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Comparative Properties of Two Clinical Preparations of Recombinant Human Tissue-Type Plasminogen Activator in Patients With Acute Myocardial Infarction

HARRY D. GARABEDIAN, SM,* HERMAN K. GOLD, MD, FACC,*
ROBERT C. LEINBACH, MD, FACC,* JENNIFER A. JOHNS, MBBS, FRACP,*
TSUNEHIRO YASUDA, MD,* MICHITO KANKE, MD,* DÉSIRÉ COLLEN, MD, PHD†·‡

Boston, Massachusetts, Burlington, Vermont and Leuven, Belgium

The biologic properties of two clinical preparations of recombinant human tissue-type plasminogen activator were studied in 52 patients with acute myocardial infarction. The first preparation (G11021) has been used in all clinical trials reported to date, whereas the second preparation (G11035) is now produced for future clinical use. When both preparations were infused intravenously for 90 minutes at rates of 4 to 11 μg/kg per min, plateau levels of the drug in plasma ranged from 0.52 ± 0.15 to 1.8 ± 0.4 μg/ml and were linearly correlated with the infusion rate. However, G11035 yielded plasma levels that were approximately 35% lower than those obtained with G11021 ($p < 0.025$).

The postinfusion disappearance rate of the drug from plasma could be described by a two compartment disposition model with the following pharmacokinetic variables. For G11021, an alpha half-life of 4.1 to 6.3 minutes, a beta half-life of 41 to 50 minutes, a central compartment volume of 3.5 to 5.4 liters, a total distribution volume of 28 to 44 liters and a plasma clearance of 450 to 640 ml/min. For G11035 these variables were 3.6 to 4.6 minutes, 39 to 53 minutes, 3.8 to 6.6 liters, 27 to 40 liters and 520 to 1,000 ml/min, respectively, indicating that G11035 is cleared more rapidly from the circulation.

G11021 at 4 μg/kg per min and G11035 at 7 μg/kg per min did not effectively produce thrombolysis. A coronary reperfusion rate of 81% (13 of 16 patients) was obtained with 5.3 μg/kg per min of G11021 and a rate of 86% (6 of 7 patients) was obtained with 9.4 μg/kg per min of G11035. At these doses, the plasma fibrinogen level decreased to 69 ± 10 (mean ± SEM) and 85 ± 12% of baseline, respectively.

A maintenance infusion of G11021 at 2 μg/kg per min for 4 hours and of G11035 at 3.3 μg/kg per min was given to nine patients each, resulting in plateau levels in plasma of 0.45 μg/ml, which effectively prevented coronary reocclusion but was associated with a moderate (approximately 20%) additional fibrinogen breakdown. Thus, G11035 produced for future clinical use requires a 25 to 50% higher infusion rate to yield similar plasma drug levels and thrombolytic efficacy but causes less fibrinogen breakdown compared with the previously used G11021.

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Human tissue-type plasminogen activator, obtained by recombinant DNA technology and expressed in a mammalian cell system (1), was indistinguishable from tissue-type plasminogen activator isolated from conditioned cell culture media with respect to biologic and thrombolytic properties (2). This recombinant human tissue-type plasminogen activator, produced on a relatively small scale for initial clinical evaluation, was used successfully for coronary thrombolysis both in animal models of coronary thrombosis (3–5) and in patients with evolving myocardial infarction (6–11). An alternative procedure for the large scale production of recombinant human tissue-type plasminogen activator for clinical use has now been developed by Genentech, Inc. and this material has been extensively investigated in animals. In this report we compare the pharmacokinetics, thrombolytic profile and hemostatic effects of this new prep-

From the *Cardiac Division and the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, the †Departments of Biochemistry and Medicine, University of Vermont College of Medicine, Burlington, Vermont and the ‡Center for Thrombosis and Vascular Research, University of Leuven, Leuven, Belgium. This study was supported in part by Ischemia SCOR Grant HL26215 and Thrombosis SCOR Grant HL35058 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland and by Genentech, Inc., South San Francisco, California and was presented in part at the American Federation for Clinical Research National Meeting, May 1986, Washington, D.C.

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Address for reprints: Hermon K. Gold, MD, ACC 3A Suite 380, Massachusetts General Hospital, Fruit Street, Boston, Massachusetts 02114.

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Table 1. Thrombolytic Dosage Regimens of 52 Patients

Dose		No. of Patients		Total Dose (mg)*	
μg/kg per min	mg/kg	G11021	G11035	G11021	G11035
4.0	0.35	6	—	30 ± 1	—
5.3	0.48	16	—	37 ± 8	—
7.0	0.63	7	5	49 ± 7	51 ± 8
9.4	0.85	—	7	—	69 ± 10
11.0	1.0	—	11	—	84 ± 14

*Mean ± SD.

aration with that of the previously used material in patients with acute myocardial infarction.

