

Differential effects of tissue plasminogen activator and streptokinase on infarct size and on rate of enzyme release: influence of early infarct related artery patency

The GUSTO Enzyme Substudy

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Background The recent international GUSTO trial of 41 021 patients with acute myocardial infarction demonstrated improved 90-min infarct related artery patency as well as reduced mortality in patients treated with an accelerated regimen of tissue plasminogen activator, compared to patients treated with streptokinase. A regimen combining tissue plasminogen activator and streptokinase yielded intermediate results. The present study investigated the effects of treatment on infarct size and enzyme release kinetics in a subgroup of these patients.

Methods A total of 553 patients from 15 hospitals were enrolled in the study. Four thrombolytic strategies were compared: streptokinase with subcutaneous heparin, streptokinase with intravenous (i.v.) heparin, tissue plasminogen activator with i.v. heparin, and streptokinase plus tissue plasminogen activator with i.v. heparin. The activity of alpha-hydroxybutyrate dehydrogenase (HBDH) in plasma was centrally analysed and infarct size was defined as cumulative HBDH release per litre of plasma within 72 h of the first symptoms (Q(72)). Patency of the infarct-related vessel was determined by angiography in 159 patients, 90 min after treatment.

Results Infarct size was $3.72 \text{ g-eq} \cdot \text{l}^{-1}$ in patients with adequate coronary perfusion (TIMI-3) at the 90 min angi-

ogram and larger in patients with TIMI-2 ($4.35 \text{ g-eq} \cdot \text{l}^{-1}$) or TIMI 0–1 ($5.07 \text{ g-eq} \cdot \text{l}^{-1}$) flow ($P=0.024$). In this subset of the GUSTO angiographic study, early coronary patency rates (TIMI 2+3) were similar in the two streptokinase groups (53 and 46%). Higher, but similar, patency rates were observed in the tissue plasminogen activator and combination therapy groups (87 and 90%). Median infarct size for the four treatment groups, expressed in gram-equivalents (g-eq) of myocardium, was 4.4, 4.5, 3.9 and 3.9 g-eq per litre of plasma ($P=0.04$ for streptokinase vs tissue plasminogen activator). Six hours after the first symptoms, respectively 5.3, 6.6, 14.0 and 13.6% of total HBDH release was complete ($P<0.0001$ for streptokinase vs tissue plasminogen activator).

Conclusions Rapid and complete coronary reperfusion salvages myocardial tissue, resulting in limitation of infarct size and accelerated release of proteins from the myocardium. Treatment with tissue plasminogen activator, resulting in earlier reperfusion was more effective in reducing infarct size than the streptokinase regimens, which contributes to the differences in survival between treatment groups in the GUSTO trial.
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Introduction

Prognosis of patients with myocardial infarction is markedly improved by timely reperfusion of the ischaemic myocardium. The GUSTO trial recently demonstrated differences in outcome from four treatment

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regimens^[1], which could be explained by different rates of early coronary reperfusion, varying between 81% for accelerated tissue plasminogen activator with i.v. heparin and 54 and 60% for streptokinase with subcutaneous or intravenous heparin^[2]. Early reperfusion of the ischaemic myocardium allows recovery and salvage of myocytes and preserves left ventricular function^[3]. This concept was confirmed by the angiographic study in GUSTO, which demonstrated small but significant improvements in regional and global left ventricular function with the accelerated tissue plasminogen activator regimen^[2]. Infarct size can be assessed from regional left ventricular function, and measured directly by assessing cumulative protein release from the infarcted myocardium^[4,5]. The cumulative amount of myocardial enzymes or other proteins that can be recovered from plasma samples correlates closely with infarct size, as determined by pathology examinations^[6-9].

In the setting of thrombolytic therapy, the pattern of protein release from the infarct changes. First, proteins such as creatine kinase appear earlier after symptom onset, reflecting either rapid washout or (more likely) accelerated disruption of myocytes after coronary reperfusion^[10]. Secondly, later release of proteins is reduced after thrombolysis. Thus, in spite of an early and often high peaking of creatine kinase and other proteins after coronary reperfusion, the total amount of proteins released from the infarcted tissue is reduced by thrombolytic therapy. Limitation of infarct size by different regimens of thrombolytic therapy ranged from 20% to 35% when compared with conventional therapy^[11] or placebo^[12].

Within the GUSTO trial, a substudy was conducted to assess the patterns of enzyme release in the four treatment groups. In particular, this substudy was designed to verify the relationships among early coronary patency, infarct size, left ventricular function and survival. A priori it was postulated that more rapid reperfusion in patients receiving tissue plasminogen activator (either the accelerated regimen or in combination with streptokinase) would result in smaller infarct size when compared with the streptokinase regimens.

Methods

Patients and thrombolytic strategies

Additional data for the enzyme substudy were collected from 553 GUSTO patients enrolled in 15 hospitals in four European countries. Inclusion criteria have been described in detail^[1]. In short, patients with onset of symptoms less than 6 h before enrolment, chest pain lasting at least 20 min and ST segment elevation ≥ 0.1 mV in two or more limb leads, or ≥ 0.2 mV in two or more precordial leads were eligible. Patients with a history of stroke, recent trauma or major surgery, active bleeding or previous treatment with streptokinase or anistreplase were excluded. After informed consent,

patients were randomized to one of four intravenous thrombolytic regimens: (1) streptokinase, 1.5 million U in 60 min, with subcutaneous heparin, 12 500 U twice daily, beginning 4 h after the start of thrombolytic therapy until day 7 or hospital discharge; (2) streptokinase 1.5 million U in 60 min, with intravenous heparin in a bolus dose of 5000 U followed by 1000 U per hour for at least 48 h, with the dose adjusted to prolong the activated partial thromboplastin time to 2–2.5 times normal; (3) accelerated alteplase, bolus of 15 mg, followed by $0.75 \text{ mg} \cdot \text{kg}^{-1}$ (not to exceed 50 mg) over 30 min and $0.5 \text{ mg} \cdot \text{kg}^{-1}$ (not to exceed 35 mg) over the next 60 min, along with intravenous heparin; (4) the combination of alteplase ($1.0 \text{ mg} \cdot \text{kg}^{-1}$, not to exceed 90 mg, over 60 min with 10% given as an initial bolus) and streptokinase (1.0 million U over 60 min), along with intravenous heparin. All patients received aspirin. Patients without contraindication to beta-blockade received intravenous atenolol ($2 \times 5 \text{ mg}$), followed by 50 to 100 mg orally per day. All other medication was left to the discretion of the attending physician.

