Effects of liver blood flow on the pharmacokinetics of tissue-type plasminogen activator (alteplase) during thrombolysis in patients with acute myocardial infarction

Background: The removal of recombinant tissue-type plasminogen activator (rt-PA; alteplase) by the liver is so rapid that liver blood flow becomes rate determining for its clearance. In patients with myocardial infarction changes in liver blood flow may result from impaired cardiac performance or drug treatment.
Objective: To estimate the effect of variations in liver blood flow on t-PA plasma concentrations during thrombolytic therapy.
Methods: Fifteen patients with acute myocardial infarction were investigated in an open single-center study at the coronary care unit of University Hospital Leiden. Patients received thrombolytic treatment with 100 mg rt-PA over 3 hours. Liver blood flow was estimated by indocyanine green clearance and by Doppler echocardiography. Concentrations of t-PA antigen, t-PA activity, indocyanine green, α_2-antiplasmin, fibrinogen, and fibrin and fibrinogen degradation products were measured.
Results: Indocyanine green clearance and clearance of both t-PA antigen ($r = 0.78$; $p < 0:01$) and t-PA activity ($r = 0.54$; $p < 0.05$) were significantly related. Significant associations between t-PA antigen and fibrin and fibrinogen degradation products and between t-PA antigen and α_2-antiplasmin were also found.
Conclusions: The liver blood flow of patients with myocardial infarction is inversely correlated with plasma concentrations of t-PA. In patients with severely impaired liver blood flow and heart failure, high t-PA plasma concentrations may occur if standard doses are given. This finding could contribute to optimization of the dosage of t-PA in certain patient groups. (Clin Pharmacol Ther 1998;63:39-47.)

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Recombinant tissue-type plasminogen activator (rt-PA; alteplase)[1] is comparable to other thrombolytic drugs, but its use is associated with a higher incidence of hemorrhagic strokes in comparison with streptokinase.[2] Accelerated rt-PA administration, when 100 mg rt-PA is given in 90 minutes instead of 160 minutes, provides a possible increased survival benefit compared with streptokinase, although the rate of hemorrhagic strokes remains higher.[3]

The liver exclusively and efficiently eliminates t-PA from the blood.[4] The blood flow to the liver is rate-determining for the clearance of t-PA. In healthy volunteers a reduction in liver blood flow induced by exercise increased t-PA plasma concen-

Table I. Clinical characteristics of the 15 patients with acute myocardial infarct

Sex	
Male	12
Female	3
Age (yr)	59 ± 13
Body weight (kg)	73 ± 11
Infarct location	
Anterior	8*
Inferior	3
Inferolateral	4
First or recurrent infarction	
First infarction	11
Recurrent infarction	4
Killip classification at admission	
I	12
II	2
III	1
IV	0
Time interval between onset of symptoms and treatment	
0 to 2 hours	4
2 to 4 hours	7
4 to 6 hours	4
Peak CK-MB (units/L)	123 ± 91

Date are mean values ± SD.
CK-MB, Creatine kinase muscle/brain fraction.
*One patient showed electrocardiographic and clinical signs of anterior transmural infarction but had no enzyme elevations after thrombolysis.

trations proportional to the achieved reduction in blood flow.[5]

Considerable interindividual differences (up to fivefold) in steady-state t-PA concentrations of patients with acute myocardial infarction have been found after infusion of standard doses of rt-PA,[6-8] whereas plasma concentrations have been less variable in healthy subjects.[9] After adjustment of the rt-PA dose for weight the variability was reduced but still persisted[8]; this remaining variability could be caused by the diversity in liver blood flow. A similar situation exists for lidocaine, which is also a high-clearance drug. High plasma lidocaine levels and reduced clearance in patients with congestive heart failure were explained by the reduced liver blood flow in this group.[10,11]

Variability in t-PA plasma concentrations may have important clinical implications because it may determine the efficacy and side effects of t-PA to a significant extent. Coronary thrombolysis[12] and the occurrence of major bleeding in nonsurgical patients[13] are dose dependent. A reduction in the variability could lead to a reduction in serious side effects by elimination of inordinately high concentrations and an increase in efficacy in patients who are expected to have low plasma concentrations.