Methods

Materials. The human tissue-type plasminogen activator was produced by recombinant deoxyribonucleic acid (DNA) technology by Genentech, Inc. The material used for initial clinical evaluation (G11021) was supplied in 10 ml vials of liquid excipient containing 5 mg of predominantly (>90%) two chain material. The material produced for future clinical use (G11035) was supplied in lyophilized form in 50 ml vials containing 50 mg of active substance in predominantly (60 to 75%) single chain form.

Study patients. The two consecutive study groups consisted of 29 patients treated with G11021 between December 1984 and July 1985 and 23 patients treated with G11035 between September 1985 and January 1986. These 52 patients were referred from the emergency ward within 6 hours of the onset of chest pain of a suspected first myocardial infarction. All patients met the following criteria: 1) ST segment elevation of 0.1 mV or greater in at least two adjacent electrocardiographic leads; 2) age ≤70 years; 3) no contraindication to thrombolytic therapy; and 4) complete coronary artery occlusion documented angiographically before treatment. Patients with cardiogenic shock and childbearing potential were excluded. Written informed consent was obtained from all patients. These studies were conducted within the framework of a protocol approved by the Bureau of Biologics of the Food and Drug Administration and by our hospital subcommittee on human studies on December 18, 1984.

Treatment protocol. Pretreatment coronary angiography by the Judkins technique was performed immediately in all patients after systemic heparinization (5,000 unit bolus followed by a continuous intravenous infusion of 1,000 units/h). After demonstration of complete coronary occlusion, recombinant human tissue-type plasminogen activator was infused intravenously using a constant rate infusion pump. All 29 patients treated with G11021 received an intravenous bolus injection over 2 minutes of 10% of the total amount of recombinant human tissue-type plasminogen activator to be infused, immediately followed by a constant rate infusion for 90 minutes of 4 μg/kg per min (0.35 mg/kg), 5.3 μg/kg per min (0.48 mg/kg) and 7 μg/kg per min (0.63 mg/kg) (Table 1). In nine patients in whom coronary reperfusion was obtained but with high grade (≥80%) residual stenosis, a maintenance infusion for 4 hours of 2 μg/kg per min (0.5 mg/kg) was administered immediately (Table 2).

The 23 patients treated with G11035 were subjected to the following protocol: In 10 of these patients, an intravenous bolus injection over exactly 1 minute of 10% of the total dose of recombinant human tissue-type plasminogen activator was followed by frequent blood sampling (at intervals of 30 seconds to 1 minute) during a 10 minute period to determine the initial disposition of the drug. Then thrombolysis was attempted in these 10 and in the other 13 patients of the group by injecting 10% of the total dose over 2

Table 2. Dosage Regimens of the 23 Patients Receiving Maintenance Therapy After the Initial Thrombolytic Dose

No. of Patients	Dose (μg/kg per min)		Dose Total (mg)*	
	Thrombolytic	Maintenance	Thrombolytic	Maintenance
G11021				
4	5.3	2.0	43 ± 10	48 ± 12
5	7.0	2.0	52 ± 5	43 ± 4
G11035				
5	9.4	2.0	67 ± 11	50 ± 7
9	11.0	3.3	80 ± 12	65 ± 10

*Mean ± SD.

minutes, immediately followed by a constant rate infusion for 90 minutes of 7 μg/kg per min (0.63 mg/kg), 9.4 μg/kg per min (0.85 mg/kg) and 11 μg/kg per min (1 mg/kg) (Table 1). In 14 patients who showed coronary reperfusion and high grade residual stenosis, a maintenance infusion for 4 hours of 2 μg/kg per min or 3.3 μg/kg per min (0.85 mg/kg) was given immediately (Table 2).

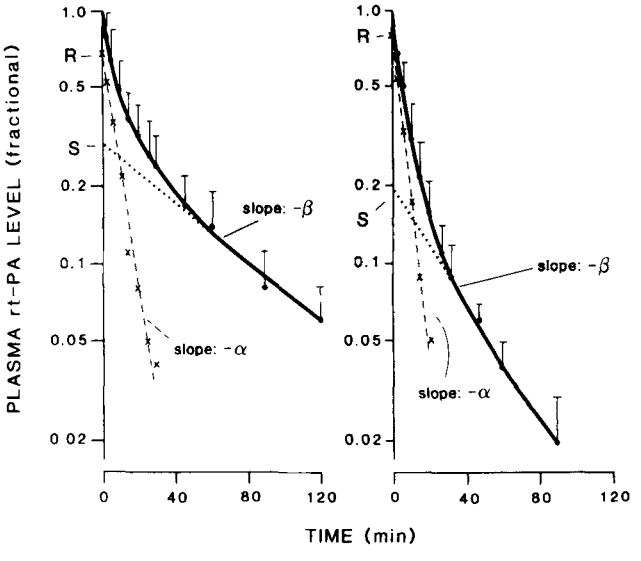
Angiographic evaluation. Coronary angiography of the occluded artery was performed at 15 minute intervals during the initial infusion and then 1 hour after completion of this therapy. Thrombolytic efficacy was assessed by angiographic criteria that included 1) the frequency of coronary reperfusion, and 2) the identification of residual intraluminal thrombus. Reperfusion was defined as complete distal opacification of the previously occluded coronary artery with washout of contrast medium after injection in four cardiac cycles or less. Treatment failure was defined as poor distal flow beyond the point of previous occlusion (washout of contrast in more than four cardiac cycles), no anterograde flow within 90 minutes or reocclusion during infusion. Residual thrombus was identified as an intraluminal filling defect linked to the site of previous occlusion. A primary angiographic end point at 90 minutes was used for identification of residual thrombus. All angiographic reviews were performed by a cardiologist who was unaware of the identity of the patient and the dosage regimen.