Angiographic substudy

In 10 out of the 15 participating hospitals, patients were also enrolled in the Angiographic Substudy^[2] and were randomly assigned to angiography at 90 min, 180 min, 24 h or 5–7 days after the start of thrombolysis. Patients randomized to angiography at 90 min were also assigned to follow-up angiography 5–7 days later. The infarct-related artery was identified by ECG and angiographic data combined. The first injection into this artery was used to determine coronary perfusion according to TIMI criteria^[13]: no flow (grade 0), minimal filling (grade 1), incomplete or delayed filling (grade 2) and normal flow (grade 3). Global left ventricular function and regional wall motion were measured^[2].

Collection of plasma samples and enzyme determination

Participating hospitals received instructions on blood collection and sample handling. Twelve out of the 15 participating hospitals obtained 11 blood samples at 0, 1, 3, 6, 12, 18, 24, 36, 48, 72 and 96 h after the start of thrombolytic therapy. The first six samples were drawn within 30 min of the intended time, later samples could be synchronized to routine phlebotomy. Three hospitals (not participating in the Angiographic Substudy) were not able to adhere to this frequent sampling schedule and collected five samples: at enrolment and between 0800 and 1000h on the following 4 days. In all cases, the exact time of sampling was recorded in the GUSTO Enzyme Case Report Form. Venous blood samples of 5–10 ml were collected in predistributed tubes containing dry heparin to prevent clotting. Blood samples from indwelling catheters were allowed provided that the first

few drops were discarded to prevent dilution with infusion fluids. After centrifugation for 10–15 min at 1000–2000 $\times g$, the supernatant plasma was collected and stored at -20°C , leaving some plasma on the sediment to prevent contamination by blood cells.

Within 8 weeks, plasma samples were transported in polystyrene boxes with dry ice to the Enzyme Core Laboratory at Maastricht, The Netherlands, for central analysis. Haemolytic samples, as detected by visual inspection, and those arriving thawed were discarded. The remaining were stored at -80°C . Plasma activity of alpha-hydroxybutyrate dehydrogenase (HBDH) was determined by spectrophotometry, using a centrifugal analyser (Cobas Bio System, Hoffmann la Roche, Basle, Switzerland) and a commercial test kit (Boehringer Mannheim, Germany). The HBDH test mainly measures the myocardial isoforms LDH1 and LDH2 of lactate dehydrogenase (EC 1.1.1.27), by means of their higher catalytic activities in the conversion of alpha-ketobutyrate. Determinations were performed in duplicate at 25°C , within 6 weeks after arrival of the samples in Maastricht. Activities were expressed in micromoles of substrate converted per minute per litre of plasma [$\text{U} \cdot \text{l}^{-1}$].

Determination of infarct size

Cumulative release of HBDH activity per litre of plasma between the onset of symptoms ($t=0$) and time t , indicated as $Q(t)$, was calculated as described^[4,5] from the expression:

$$Q(t) = C(t) + \text{TER} \cdot \int_0^t e^{\text{ERR}(\tau-t)} \cdot C(\tau) d\tau + \text{FCR} \cdot \int_0^t C(\tau) d\tau$$

with $C(t)$ the plasma activity of HBDH, TER the fractional transcapillary escape rate constant, ERR the fractional extravascular return rate constant and FCR the fractional catabolic rate constant for the elimination of HBDH activity from plasma. Values of $C(t)$ were obtained by subtraction of the normal steady-state plasma activity C_s from the actually measured activities. If the first measurement of plasma HBDH activity was performed within 3 h of the first symptoms, the obtained value was used for C_s ; otherwise a fixed mean value of $82 \text{ U} \cdot \text{l}^{-1}$ was used. Values used for TER, ERR and FCR were respectively 0.014 h^{-1} , 0.018 h^{-1} and 0.015 h^{-1} ^[4,5]. Infarct size was defined as $Q(t)$ calculated over 72 h, that is $Q(72)$. $Q(72)$ was divided by the normal HBDH content of human myocardium determined with the same assay, that is, 123 U per gram of wet weight^[14,15] to obtain infarct size in gram-equivalents of myocardium per litre of plasma ($\text{g-eq} \cdot \text{l}^{-1}$).

To assess differences in release pattern, the proportion of early HBDH release in the first 6 or 12 h was calculated as $Q(6)/Q(72)$ and $Q(12)/Q(72)$.

Statistical analysis

Results were analysed on an intention-to-treat basis with imputation for missing data. To this end, a value of $11.0 \text{ g-eq} \cdot \text{l}^{-1}$ for $Q(72)$ was allotted to patients who died early, and a value of $4.9 \text{ g-eq} \cdot \text{l}^{-1}$ to 39 remaining cases with missing data. The first value was the mean infarct size found in six patients who died within 4 days after the first symptoms, and the second value was the overall mean infarct size in the 502 cases that allowed calculation of infarct size. At the start of the Enzyme Substudy it was appreciated that the sample size would not allow detection of differences in infarct size among all four GUSTO treatment groups. Therefore, it was decided to combine the two tissue plasminogen activator groups as well as the two streptokinase regimens in the primary analysis. Furthermore, the intention was to enroll consecutive patients in order to avoid systematic bias in subsequent comparisons of the enzyme results.