We investigated the variability in liver blood flow in patients with acute myocardial infarction and the effect on plasma concentrations of t-PA by measuring liver blood flow by continuous infusion of indocyanine green. As a secondary objective we attempted to identify potential clinically useful markers for a reduction in liver blood by applying Doppler echographic measurements of liver blood flow.

METHODS

Patients

Patients who came to the hospital less than 6 hours after the onset of symptoms of acute myocardial infarction, with chest pain lasting at least 30 minutes despite the use of sublingual nitrates and accompanied by characteristic electrocardiographic signs, were eligible for enrollment. The standard exclusion criteria for thrombolytic therapy were applied. Patients gave oral informed consent for participation, and the protocol and this consent procedure was approved by the Hospital Ethical Committee of the Amsterdam Academic Medical Center.

Trial design and treatment

Medication. Patients were treated at a single center and received thrombolytic treatment with a bolus injection of 10 mg rt-PA (alteplase, Boehringer Ingelheim, Alkmaar, The Netherlands), followed by a continuous infusion of 50 mg over 60 minutes and 40 mg over the next 120 minutes. A 5000 IU bolus injection of heparin was given before the rt-PA bolus and a 1000 IU/hr heparin infusion was started, with the dose adjusted to raise the activated partial thromboplastin time 1.5 to 2.5 times the control and continued for 48 hours. Acetylsalicylic acid (250 mg) was given orally and continued at a dose of 80 mg per day.

Measurement of liver blood flow. Indocyanine green (Hynson, Westcott & Dunning, Baltimore, Md.) was administered as a continuous infusion of 90 mg over 180 minutes simultaneously with the rt-PA. Indocyanine green is a tricarbocyanine dye that is commonly used to estimate liver blood flow.[14] It is exclusively removed by the liver without biotransformation[15] and does not undergo enterohepatic circulation. The hepatic extraction ratio in humans is high ($\geq$0.7).[16]

