by results of experimental studies showing that rt-PA and scuPA are synergistic in fibrinolysis.19–23 A mechanism for synergy has been proposed, which is based on in vitro studies showing that rt-PA and scuPA are complementary in their activation of fibrin-bound plasminogen.43, 44 These experimental findings indicate that rt-PA or scuPA, at fibrin-specific doses, activate distinctly different portions of the available fibrin-bound plasminogen. Accordingly, neither activator alone can activate all the available fibrin-bound plasminogen. This seriously limits efficacy at fibrin-specific doses and, therefore, requires the administration of much higher doses to effect nonspecific activation of plasminogen. Alternatively, the activators can be combined, as in the present study, to improve efficacy without sacrificing specificity.

A relatively high total dosage was chosen in this study to ensure that a therapeutic effect, at least comparable with that of existing regimens, would be achieved. The fractional dosages used, however, were only the equivalent of 50% of the dosage reported for scuPA and 12% of the standard dosage of rt-PA. The dosage chosen was estimated to be just below the threshold of nonspecificity to maximize efficacy. The laboratory data showing virtually no change in fibrinogen and a patency rate of 81.8% indicate that these objectives were attained although the best combination and ratio of t-PA and scuPA are yet to be determined.

A high dose of heparin (12,500 units) was used to prevent cleavage of the small amounts of scuPA by thrombin to an inactive double-chain form.45, 46 Whether heparin has other potentiating effects47–49 on the presented thrombolytic regimen is the subject of ongoing investigations. Until these results are available, the efficacy of the proposed regimen has to be considered in context with the heparin dose used.

A low incidence of complications was found. No bleeding necessitating transfusion occurred, as compared with a 17% incidence of transfusions for high-dose rt-PA and 12% for an rt-PA–UK combination.52 Two patients died, giving an in-hospital mortality of 5.3%. The incidence of reocclusion could not be adequately assessed because the majority of patients underwent immediate PTCA.

Coronary thrombolysis by an adequate combination of rt-PA and scuPA was found to be at least as effective, more efficient, and more specific than that reported for standard monotherapy with either activator. The frequency of reocclusion in combination with PTCA remains high and requires an alternative solution. More extensive trials are required to determine if combinations of tPA and scuPA should be substituted for monotherapy in the treatment of acute myocardial infarction and to establish the optimal dosage combination of the activators.

Acknowledgment

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References


**KEY WORDS** • plasminogen activators • myocardial infarction • thrombolysis • fibrinogenolysis
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