Data analysis was performed with standard software packages (BMDP Statistical Software, Inc., Los Angeles, version 1990 and SAS Institute Inc., Cary release 6 08). Median values were calculated. The significance of differences was tested two-tailed according to Kruskal–Wallis or Mann–Whitney. Multiple linear regression analysis was performed with BMDP routine 1R. Regression lines were computed in SAS.

Results

A total of 553 consecutive patients were enrolled into the GUSTO Enzyme Substudy, of whom 502 patients had enzyme data allowing calculation of $Q(72)$. Twelve patients died before $Q(72)$ could be determined and 39 cases could not be analysed because of haemolytic plasma samples, arrival of thawed plasma samples in Maastricht, or administrative errors. Baseline characteristics of the 553 patients did not differ from the 41 021 patients in the GUSTO main trial (Table 1). There were also no significant differences in baseline characteristics among the four treatment groups within the Enzyme Substudy.

Coronary patency, infarct size and enzyme release

Coronary angiography was performed in 159 patients within 2 h of the start of treatment (Table 1). In this subgroup of the GUSTO angiographic study, complete perfusion (TIMI-3) was observed in similar proportions of patients receiving accelerated alteplase as the combination regimen. As in the larger study, TIMI scores were worse in patients treated with the streptokinase regimens. A marked association between early TIMI scores and infarct size was observed. Median infarct size was 3.72 (25th and 75th percentile respectively 1.43 and 6.48) $\text{g-eq} \cdot \text{l}^{-1}$ in patients with normal coronary

Table 1 Baseline data and selected outcome parameters

	Main trial	Enzyme cohort	SK s.c. hep.	SK i.v. hep.	tPA i.v. hep.	tPA + SK i.v. hep.
Baseline characteristics						
Number of patients	41 021	553	139	136	137	141
Age (years)	62	62 (54–69)	61 (52–69)	63 (55–70)	62 (52–70)	62 (55–69)
Female sex (%)	25	23	21	28	22	19
Infarct location						
Anterior (%)	39	40	40	36	43	42
Inferior (%)	58	56	56	60	54	54
Other (%)	3	4	4	4	4	4
Previous infarction (%)	16	15	17	10	19	15
Previous coronary surgery (%)	4	2	3	4	2	1
Time to treatment (h)	2.8	2.8 (2.0–3.8)	2.8 (2.2–3.8)	2.6 (1.8–3.8)	2.8 (2.1–3.7)	2.8 (2.0–3.8)
Selected clinical events						
24 h mortality (%)	2.7	1.6	1.4	2.9	0.7	1.4
30 day mortality (%)	7.0	4.5	2.9	8.1	2.9	4.3
All strokes (%)	1.5	1.1	0.7	1.5	0.7	1.4
Coronary angiography at 90 min						
Number of patients		159	40	26	52	41
TIMI 3 (patients)		65	7	7	29	22
2		50	14	5	16	15
0–1		44	19	14	7	4
patent TIMI 3 (%)		41	18	27	56	54
TIMI 2+3 (%)		72	53	46	87	90

SK=streptokinase; s.c. hep=subcutaneous heparin; i.v. hep=intravenous heparin; tPA=alteplase. Values followed by numbers in parentheses are medians, with the 25th and 75th percentiles shown inside the parentheses.