Liver blood flow was measured independently by

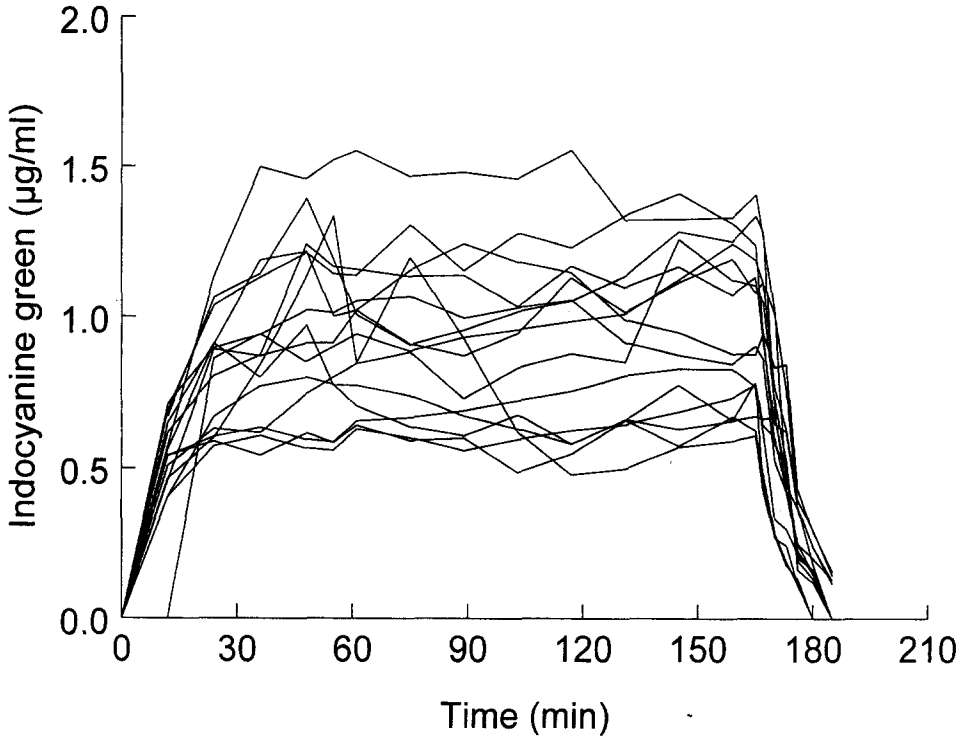

Fig. 1. Individual indocyanine green plasma concentration curves of 15 patients with acute myocardial infarct after infusion of 90 mg indocyanine green over 180 minutes.

echography of the portal venous system during drug administration (every half hour when possible) using an echocardiograph (Hewlett-Packard Sonos 1500) equipped with a 3.5/2.7 MHz pulsed-wave transducer. The velocity spectrum was recorded after visualization of the portal vein or the intrahepatic right portal vein branch. Vessel diameter and maximal blood flow velocity (V_{max}) were measured from the recorded images. The cross-sectional area (CSA) of the portal vein or branch was calculated from the vessel diameter, assuming circular geometry. Blood flow volume (Q; ml/min) was calculated as follows: $Q = CSA \cdot V_{max}$.

Study protocol

After determination of eligibility of the subjects, the cannulas for administration of the medication and the indocyanine green were inserted into a convenient vein of one forearm and a cannula for blood collection in the contralateral arm. Blood samples for routine hematology, biochemistry, and cardiac enzymes were collected. Patients received heparin and acetylsalicylic acid, and the thrombolytic therapy and indocyanine green infusion were started. Blood samples were collected shortly before and 2, 6, 12, 24, 36, 48, 55, 61, 75, 89, 103, 117, 131, 145, 159, 165, 167, 170, 176, 185, 195, and 205 minutes after the start of the bolus injection of rt-PA for the determination of t-PA antigen and activity; before and 12, 24, 36, 48, 55, 61, 75, 89, 103, 117, 131,145, 159, 165, 167, 170, 173, 176, 180, and 185 minutes after the start of the bolus injection of rt-PA for indocyanine green measurements; and before and 36, 103, 165, and 225 minutes after the start of the bolus injection of rt-PA for the hemostatic parameters. Heart rate and systolic and diastolic blood pressure were monitored before administration and 10, 30, 50, 65, 95, 125, 155, 170, 195, and 225 minutes after the start of the infusion. Killip class[17] was determined on arrival of the patient, 133 and 225 minutes after start of the thrombolytic treatment, and when clinical changes of the patient were observed.

Blood sampling

The cannula for blood collection was kept patent by a slow continuous infusion of saline solution (total volume <200 ml/24 hr) without heparin. Blood was taken after the saline infusion was interrupted and after the contents of the cannula were