Processing of arterial blood samples. Arterial blood samples (4.5 ml) for measurement of fibrinogen, fibrinogen degradation products, plasminogen and alpha$_2$-antiplasmin were collected in 0.5 ml (0.1 *M*) sodium citrate before, during and after infusion of recombinant human tissue-type plasminogen activator. All samples were placed immediately on ice and were promptly centrifuged at 2,300 × *g* for 5 minutes. To inhibit activation of the fibrinolytic system in vitro, the plasma was transferred to polystyrene test tubes supplemented with either aprotinin (final concentration 200 kallikrein inhibitor units/ml plasma) or a solution of a monoclonal antibody (final concentration 200 μg/ml plasma) that interferes with the binding of recombinant human tissue-type plasminogen activator to fibrin (12). Plasma samples were kept frozen at −20°C until assayed. To determine the disposition of recombinant human tissue-type plasminogen activator antigen, frequent additional 1 ml blood samples were collected into 0.05 ml (15%) liquid ethylenediaminetetraacetate and the plasma was separated by centrifugation. These samples were collected every 15 minutes during infusion of the drug and, in those patients not receiving the maintenance infusion, at 1 to 5 minute intervals for the first 30 minutes after its completion and then every 15 minutes for an additional 210 minutes. During maintenance therapy, plasma levels of recombinant human tissue-type plasminogen activator were obtained at 30 minute intervals.

Blood sample assays. Fibrinogen levels were determined by a modified version (13) of the clotting rate assay of Clauss (14) and fibrinogen degradation products by latex agglutination (Thrombo-Wellcotest, Wellcome Research Laboratories) on blood samples collected into aprotinin. Interference of heparin is minimized by 10-fold dilution of the sample in the clotting rate assay for fibrinogen. Results for fibrinogen were expressed as a percent of pretreatment values. Alpha$_2$-antiplasmin and plasminogen were assayed on blood samples collected into monoclonal antibody using the chromogenic substrate S-2251 (KabiVitrum, Stockholm) (15,16). These values were expressed in percent by comparison with a standard curve obtained by serial dilution of a normal plasma pool. Recombinant human tissue-type plasminogen activator antigen was measured with an enzyme-linked immunosorbent assay (17).

Analysis of data. The experimental data describing the disappearance of recombinant human tissue-type plasminogen activator antigen from plasma after cessation of infusion were fitted with a sum of two exponential (exp) terms: $C(t) = R \exp(-\alpha t) + S \exp(-\beta t)$ with α and β representing the alpha and beta half-lives, respectively, of the drug in plasma. The coefficients (R and S) and exponents (α and β) of this function were obtained from semilogarithmic plots by graphic curve peeling (see Fig. 1, legend).

Figure 1. Disappearance rate of recombinant human tissue-type plasminogen activator (rt-PA) from plasma after cessation of its intravenous infusion. **Left panel,** G11021; **right panel,** G11035. The data, obtained in 12 patients treated with G11021 and in 6 patients with G11035, represent mean ± SD of levels expressed as a fraction of the first sample. The values of the coefficients (C) and exponents (exp) of the plasma disappearance curve $C(t) = R \exp(-\alpha t) + S \exp(-\beta t)$ were obtained by graphic curve peeling. Therefore, the terminal phase was fitted with a straight line yielding the intercept S and the slope $-\beta$. The extrapolated values were subtracted from the values obtained during the initial phase and these data were fitted with a straight line yielding the intercept R and the slope α. The values of R and S were then normalized to R + S = 1.



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The disposition of recombinant human tissue-type plasminogen activator was therefore represented by a two-compartment mammillary model composed of one central and one peripheral compartment with elimination occurring from the central compartment (18,19). Pharmacokinetic variables were calculated from these coefficients and exponents using standard formulas derived by Gibaldi and Perrier (18). The variables A and B were first calculated, assuming steady state at the end of the infusion, using the formulas $A = RX_0 \alpha/k_0$ and $B = SX_0 \beta/k_0$ where X_0 = total administered dose and k_0 = the rate of infusion.