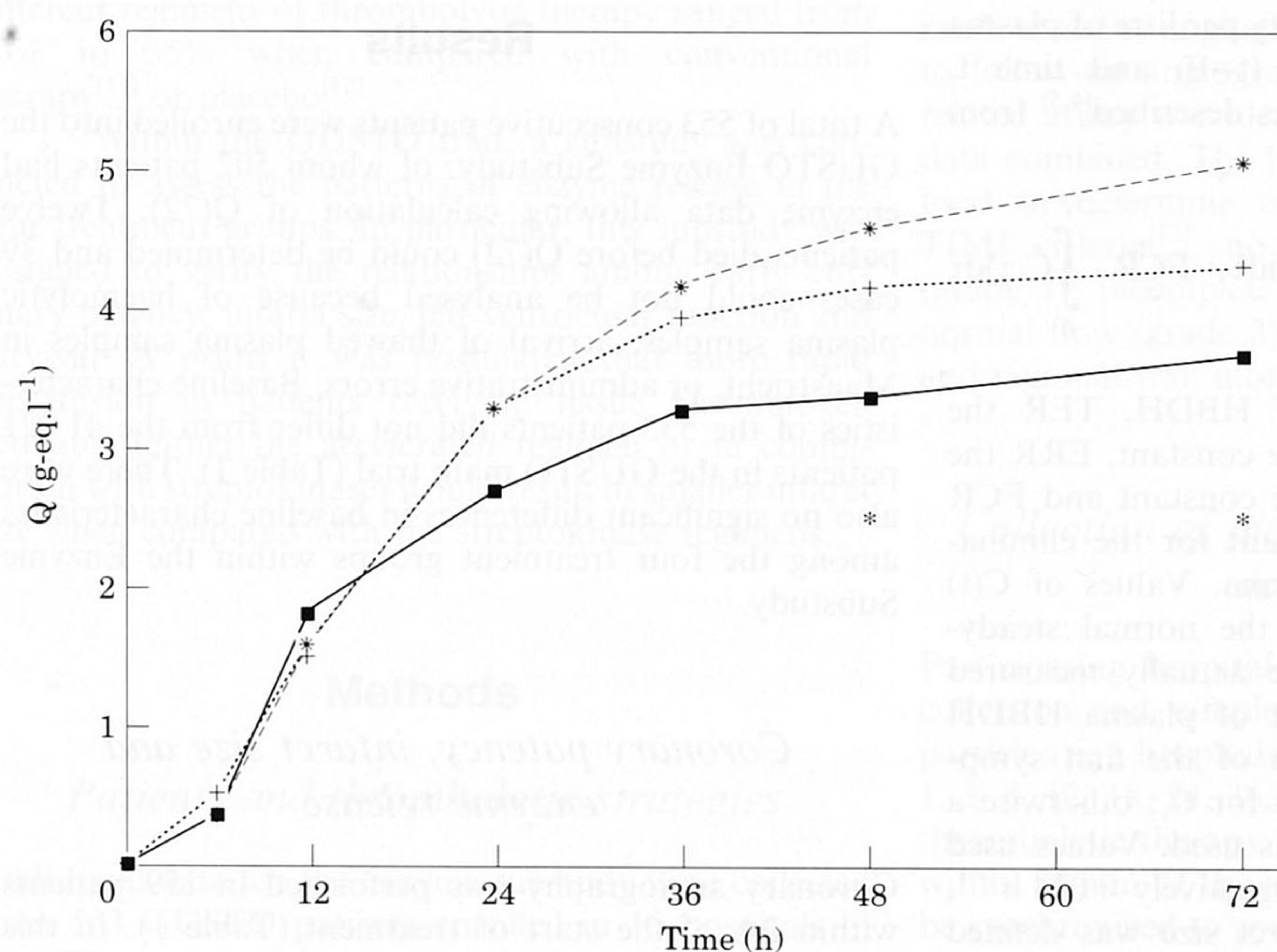


Figure 1 HBDH release patterns (Q) over 72 h for patients with TIMI 3 (■, n=65), 2 (+, n=50) or 0/1 (*, m=44) at the 90 min angiogram. *Indicates a significant difference between the groups ($P<0.05$).

perfusion (TIMI-3), 4.35 (2.63, 6.40) g-eq.l⁻¹ in patients with incomplete perfusion (TIMI-2) and 5.07 (3.73, 8.22) g-eq.l⁻¹ in those with minimal or no perfusion (TIMI-0,1) (Fig. 1). The differences among the

three groups were significant at 48 h ($P=0.047$) and at 72 h ($P=0.024$).

The overall pattern of enzyme release varied among the three patient groups. In patients with normal

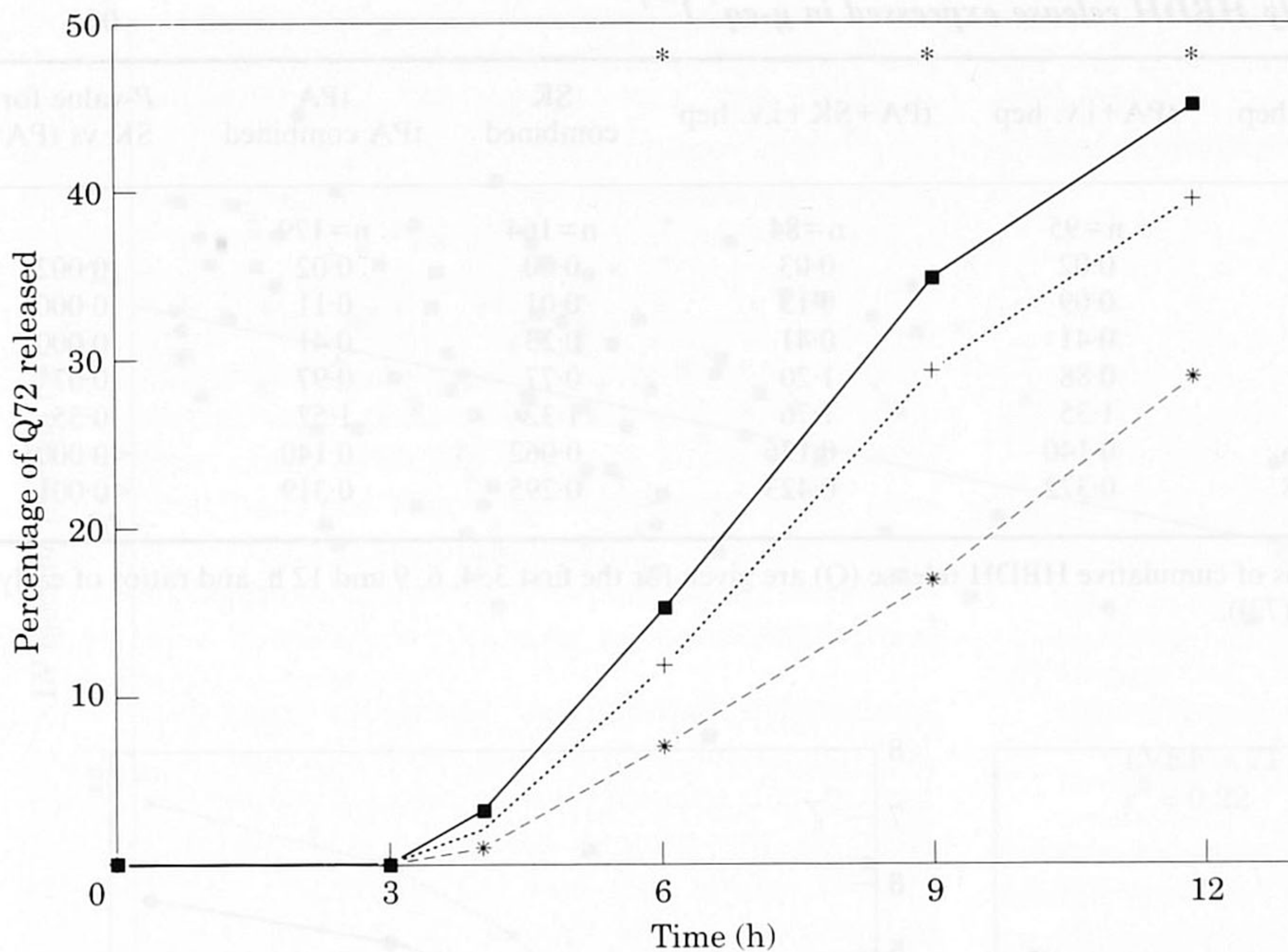


Figure 2 Percentage of Q72 released for patients with TIMI 3 (■, n=65), 2 (+, n=41), and 0/1 (*, n=42) at the 90 min angiogram. Q72 denotes the cumulative release of HBDH at 72 h. *Indicates a significant difference between the groups ($P<0.05$).