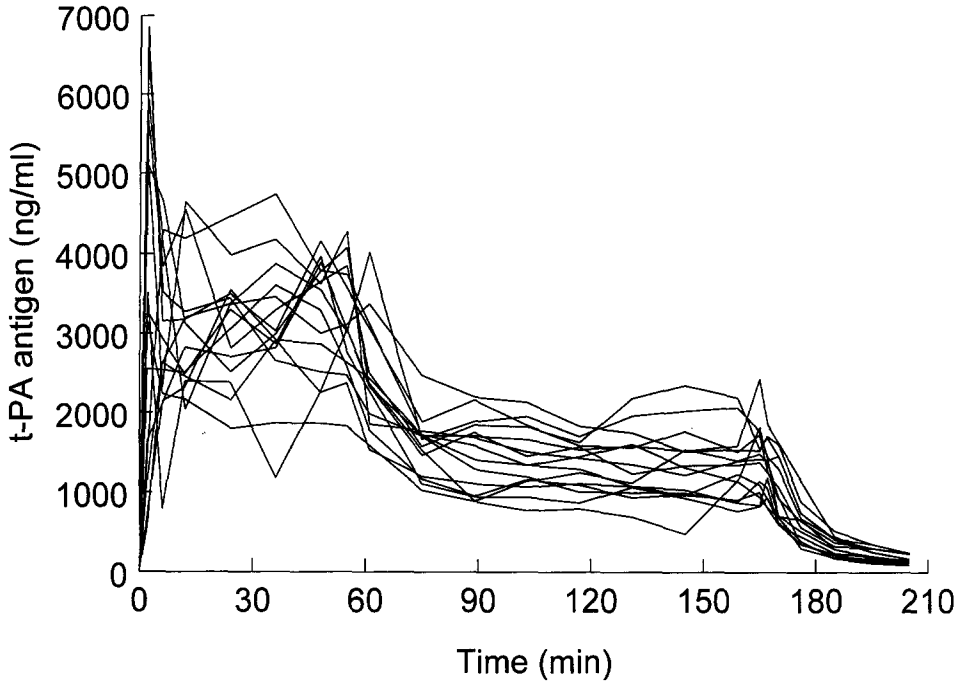

Fig. 2. Individual tissue-type plasminogen activator (t-PA) antigen curves after a dose regimen of a bolus injection of 10 mg, followed by a continuous infusion of 50 mg over 60 minutes and 40 mg over the next 120 minutes. A similar variation was observed for the t-PA activity concentrations.

discarded. All samples were immediately put on ice. For the t-PA antigen measurement, 4.5 ml blood was collected in CTAD tubes (1/10 volume of 0.11 mmol/L citric acid, 15 mmol/L theophylline, 3.7 mmol/L adenosine, 0.198 mmol/L dipyridamole; Becton Dickinson, Frembodegem, Belgium). For the t-PA activity determination, blood was collected in Stabilyte Vacutainer tubes (0.5 ml acidic citrate; Biopool, Umeå, Sweden). Blood (3 ml) for the indocyanine green assay was collected in lithium heparin tubes (Sarstedt, Nümbrecht, Germany). Determinations of fibrinogen, total fibrin and fibrinogen/fibrin degradation products, and α_2-antiplasmin were performed after blood was collected (3 ml) in 0.1 volume of 3.8% ice cold citrate. The samples contained prostaglandin E_2, theophylline, and PPACK (final concentrations in blood was 0.09 μmol/L, 1 mmol/L, and 10 μmol/L, respectively). After centrifugation of the tubes at 3000*g* for 10 minutes at 4° C, platelet-poor plasma was collected, snap frozen, and stored at –20° C until analysis.

Assays

Plasma concentrations of t-PA antigen were determined by an enzyme-linked immunosorbent assay technique,[18] used as described previously.[19] For the activity assay plasma samples were acidified to neutralize inhibitors and diluted as described previously[19] with use of a spectrophotometric plasmin generation assay.[20] The limit of detection was 0.1 μg/ml, and the maximal intraday precision was 2.0%. Plasma levels of indocyanine green were determined by HPLC according to Rappaport and Thiessen[21] in a slightly modified form with use of diazepam as the internal standard. The α_2-antiplasmin was determined by the immediate plasmin inhibition test with use of H-D-Val-Leu-Lys-pNA as the plasmin substrate according to an improved automated method of Friberger.[22] Fibrinogen was measured according to Clauss.[23] Before fibrinogen determination, total fibrin and fibrinogen/fibrin degradation products were measured in all samples with an enzyme-linked immunosorbent assay method that equally detects both types of degradation products.[24]

Pharmacokinetic analysis and statistics

Pharmacokinetic parameters were calculated with a standard monoexponential pharmacokinetic model for indocyanine green and a biexponential model for t-PA (Siphar software package; Simed,

Table II. Pharmacokinetic parameters of indocyanine green, t-PA antigen, and t-PA activity

	*AUC (units/ml · min)**	*CL (ml/min)**	V_C *(L)*	$t_{1/2}\lambda_1$ *(min)*
Indocyanine green (μg)	149 ± 37	585 ± 144	5.4 ± 2.1	7.0 ± 1.7
t-PA antigen (μg)	360 ± 74	269 ± 63	2.9 ± 1.2	4.2 ± 1.5
t-PA activity (IU)	84 ± 21	470 ± 138	3.8 ± 1.0	4.4 ± 1.2

Data are mean values ± SD.
*Model independent parameters.
t-PA, Tissue-type plasminogen activator; AUC, area under the plasma concentration–time curve; CL, clearance; V_c, volume of distribution; $t_{1/2}\lambda_1$, half-life of the first exponential phase.