From these constants the following drug disposition variables were derived: 1) volume of the central compartment (V_c) = $X_0/(A + B)$; 2) total volume of distribution (V_B) = $V_c k_{10}/\beta$; 3) extrapolated area under the curve (AUC) = $A/\alpha + B/\beta$; and 4) plasma clearance (Cl_p) = X_0/AUC. The fractional efflux rate constant from the central to the peripheral compartment (k_{12}), the fractional reflux rate constant (k_{21}) and the fractional catabolic rate constant (k_{10}) were calculated using the following formulas: $k_{21} = (A\beta + B\alpha)/(A + B)$; $k_{10} = \alpha\beta/k_{21}$; and $k_{12} = \alpha + \beta - k_{21} - k_{10}$. In the 10 patients who received an initial bolus injection of G11035 followed by frequent blood sampling over the next 10 minutes, the central compartment volume was calculated by dividing the amount of the bolus dose by the plasma level at time zero, determined by back extrapolation of the concentration-time curves (18).

Statistical methods. Results for hemostatic factors are expressed as mean ± SEM. All other results are presented as mean ± SD. Statistical analysis of the data for comparison of the two treatment groups was performed by the *t* test for paired and unpaired analysis, as appropriate. The relation between plasma levels of recombinant human tissue-type plasminogen activator and the infusion rate was analyzed by regression analysis with a least squares algorithm in the RS/1 program (Bolt Beranek and Newman) with statistical comparison between the equations of the line performed by the *t* test on parameter estimation. A probability (p) value of less than 0.05 was considered significant.

Results

Pharmacokinetics. *Plateau levels.* A linear relation between the infusion rate and the plateau level of recombinant human tissue-type plasminogen activator in plasma was obtained for both G11021 and G11035 (Fig. 2), indicating that the clearance mechanisms do not become saturated within this range of infusion rates. Intravenous infusion of G11021 at a fixed rate of 4 to 7 μg/kg per min resulted in a plateau level ranging from 0.52 ± 0.15 to 1.1 ± 0.18 μg/ml (mean ± SD). G11035 infused at a rate of 7 to 11 μg/kg per min yielded plateau levels ranging from 0.7 ± 0.14 to 1.8 ± 0.4 μg/ml. Substantial variation among individuals was noted, as indicated by the large standard deviation. In addition, infusion of G11035 yielded plasma levels that were approximately 35% lower than those obtained with comparable amounts of G11021 ($p < 0.025$).

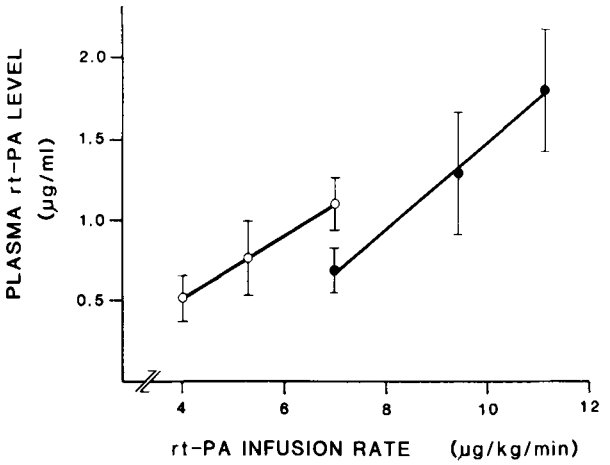

Figure 2. Linear relation between the plateau level of recombinant human tissue-type plasminogen activator (rt-PA) and the infusion rate. The plasma level of the drug was measured by an enzyme-linked immunosorbent assay. (○): G11021; (●): G11035. The data represent mean ± SD of values obtained in all patients and were fit with least squares regression analysis (see Methods) with r = 0.99 obtained for both lines. Despite the linearity on average, substantial variability in the plasma drug level exists at any given infusion rate.