Table 2 Effect of treatment on median infarct size ($Q(72)$) expressed in $\text{g-eq} \cdot \text{l}^{-1}$

SK+s.c. hep	SK+i.v. hep	tPA+i.v. hep	tPA+SK+i.v. hep	SK combined	tPA combined	P-value for SK vs tPA
n=125	n=120	n=130	n=127	n=245	n=257	
4.36	4.52	3.92	3.88	4.39	3.88	0.043
2.5–6.7	2.6–7.1	1.6–6.4	1.9–6.6	2.5–6.8	1.7–6.6	
With imputation for missing cases (see text):						
n=139	n=136	n=137	n=141	n=275	n=278	
4.90	4.90	4.39	4.17	4.90	4.35	0.034
2.6–6.5	2.8–7.1	1.7–6.3	2.1–6.7	2.7–6.8	2.0–6.6	

Abbreviations as in Table 1, n=number of patients. Medians with the 25th and 75th percentiles are given.

perfusion at early angiography a greater proportion was released within the first 6 or 12 h (Fig. 2, $P\leq 0.002$). Because the proportion of early HBDH release can be meaningfully calculated only in patients with a complete series of early samples, only 343 patients who had at least 10 samples and $Q(72)$ exceeding $0.5 \text{ g-eq} \cdot \text{l}^{-1}$ were used for this analysis.

Effect of treatment on infarct size and enzyme release kinetics

Table 2 presents the median values of $Q(72)$ for the four treatment groups, without and with imputation for missing cases, and for the combined tissue plasminogen activator and streptokinase groups. Patients treated with

accelerated tissue plasminogen activator or combination therapy had infarcts that were 12% smaller than those who received streptokinase therapy. These differences were apparent, but not statistically significant in a four-group comparison (Kruskal–Wallis, $P=0.19$). However, the pooled patients receiving streptokinase only differed significantly ($P=0.043$) from the patients receiving either tissue plasminogen activator or tissue plasminogen activator plus streptokinase. Because of the high value of $11.0 \text{ g-eq} \cdot \text{l}^{-1}$ used for the patients who died early (see Methods), imputation raised the median values by 10–15%, but these trends remained unchanged. Due to the higher number of deaths in the streptokinase patients (see Table 1) imputation resulted in a slightly more significant difference ($P=0.034$) between the combined tissue plasminogen activator and the combined streptokinase groups.

Table 3 Effect of treatment on early HBDH release expressed in $\text{g-eq} \cdot \text{l}^{-1}$

	SK+s.c. hep	SK+i.v. hep	tPA+i.v. hep	tPA+SK+i.v. hep	SK combined	tPA combined	P-value for SK vs tPA
	n=87	n=77	n=95	n=84	n=164	n=179	
Q(3)	0.00	0.00	0.02	0.03	0.00	0.02	0.002
Q(4)	0.01	0.02	0.09	0.13	0.01	0.11	0.0001
Q(6)	0.15	0.30	0.41	0.41	0.23	0.41	0.0001
Q(9)	0.74	0.87	0.88	1.20	0.77	0.97	0.07
Q(12)	1.29	1.34	1.35	1.76	1.32	1.57	0.55
Q(6)/Q(72)	0.053	0.066	0.140	0.136	0.062	0.140	<0.0001
Q(12)/Q(72)	0.282	0.318	0.372	0.423	0.295	0.319	<0.001

Abbreviations as in Tables 1 and 2. Medians of cumulative HBDH release (Q) are given for the first 3, 4, 6, 9 and 12 h, and ratios of early release are given (Q(6)/Q(72) and Q(12)/Q(72)).