Créteil, France). These models were chosen to provide the best fit to the data. The pharmacokinetic parameters clearance, half-life of the first exponential phase ($t_{1/2}\lambda_1$), and volume of distribution of the central compartment (V_C) were calculated from the fitted functions according to conventional techniques. The half-life of the second exponential phase ($t_{1/2}\lambda_2$) for t-PA describes only a small percentage of the area under the plasma concentration–time curve and was not reported. The area under the plasma concentration curve [AUC(0-t)] was determined by means of the trapezoidal rule without extrapolation to infinity. Model-independent clearance of t-PA and indocyanine green was calculated by the formula: Clearance = Dose/AUC(0-t).

The area under the effect curves of the hemostatic parameters fibrinogen, α_2-antiplasmin, and total fibrin and fibrinogen/fibrin degradation products were calculated by the same method and related to the AUC(0-t) of t-PA activity. The Pearson correlation coefficients between measurements were evaluated. Results are given as mean values ± SD.

RESULTS

The clinical characteristics of the 15 patients who were included in the study are summarized in Table I.

Measurements of liver blood flow

The individual plasma concentration–time profiles of indocyanine green are shown in Fig. 1. Peak plasma indocyanine green levels varied from 776 to 1553 ng/ml. The calculated clearance ranged from 366 to 809 ml/min. A similar interindividual variability was observed in clearances of t-PA antigen (Fig. 2) and t-PA activity. For t-PA antigen the clearance ranged from 207 to 421 ml/min and for t-PA activity from 304 to 816 ml/min. The pharmacokinetic parameters of indocyanine green and t-PA derived from individual curves are displayed in Table II. A significant correlation was observed between indocyanine green clearance, as a measure for hepatic blood flow, and clearances of both t-PA antigen ($r = 0.78$; $p < 0.01$) and t-PA activity ($r = 0.54$; $p < 0.05$; Fig. 3).

For four subjects no Doppler echographic data were available because of the condition or cooperation of the patient. A significant correlation was found between indocyanine green clearance and both the right portal vein branch flow ($r = 0.63$; $n = 6$) and flow measured at the main portal vein ($r = 0.83$; $n = 5$).

Clinical observations

Twelve patients were in the Killip I category at admission to the hospital, and all of these patients remained in this category during the study. One patient remained in Killip class II during the entire observation period. One patient was in Killip class III at admission but recovered and was in class I at the second determination at 133 minutes after drug administration. One patient was in Killip class II at admission, slowly deteriorating to class III. After ±4 hours she was again in the Killip II category (Fig. 4). High plasma concentrations of t-PA and indocyanine green were observed in this patient.

Coagulation parameters

The average fibrinogen concentration before thrombolytic treatment was 2.4 ± 0.6 gm/L, and total fibrin and fibrinogen degradation product concentration was 0.7 ± 0.3 μg fibrinogen equivalents/ml. At the last measurement, approximately 4 hours after drug administration, the average lowest fibrinogen level of 2.0 ± 0.5 gm/L and the highest total fibrin and fibrinogen degradation product level of 49.2 ± 54.9 μg fibrinogen equivalents/ml were found. No significant correlations between the area under the effect curve of fibrinogen and the area under the curve of t-PA antigen and activity were observed. The area under the effect curve of total fibrin and fibrinogen degradation products and the t-PA antigen area under the curve ($r = 0.55$; $p <$