Disappearance rate. After cessation of the thrombolytic infusion in patients not receiving maintenance therapy, the disappearance rate of recombinant human tissue-type plasminogen activator from plasma was biphasic (Fig. 1). A fit of the experimental data, after normalization of coefficients to R + S = 1 to allow for pooling of data, with a sum of two exponential (exp) terms: $C(t) = R \exp(-\alpha t) + S \exp(-\beta t)$ for the 12 patients treated with G11021 yielded the following values: R = 0.71 ± 0.12; α = 0.13 ± 0.04; S = 0.29 ± 0.12; and β = 0.015 ± 0.003. From these coefficients and exponents, the following disposition variables were calculated: A = 8.6 ± 3.3 μg/ml; B = 0.41 ± 0.23 μg/ml; k_{10} = 0.11 ± 0.04 min^{-1}; k_{12} = 0.03 ± 0.01 min^{-1}; and k_{21} = 0.02 ± 0.01 min^{-1}. For the six patients treated with G11035, the corresponding values were: R = 0.88 ± 0.05; α = 0.16 ± 0.04; S = 0.12 ± 0.05; β = 0.019 ± 0.007; A = 12.8 ± 3.3 μg/ml; B = 0.23 ± 0.17; k_{10} = 0.14 ± 0.03 min^{-1}; k_{12} = 0.02 ± 0.01 min^{-1}; and k_{21} = 0.02 ± 0.01 min^{-1}. The differences in mean fractional catabolic rate constants (k_{10}) of G11021 and G11035 are of borderline significance (p = 0.06). Detailed pharmacokinetic variables obtained in the subgroups of patients according to the dose administered are summarized in Tables 3 and 4. These data confirm that the elimination rate constant (k_{10}) and the plasma clearance rate (Cl_p) are larger for G11035 than for G11021.

Table 3. Variables Describing the Disposition of Recombinant Human Tissue-Type Plasminogen Activator From Plasma

No. of Patients	k_0	$C = Re^{-\alpha t} + Se^{-\beta t}$				Intercepts		C_{max}
		R	α	S	β	A	B	
G11021								
3	4.0	0.41 ± 0.14	0.16 ± 0.03	0.16 ± 0.06	0.016 ± 0.003	5.9 ± 2.0	0.24 ± 0.14	0.5 ± 0.21
2	5.0	0.40 ± 0.07	0.12 ± 0.03	0.20 ± 0.08	0.014 ± 0.001	4.4 ± 0.5	0.26 ± 0.13	0.6 ± 0.12
5	5.5	0.60 ± 0.30	0.11 ± 0.04	0.27 ± 0.14	0.014 ± 0.004	5.9 ± 3.0	0.38 ± 0.26	0.85 ± 0.11
2	7.0	0.82 ± 0.03	0.17 ± 0.04	0.21 ± 0.01	0.017 ± 0.001	12.4 ± 2.5	0.32	1.0 ± 0.1
G11035								
2	7.0	0.5 ± 0.07	0.19 ± 0.06	0.08 ± 0.03	0.02 ± 0.007	8.8 ± 3.7	0.17 ± 0.1	0.7 ± 0.2
2	9.4	1.1 ± 0.32	0.15 ± 0.04	0.19 ± 0.03	0.024 ± 0.002	15.1 ± 8.4	0.42 ± 0.1	1.3 ± 0.06
2	11.0	2.0 ± 0.7	0.15 ± 0.02	0.25 ± 0.06	0.013	27 ± 6	0.3 ± 0.1	2.2 ± 0.6

A,B = recalculated intercepts (18); C_{max} = plateau level of recombinant human tissue-type plasminogen activator measured in plasma (μg/ml); k_0 = infusion rate (μg/kg per min); R,S α, β = coefficients and exponents describing the disappearance curve of recombinant human tissue-type plasminogen activator concentration from plasma.

The disappearance of recombinant human tissue-type plasminogen activator from plasma after an initial bolus injection of 8 ± 1.6 mg of G11035 in 10 patients was very rapid (Fig. 3). The decline in plasma concentration as a function of time could be approximated by a sum of two exponential terms: $C(t) = 0.55\exp(-1.1t) + 0.45\exp(-0.18\,t)$. The alpha half-life of the drug in plasma was 0.6 ± 0.15 minute. From these values, the following disposition variables were determined: k_{12} = 0.06 ± 0.02/min; k_{21} = 0.04 ± 0.01/min; and k_{10} = 0.08 ± 0.02/min. The central compartment volume, calculated from the initial bolus injection of G11035 in these patients, was 3.2 ± 1.2 liters.

Coronary reperfusion. The time from onset of symptoms to the start of the infusion averaged 3.9 ± 1.0 and 4.1 ± 1.3 h for G11021 and G11035, respectively (p = 0.33). No significant differences were found between treatment groups with respect to age, sex, blood pressure on admission, heart rate, time from onset of chest pain to reperfusion, localization of coronary thrombus or myocardial infarction or presence of collateral blood flow to the area supplied by the occluded artery.