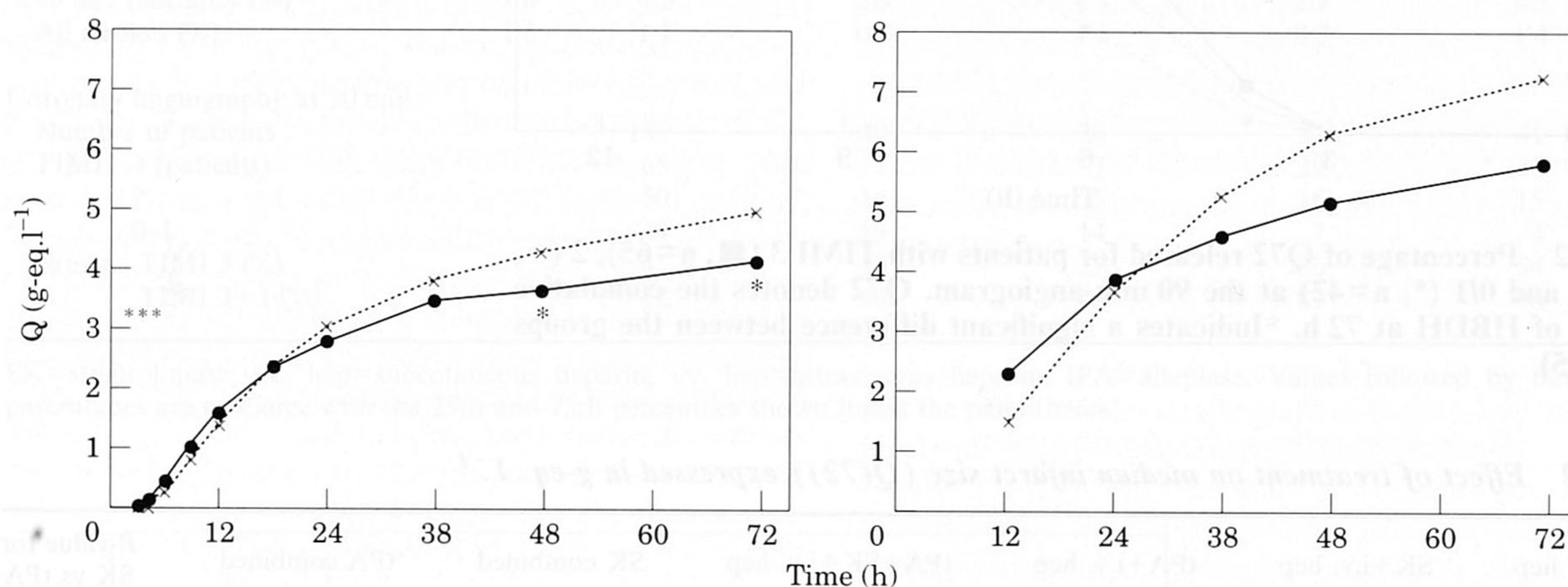


Figure 3 HBDH release patterns (Q) of patients treated with streptokinase (X) (and intravenous or subcutaneous heparin) or with alteplase (●) (and intravenous heparin or with streptokinase and intravenous heparin). * indicates a significant difference between the groups ($P < 0.05$). For comparison, data of the European Cooperative Study Group (rt-PA/placebo trial), are presented (right panel). Note accelerated (early) release for tissue plasminogen activator (●) in comparison with both placebo (X, right panel) as well as streptokinase (X, left panel).

Early release of HBDH, expressed in $\text{g-eq} \cdot \text{l}^{-1}$, within the first 12 h after onset of symptoms is presented in Table 3 for 343 patients with at least 10 samples. In spite of the larger ultimate infarct size in the streptokinase groups (Table 2), release up to 6 h after first symptoms was significantly lower in the combined streptokinase groups, compared with the combined tissue plasminogen activator groups. Differences between ratios Q6/Q72 and Q12/Q72 were more significant than differences between absolute values of Q6 and Q12 (Table 3). This reflects two distinct effects on release kinetics: enhancement of early release and reduction of late release in patients treated with tissue plasminogen activator vs patients treated with streptokinase. This is also illustrated in Fig. 3.

In order to verify whether the effect of treatment could be caused by factors other than infarct-related artery patency, multiple linear regression analysis was performed using Q(72) as the dependent variable and treatment (streptokinase vs tissue plasminogen activa-

tor) as well as TIMI score (3 vs 2 vs 0 or 1) as independent variables. In this analysis, the contribution of treatment became totally insignificant ($P = 0.97$) while the effect of TIMI score remained highly significant ($P < 0.01$). This indicates that the effect of treatment on infarct size may be explained by its effect on 90-min infarct-related artery patency. Similarly, multiple linear regression analysis with Q(12)/Q(72) as the dependent variable and treatment (streptokinase vs tissue plasminogen activator) and TIMI score (3 vs 2 vs 0 or 1) as independent variables demonstrated that the effect of treatment on the acceleration of enzyme release was explained by early coronary patency.

Infarct size and left ventricular function

Both infarct size and left ventricular function measurements at 5–7 days after allocation were available in 156 patients with a first myocardial infarction. There was an