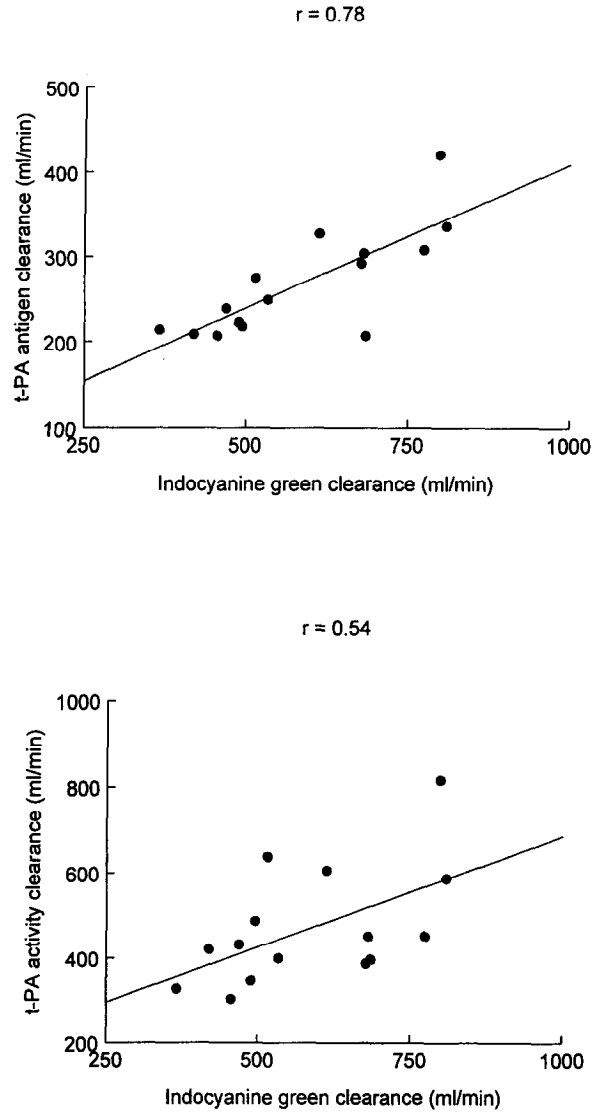

Fig. 3. Significant correlations between indocyanine green clearance and t-PA antigen **(upper panel)** and indocyanine green clearance and t-PA activity clearance **(lower panel).**