Infusion of G11021 at a rate of 4 μg/kg per min for 90 minutes did not produce coronary reperfusion in any of six patients. This therapy was not effective in two of these patients because their arteries had transient openings and reoccluded before completion of the infusion. With 5.3 μg/kg per min, reperfusion occurred in 13 (81%) of 16 patients within 63 ± 19 minutes. Intraluminal filling defects persisting after 90 minutes of infusion were observed in 8 of 12 patients. In one case of treatment failure, anterograde flow was transient and reocclusion occurred before the infusion was completed. Infusion rates of 7 μg/kg per min for 90 minutes yielded 86% reperfusion (six of seven pa-

Table 4. Pharmacokinetic Variables of the Disposition of Recombinant Human Tissue-Type Plasminogen Activator

No. of Patients	k_0	V_c (liters)	V_B (liters)	k_{10} (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	Cl_p (ml/min)	AUC	C_{max} (μg/ml)
G11021									
3	4.0	5.4 ± 2.2	44 ± 19	0.13 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	636 ± 195	51 ± 17	0.59 ± 0.08
2	5.0	6.5 ± 0.5	43 ± 13	0.09 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	573 ± 146	54 ± 14	0.57 ± 0.1
5	5.5	7.1 ± 3.2	42 ± 22	0.08 ± 0.03	0.02 ± 0.02	0.02 ± 0.01	541 ± 211	78 ± 23	0.84 ± 0.2
2	7.0	3.4 ± 1.1	28 ± 3	0.14 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	451 ± 52	93 ± 2	0.97 ± 0.04
G11035									
2	7.0	6.6 ± 4.4	53 ± 37	0.17 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	998 ± 432	53 ± 9	0.57 ± 0.1
2	9.4	5.7 ± 3.4	27 ± 6	0.13 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	682 ± 204	114 ± 26	1.25 ± 0.2
2	11.0	3.8 ± 0.7	40 ± 9	0.13 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	516 ± 122	205 ± 55	2.2 ± 0.6

AUC = area under the plasma-time curve (μg-min/ml); C_{max} = plateau level of recombinant human tissue-type plasminogen activator; Cl_p = clearance rate of recombinant human tissue-type plasminogen activator from plasma; k_0 = infusion rate (μg/kg per min); k_{10} = fractional elimination rate constant; k_{12} = fractional transfer rate constant from central to peripheral compartment; k_{21} = fractional transfer rate constant from peripheral to central compartment; V_B = total volume of distribution; V_c = volume of central compartment. These constants were calculated from the variables in Table 3 using the formulas given in the Methods section

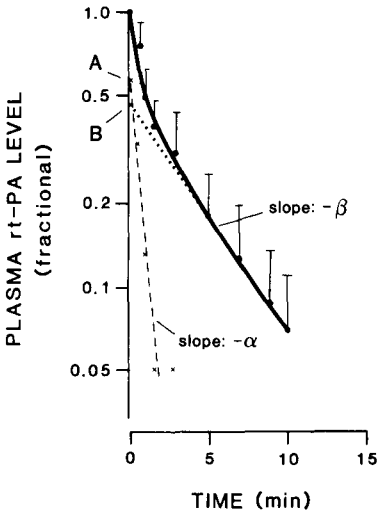

Figure 3. Disappearance rate of recombinant human tissue-type plasminogen activator (rt-PA) from plasma after an initial intravenous bolus injection of 8 ± 1.6 mg of G11035 in 10 patients. The data represent mean ± SD of levels expressed as a fraction of the first sample. For details see legend of Figure 1. A and B = recalculated intercepts.

tients) within 54 ± 17 minutes, and intraluminal filling defects at 90 minutes were observed in two of six patients.

With G11035, infusion of 7 μg/kg per min for 90 minutes caused reperfusion in only two of five patients within 82 ± 11 minutes, and large intraluminal filling defects were observed in the coronary arteries of both patients. This therapy was not considered to be effective in one patient because transient opening of the artery was followed by reocclusion before cessation of therapy. With 9.4 μg/kg per min, reperfusion occurred in six (86%) of seven patients within 60 ± 25 minutes. Intraluminal filling defects persisted in two of six patients. Infusion rates of 11 μg/kg per min for 90 minutes yielded 82% reperfusion (9 of 11 patients) within 48 ± 17 minutes with intraluminal filling defects in 2 of 9 patients. In one case of treatment failure, poor distal flow with large residual thrombus occurred immediately before cessation of the infusion.

Effects on the fibrinolytic and hemostatic systems. With both preparations of recombinant human tissue-type plasminogen activator, the extent of fibrinogen degradation at the end of the infusion was proportional to dose (Table 5). At an infusion rate of 7 μg/kg per min, G11035 induced significantly less fibrinogen breakdown than did G11021 ($p = 0.01$). Overall, the extent of fibrinogenolysis was also less pronounced for G11035. The concentration of fibrinogen degradation products in serum increased slightly toward the end of the recombinant human tissue-type plasminogen activator infusion but remained below 150 μg/ml, representing less than 6% of the plasma fibrinogen (Table 6).