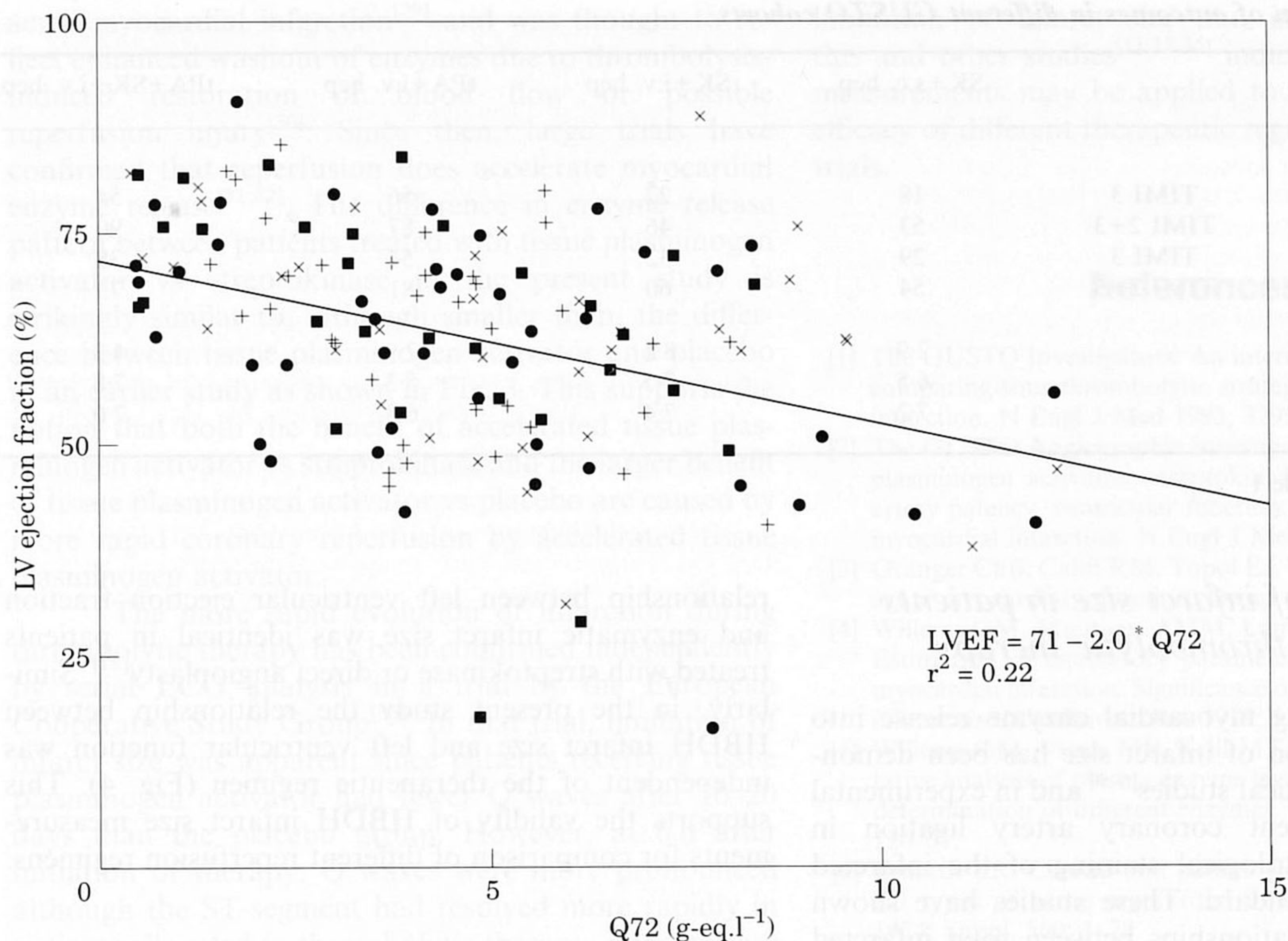


Figure 4 Left ventricular (LV) ejection fraction (LVEF) 5–7 days after a first myocardial infarction against Q72. Q72 denotes the cumulative release of HBDH at 72 h. The linear regression line for all patients is given in the figure. The regression lines for the four treatment groups did not differ (intercept and slope for alteplase+intravenous heparin (●) were, respectively, 70 and -1.9 ; for streptokinase+intravenous heparin (+) 72, -2.2 ; for alteplase+streptokinase+intravenous heparin (■) 72, -2.2 and for streptokinase and subcutaneous heparin (×) 72, -1.8).

inverse relationship between infarct size and both left ventricular ejection fraction and regional wall motion of the infarct zone. This relationship for left ventricular ejection fraction is shown in Fig. 4. Regression lines were computed as shown in Fig. 4, indicating consistent relationships between infarct size and global or regional left ventricular function, independent of the treatment regimens.

Discussion

In GUSTO, treatment with accelerated tissue plasminogen activator yielded improved survival compared with two streptokinase regimens. The absolute difference in 30 day mortality was 1%, which corresponded to a 14% relative mortality reduction^[1]. Intermediate results were obtained with the combined regimen of tissue plasminogen activator with streptokinase. These differences could be explained by more frequent early reperfusion in patients receiving tissue plasminogen activator, as documented by the GUSTO angiographic substudy^[2]. The present GUSTO enzyme substudy extends these findings and confirms the concept that the improved survival after treatment with accelerated tissue plasminogen activator in comparison with streptokinase^[1] is related to

myocardial salvage by more rapid coronary reperfusion. Indeed, infarct size in patients treated with tissue plasminogen activator appeared to be 12% smaller than in those receiving streptokinase.

It should be appreciated that the mortality and patency results in this substudy are compatible with, but not identical to, those in the larger trial (Table 4). In particular, early coronary patency was almost identical among patients receiving accelerated tissue plasminogen activator or the combination regimen (tissue plasminogen activator within streptokinase) in this subset of the angiographic substudy. This is in agreement with the infarct size measurements, which were almost identical in these two groups. Accordingly, the predefined intention to compare the two tissue plasminogen activator regimens (accelerated tissue plasminogen activator and combined tissue plasminogen activator and streptokinase) with the two streptokinase regimens appeared justified. In contrast, in the larger angiographic substudy and in the mortality study, both early patency and survival for the combination regimen were intermediate between the figures for accelerated tissue plasminogen activator and the streptokinase groups, and combination of the two regimens containing tissue plasminogen activator would not be appropriate.

Table 4 Comparison of outcomes in different GUSTO cohorts

		SK+s.c. hep	SK+i.v. hep	tPA+i.v. hep	tPA+SK+i.v. hep
90 min patency (%)					
Enzyme substudy	TIMI 3	18	27	56	54
	TIMI 2+3	53	46	87	90
Angio substudy	TIMI 3	29	32	54	38
	TIMI 2+3	54	60	81	73
30 day mortality (%)					
Enzyme substudy		2.9	8.1	2.9	4.3
Angio substudy		6.5	7.5	5.3	7.8
GUSTO main trial		7.2	7.4	6.3	7.0

Abbreviations as in Table 1.