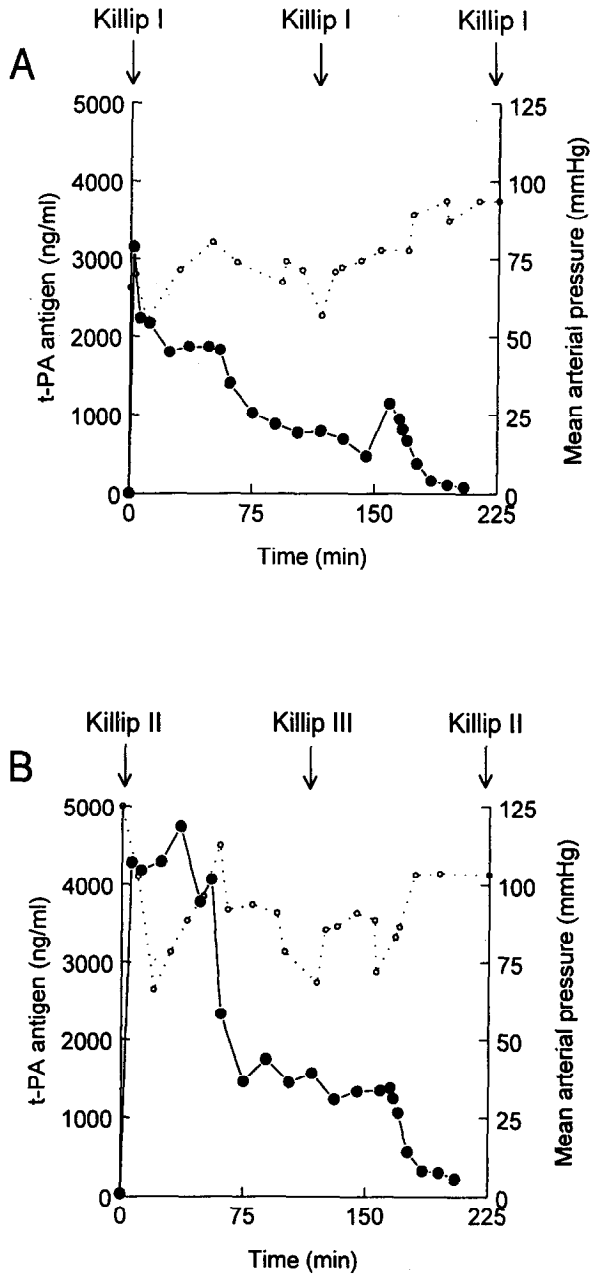

Fig. 4. t-PA antigen concentrations *(solid circles)* and mean arterial pressure *(open circles)* of a patient **(A)** who remains in Killip I category and a patient **(B)** with congestive heart failure (Killip II and III). Patient B received a 60-minute nitroprusside infusion 20 minutes after starting the thrombolytic treatment.

0.05) were significantly related. The functional α_2-antiplasmin decreased to 13% ± 9% of the normal value after 165 minutes. A negative correlation was observed between the area under the effect curve of α_2-antiplasmin and the t-PA antigen area under the curve ($r = -0.70$; $p < 0.01$).

DISCUSSION

This study has shown that the variability in plasma concentrations of t-PA is at least partly explained by liver blood flow in accordance with the theory. This correlation could already be detected in a small patient group that did not include a large number of patients with severely impaired cardiac function. A similar correlation between liver blood flow and

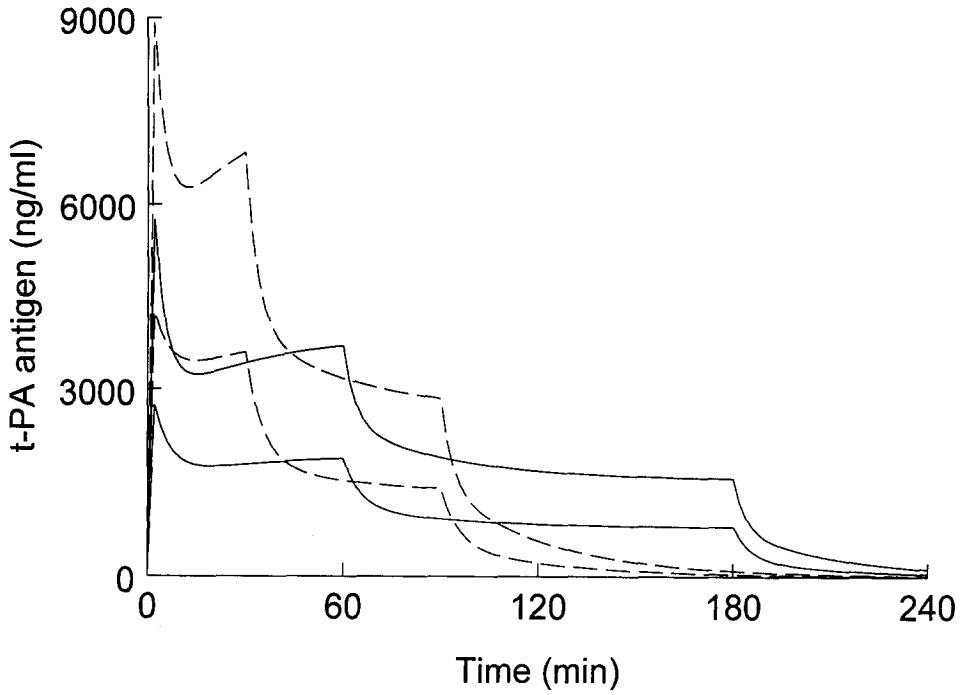

Fig. 5. Comparison of simulated plasma profiles after the conventional *(solid line)* and the "accelerated" *(broken line)* recombinant tissue-type plasminogen activator (rt-PA) dosing scheme, displaying extremes of clearance *(lower line,* high clearance; *upper line,* low clearance) as found in this study for each regimen.

clearance of lidocaine was shown earlier by Zito et al.[10] in a group of patients with a higher incidence of congestive heart failure.