After infusion of G11021 at 7 μg/kg per min for 90 minutes, plasminogen and alpha$_2$-antiplasmin levels, measured on plasma samples collected in the monoclonal antibody, declined to 57 ± 14 (mean ± SEM) and 20 ± 10% of the preinfusion value, respectively. With G11035 infused at 11 μg/kg per min, these respective values were 75 ± 4 and 6 ± 3%. In plasma samples collected in citrate only, extensive in vitro activation of the fibrinolytic system occurred, frequently resulting in unmeasurable levels of fibrinogen, plasminogen and alpha$_2$-antiplasmin.

Maintenance infusion after reperfusion. In nine patients with coronary reperfusion and high grade residual stenosis after infusion of G11021, a maintenance infusion of 2 μg/kg per min for 4 hours was given after the initial thrombolytic dose (Table 2). The plasma concentration of recombinant human tissue-type plasminogen activator decreased from a plateau level of 1.0 ± 0.32 μg/ml during the initial infusion to a plateau level of 0.43 ± 0.14 μg/ml during maintenance. Acute coronary reocclusion was not observed during this maintenance therapy. Five patients whose artery was reperfused with 9.4 μg/kg per min of G11035 for 90 minutes and with high grade residual stenosis were given a maintenance infusion of 2 μg/kg per min for 4 hours. The plasma concentration decreased from a plateau level of 1.3 ± 0.45 μg/ml during the initial infusion to 0.34 ± 0.08 μg/ml during maintenance. However, coronary reocclusion was documented angiographically within 60 minutes

Table 5. Plasma Fibrinogen Levels During Infusion of Recombinant Human Tissue-Type Plasminogen Activator

Dose (μg/kg per min)	G11021		G11035	
	A	B	A	B
4.0	75 ± 3* (6)†	NI		
5.3	69 ± 10 (16)	42 ± 15 (4)		
7.0	53 ± 12 (7)	31 ± 10 (5)	94 ± 6 (4)	NI
9.4			85 ± 12 (7)	57 ± 26 (4)
11.0			71 ± 5 (11)	58 ± 6 (9)

*The data represent mean ± SEM; †the number of patients is in parentheses. A = at the end of the 90 minute infusion; B = at the end of the maintenance infusion; NI = no maintenance infusion was administered.

Table 6. Serum Fibrinogen Degradation Products (μg/ml) During Infusion of Recombinant Human Tissue-Type Plasminogen Activator

Dose (μg/kg per min)	G11021		G11035	
	A	B	A	B
4.0	8.7 ± 1.6* (6)†	NI		
5.3	13 ± 2.9 (16)	38 ± 12 (4)		
7.0	90 ± 36 (7)	144 ± 48 (5)	15 ± 9 (5)	NI
9.4			28 ± 6 (7)	66 ± 36 (4)
11.0			16 ± 5 (11)	32 ± 13 (9)

*The data represent mean ± SEM; †the number of patients is in parentheses. Pretreatment values averaged 5.1 ± 0.5 μg/ml for G11021 and 4.6 ± 0.6 μg/ml for G11035. A = at the end of the 90 minute infusion; B = at the end of the maintenance infusion; NI = no maintenance infusion was administered.

after initiation of maintenance therapy in two patients, both of whom demonstrated large intraluminal filling defects after the initial infusion. Nine patients treated with 11 μg/kg per min of G11035 received a maintenance infusion of 3.3 μg/kg per min for 4 hours, which resulted in a decrease of the plasma concentration of recombinant human tissue-type plasminogen activator from a plateau level of 1.8 ± 0.4 μg/ml during the initial infusion to 0.45 ± 0.16 μg/ml during the maintenance infusion. Acute coronary reocclusion was not observed with this maintenance dose. All maintenance infusions were associated with moderate additional fibrinogen breakdown ranging from 13 to 28% of pretreatment values (Table 5).

Bleeding complications. The frequency of bleeding complications during and after infusion of G11021 or G11035 in patients receiving heparin are summarized in Table 7. Bleeding occurred most frequently at intervention sites.

Discussion

Recent clinical studies (6–11) have established that recombinant human tissue-type plasminogen activator is an effective and safe thrombolytic agent in patients with acute myocardial infarction due to coronary artery thrombosis. The recombinant human tissue-type plasminogen activator used in all studies reported to date (G11021) was produced on a relatively small scale for initial clinical evaluation. For future clinical use, material is now produced on an industrial scale (G11035). These two preparations are, however, synthesized by complex biologic techniques differing in significant aspects, and their biologic identity is not evident a priori. Therefore, we have performed a comparative evaluation of the pharmacokinetics, thrombolytic efficacy and effects on the hemostatic system of these two clinical preparations of recombinant human tissue-type plasminogen activator in two consecutively treated groups of patients with acute myocardial infarction.