Estimation of infarct size in patients receiving thrombolytic therapy

The validity of using myocardial enzyme release into plasma for estimation of infarct size has been demonstrated in several clinical studies^[6-9] and in experimental studies on permanent coronary artery ligation in dogs^[16-18] using histological staining of the infarcted area as the gold standard. These studies have shown highly significant relationships between total infarcted muscle volume and peak values or cumulative release of enzymes such as creatine kinase, lactate dehydrogenase and aspartate aminotransferase. In patients treated with thrombolytic therapy, however, some clinical studies have indicated altered relationships between the release of creatine kinase, or creatine kinase-MB into plasma and myocardial injury as estimated from an independent parameter such as left ventricular ejection fraction^[19-21] or thallium-201 defect^[22]. In these studies, thrombolysis caused extra release of creatine kinase, creatine kinase-MB compared with control patients for the same degree of damage as determined from the independent variable. Reperfusion also resulted in enhanced creatine kinase recovery in plasma after experimental infarction in the dog^[23]. Currently, this remains a controversial issue because complete recovery of creatine kinase in plasma has been reported after permanent coronary occlusion^[24], while recovery was found to be decreased after reperfusion^[25]. It should be appreciated that this controversy does not invalidate the conclusion of the present study, that infarcts remain smaller after tissue plasminogen activator therapy. All clinical studies suggesting an effect of thrombolytic therapy on enzyme release have claimed reperfusion-induced *enhancement* of release. This implies that the 12% reduction of infarct size in patients treated with tissue plasminogen activator could only be under-estimated, and that the true benefits might be greater.

For HBDH, complete recovery in plasma after permanent coronary occlusion has been reported^[24]. In a large clinical trial, the relationship between release of HBDH and left ventricular ejection fraction remained unaltered by thrombolytic therapy^[26], indicating that no reperfusion-induced extra release of HBDH occurs. The

relationship between left ventricular ejection fraction and enzymatic infarct size was identical in patients treated with streptokinase or direct angioplasty^[27]. Similarly, in the present study the relationship between HBDH infarct size and left ventricular function was independent of the therapeutic regimen (Fig. 4). This supports the validity of HBDH infarct size measurements for comparison of different reperfusion regimens.

Effect of infarct artery patency on infarct size

The reduction of infarct size by the tissue plasminogen activator regimens, compared to streptokinase, could be explained by improved 90 min patency of the infarct-related artery. It is also interesting to note that reduction of infarct size was not restricted to patients with early complete perfusion (TIMI-3). Patients with partial perfusion (TIMI-2) also had smaller infarcts than those with an occluded infarct related artery (TIMI-0-1). Others have reported a lack of infarct size reduction in patients with TIMI-2 flow^[28]. A possible explanation for this discrepancy could be the somewhat later angiography in the TEAM-2 study: a mean delay of 2.4 h vs 1.6 h in the present study. This is supported by data from 72 patients in the present study who had angiography between 2 and 4 h after initiation of thrombolytic therapy. In these patients, infarct size was $3.41 \text{ g-eq} \cdot \text{l}^{-1}$, $5.29 \text{ g-eq} \cdot \text{l}^{-1}$ and $4.99 \text{ g-eq} \cdot \text{l}^{-1}$ for TIMI perfusion grades, 3, 2 and 0-1 respectively ($P=0.42$), so no benefit was apparent in patients with TIMI-2 flow between 2 and 4 h. Thus, early incomplete perfusion (TIMI-2) might be adequate for some recovery of ischaemic myocytes. Alternatively, some of these patients may have further clot resolution with complete perfusion (TIMI-3) before necrosis has been completed.

Effect of infarct artery patency on acceleration of enzyme release

Earlier release of myocardial enzymes into plasma was already noted in the first study on thrombolysis after

acute myocardial infarction^[29] and was thought to reflect enhanced washout of enzymes due to thrombolysis-induced restoration of blood flow or possible reperfusion injury^[30]. Since then, large trials have confirmed that reperfusion does accelerate myocardial enzyme release^[31,32]. The difference in enzyme release pattern between patients treated with tissue plasminogen activator vs streptokinase in the present study is strikingly similar to, although smaller than, the difference between tissue plasminogen activator and placebo in an earlier study as shown in Fig. 3. This supports the notion that both the benefit of accelerated tissue plasminogen activator vs streptokinase and the larger benefit of tissue plasminogen activator vs placebo are caused by more rapid coronary reperfusion by accelerated tissue plasminogen activator.

The more rapid evolution of infarction during thrombolytic therapy has been confirmed independently by serial ECG analysis in a trial by the European Cooperative Study Group^[33]. In that trial, limitation of infarct size was apparent since patients receiving tissue plasminogen activator had fewer Q waves after 10–20 days than the placebo group. However, at 6 h after initiation of therapy, Q waves were more pronounced although the ST-segment had resolved more rapidly in patients allocated to thrombolytic therapy. Early release of various myocardial marker proteins is now widely used as a non-invasive indicator of successful reperfusion in individual patients, although the sensitivity and specificity of this method are not established. In the evaluation of thrombolytic therapy, acceleration of enzyme release is a more sensitive parameter than reduction of infarct size.

Limitation of infarct size and reduced mortality

Collectively, the large placebo-controlled or open trials of thrombolytic therapy have demonstrated a marked reduction in mortality for patients presenting to hospital within 6 to 12 h of the onset of symptoms^[38]. In trials that studied the release of HBDH (or LDH1), reductions of infarct size between 20% and 35% were consistently found^[11,12,34,35]. Both reduction of mortality^[1] as well as reduction of HBDH release^[36,37] were most impressive in patients treated within 1 h of the first symptoms. Similar trends were observed in the GUSTO study, with a 14% reduction in mortality for patients treated with tissue plasminogen activator reported in the main trial^[1] which corresponded to a 12% reduction in infarct size as shown in this study.

To conclude: the presented data are in full agreement with and support the concept that more rapid reperfusion (by accelerated tissue plasminogen activator, in comparison with the streptokinase regimens in GUSTO) salvages myocytes, resulting in limitation of infarct size, preservation of left ventricular function and improved survival. The observed association between

limitation of infarct size and improved survival in this and other studies^[11,12,35] indicates that infarct size measurements may be applied to compare the clinical efficacy of different therapeutic regimens in medium size trials.

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Appendix

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