The data suggest that even patients with moderate impairment of cardiac function and congestive heart failure may have reduced liver blood flow. Perfusion of vital organs may be preserved at the expense of splanchnic blood flow, resulting in reduced liver perfusion, while left ventricular systolic and end diastolic pressure are still in the normal range. It can be predicted that patients in cardiogenic shock may have even higher t-PA levels.

High t-PA antigen levels may be associated with the occurrence of bleeding complications[13] and the probability of having any bleeding event is related to the total dose of rt-PA administered.[19] The relationship between measurements of t-PA activity and liver blood flow was less pronounced than that for t-PA antigen. This interindividual difference is reflected in a large variability of the ratio between activity and antigen values and shows that liver blood flow is not the only factor that determines variability.

The practical applicability of our findings is dependent on the presence of a technique for rapid bedside evaluation of either liver blood flow or t-PA or a clinical correlate for these measures. A reliable measurement of t-PA concentrations requires complicated sample handling and frequency of measurement that cannot be carried out in routine coronary care. Bedside determination of t-PA plasma concentrations would obviously be preferable but, although such techniques are being developed, none are currently marketed.

The Killip classification can discriminate various levels of heart failure and has the advantage of being readily available. Our study was too small to indicate whether this method can identify those patients with increased t-PA concentrations, but it was noteworthy that the patient who deteriorated to Killip III had higher than average t-PA concentrations and the highest indocyanine green concentrations. Doppler echographic measurements of liver blood flow could be an alternative. Measurement of flow with Doppler echography is difficult and depends on the patient's condition, habitus, and cooperation. However, it can be concluded that Doppler echography has some potential to assess liver blood flow

changes during the acute phase of a myocardial infarction, and its use should be evaluated further.

The hemodynamic changes induced by the infarction itself are not the only influence on liver blood flow. Other drugs that affect liver blood flow independently may be administered together with rt-PA and unexpected interactions may result. For example, short-term administration of nitrates[25,26] and β-blockers[27] decrease liver blood flow and calcium antagonists increase liver blood flow.[28] The effect of angiotensin converting enzyme inhibitors is unknown. On theoretical grounds an increase in liver blood flow will influence the t-PA concentrations to a lesser extent than a decrease.[29] For example, a combination of rt-PA and a stable analog of prostacyclin (iloprost) did not improve coronary artery patency or left ventricular functional recovery compared with that achieved with rt-PA alone.[30] This treatment failure was suggested to be caused by an increased t-PA clearance.

We compared the simulated plasma concentrations that resulted from the extremes of clearance values obtained in this study after the conventional rt-PA dosing scheme (10 mg bolus, 50 mg in 1 hour, and 40 mg in the next 2 hours) and the more recently introduced "front-loaded" rt-PA dosing scheme (15 mg bolus, 50 mg in 30 minutes, and 35 mg in the next hour; Fig. 5). The main difference in both t-PA activity and antigen concentrations occurs at the start when extremely high t-PA plasma levels may occur in patients with impaired liver blood flow. The GUSTO investigators reported that the new dosage regimen of rt-PA given with intravenous heparin provides a survival benefit over previous standard thrombolytic regimens.[3] The superiority of this rt-PA dose was suggested to be related to faster recanalization of the infarct related vessel achieved by rapid administration. This may be related to the high concentrations in some patients but could also be the cause of an excess of bleeding complications. The lack of a head-to-head comparison of the two rt-PA regimens in the GUSTO study leaves this as an open possibility. We propose that caution should be applied when rt-PA in standard dosages is given to patients with severely impaired liver blood flow or in combination with drugs that may affect liver blood flow and suggest that dose adjustments should be considered.

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