Table 7. Bleeding Episodes During and After Infusion of Recombinant Human Tissue-Type Plasminogen Activator

Site of Bleeding	Total Bleeding Episodes	
	G11021	G11035
Catheterization puncture site	11	4
Other vascular puncture sites	14	7
Gingival	1	1
Guaiac positive stool	1	1

Pharmacokinetics. Within the range of applied infusion rates, a linear correlation is observed between the rate of intravenous infusion and the plasma concentration of recombinant human tissue-type plasminogen activator for both G11021 and G11035. However, because of the large intersubject variability, accurate prediction of the plateau level of the drug in plasma from the rate of infusion is not possible for any given patient. In addition, infusion of G11035 results in plasma levels of recombinant human tissue-type plasminogen activator that are approximately 35% lower than those obtained with comparable doses of G11021.

Pharmacokinetic analysis of the postinfusion disappearance rate of recombinant human tissue-type plasminogen activator from plasma indicated that G11035 was cleared more rapidly from the blood than was G11021. This conclusion is consistent with the observation that higher infusion rates of G11035 are required to attain plasma drug levels similar to those attained with G11021.

The cause for the faster disposition rate of G11035 compared with that of G11021 is not readily apparent. It is possible but unlikely that it is due to the different ratios of one chain and two chain forms of recombinant human tissue-type plasminogen activator in the two preparations. Indeed, the disposition rates in rabbits of one-chain and two-chain forms of natural tissue-type plasminogen activator, isolated from conditioned cell culture media, are identical (20). A variable degree of glycosylation may be responsible for the differences in clearance rate by the liver, but data supporting differences in carbohydrate composition of G11021 and G11035 are not available.

The very rapid disappearance rate (alpha half-life of 0.6 minute) of a bolus injection of G11035 observed in 10 patients suggests that the initial amounts of injected recombinant human tissue-type plasminogen activator are distributed between plasma and a rapidly exchanging saturable compartment, which may be constituted of liver or endothelial cell receptors.

Coronary reperfusion. Although pretreatment coronary angiography was required, the time from onset of symptoms to infusion of recombinant human tissue-type plasminogen activator in our patients is similar to that in previous studies (6–9). Equally stringent angiographic criteria, similar to those of previous trials (8,9), were used to define coronary reperfusion. Our results show that the efficacy of both G11021 and G11035 for coronary thrombolysis was dose dependent with a trend toward earlier reperfusion with increasing rates of infusion.

In our study, the minimal effective dose was 5.3 μg/kg per min for G11021 and 9.4 μg/kg per min for G11035, when both were infused for 90 minutes. At these or higher doses, reperfusion frequencies > 80% were achieved. However, residual thrombus was more frequently identified at these doses, than with infusion rates of 7 μg/kg per min of G11021 or 11 μg/kg per min of G11035. Yet, probably because of the small sample size, these differences were not statistically significant. Identification of intraluminal filling defects in this study was not strictly quantitative and was likely to be an underestimate. However, our results suggest a trend toward more complete clot lysis at the higher doses of recombinant human tissue-type plasminogen activator. This finding may be relevant for minimizing the likelihood of acute coronary reocclusion.

Systemic activation of the fibrinolytic system. The extent of in vivo fibrinogen breakdown was proportional to the infusion rate, confirming that the fibrin specificity of recombinant human tissue-type plasminogen activator is a relative property. However, G11035 induced substantially less fibrinogen degradation, although, in agreement with previous observations (21), a large interindividual variability in the extent of fibrinogenolysis was noted at any given dose.

Maintenance therapy. Previous studies have indicated that early coronary reocclusion after thrombolytic therapy for myocardial infarction with streptokinase or recombinant human tissue-type plasminogen activator is precipitated by high grade residual coronary stenosis (10,22). A maintenance infusion of recombinant human tissue-type plasminogen activator (G11021) has been shown to adequately prevent coronary reocclusion in such patients (10). The results of our study show that infusion of G11035 at a rate of 2 μg/kg per min for 4 hours, yielding plateau levels of recombinant human tissue-type plasminogen activator in plasma of 0.34 ± 0.08 μg/ml, was insufficient to prevent reocclusion in two patients with high grade residual coronary artery stenosis. This finding indicates that a relatively higher maintenance dose is required to maintain a thrombolytic state. Indeed, we show that a maintenance infusion of 2 μg/kg per min of G11021 or of 3.3 μg/kg per min of G11035 for 4 hours, resulting in plateau drug levels in plasma of 0.45 μg/ml, adequately prevented coronary reocclusion. Both infusions were, however, associated with moderate additional fibrinogen breakdown.

Conclusions. Our study indicates that the new preparation of recombinant human tissue-type plasminogen activator produced on a large scale for future clinical use (G11035) is removed from the systemic circulation at a faster rate than is the material previously used for initial clinical evaluation (G11021). Therefore, 25 to 50% higher infusion rates with G11035 are required to obtain similar plasma levels. Under those conditions, the thrombolytic efficacy is comparable with that observed with G11021, whereas the extent of fibrinogen breakdown is less pronounced.

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