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Thrombolysis-induced coronary reperfusion causes acute and massive interstitial release of cardiac muscle cell proteins

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Abstract

Objective: Reperfusion of the infarct-related artery in patients with acute myocardial infarction limits infarct size, but also causes accelerated release into plasma of cardiac tissue proteins. The latter effect could reflect either enhanced protein washout from the heart or abrupt disruption of myocyte membranes. The present study indicates that the latter mechanism prevails. **Methods:** In 26 patients, patency of the infarct-related artery was determined by coronary angiography 90 min and 5–7 days after thrombolytic treatment. Continuous electrocardiography was performed during the first 24 h after admission. Cumulative release of myoglobin (Mb) and creatine kinase (CK) into plasma was calculated from frequently sampled plasma concentrations. **Results:** In patients with a patent infarct-related artery after 90 min, the onset of a rapid (> 50%) decrease in ST-vector magnitude coincided with an equally rapid increase in QRS-vector magnitude, and with a sudden onset of release into plasma of Mb as well as CK. In these patients, a maximal initial release rate was observed and cumulative release conformed closely to a simple model for sudden interstitial liberation of proteins. In contrast, protein release started more gradually and could not be fitted to this model, in patients with persistent occlusion of the infarct-related artery at 90 min and absence of ST-vector normalisation. **Conclusions:** Previous studies have demonstrated significant myocardial salvage by timely reperfusion therapy. Nevertheless, this study indicates that the moment of recanalisation of the infarct-related artery coincides with sudden and massive disruption of myocyte membranes. Attenuation of this effect, if possible, could further improve the benefits of reperfusion therapy.

Keywords: Myocardial infarction; Reperfusion; Electrocardiography; Thrombolysis; Human

1. Introduction

Restoration of blood flow in the ischemic area by thrombolytic therapy or angioplasty has been shown to salvage myocardium, to preserve left ventricular function and to increase survival in patients with acute myocardial infarction (AMI). Nevertheless, experimental studies have demonstrated the existence of so-called reperfusion injury, a sudden and paradoxical exacerbation of myocyte injury following reperfusion of ischemic myocardium [1,2]. Such injury has been related to an acute inflammatory response with activation of leukocytes, production of free radicals and oxidative stress [3]. In other studies, however, no evidence of reperfusion injury was found [4,5]. Accordingly, the existence of reperfusion injury remains controversial and its clinical relevance is unknown.

Reperfusion injury could render thrombolytic therapy a 'double-edged sword' [6], and understanding the role of reperfusion injury in the clinical setting of recanalisation therapy for acute myocardial infarction is therefore important. Such understanding is hampered by the circumstance that in most clinical studies neither the exact moment of reperfusion nor the exact timing of the onset of cellular injury could be determined. In the present study an attempt

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was made to overcome these limitations. Restoration of coronary patency was verified by coronary angiography, 90 min after the start of therapy and 5–7 days later. The exact moment of reperfusion was estimated from the onset of a rapid normalisation of ST-segment elevation in a continuous 24-h multilead ECG recording. The moment of onset of cellular damage was estimated from frequent measurements of the onset of myocyte protein release into plasma.

2. Methods

2.1. Patient population

Patients with evolving myocardial infarction, who participated in the GUSTO trial, and who were randomised for coronary-angiography at 90 min after attempted thrombolysis [7], were included in this study. Data were collected at the Department of Cardiology, Gasthuisberg Hospital, Leuven, Belgium, and at the Thorax Center, Erasmus University, Rotterdam, The Netherlands. Patients received one of 4 different intravenous thrombolytic regimens, according to the GUSTO protocol [7]: (1) streptokinase with subcutaneous heparin; (2) streptokinase with intravenous heparin; (3) front-loaded t-PA with intravenous heparin; or (4) the combination of streptokinase and t-PA with intravenous heparin. The investigation conformed with the principles outlined in the Declaration of Helsinki.

2.2. Coronary angiography

Early coronary angiography was performed 90 min after initiation of thrombolytic therapy [7]. The first contrast injection was used to score coronary perfusion status, according to the Thrombolysis in Myocardial Infarction (TIMI) criteria [8]. If rescue balloon dilatation was performed, the perfusion following this procedure was also scored and used in the present study. In most patients, a second TIMI score was obtained by coronary angiography, between 5 and 7 days after first symptoms.

2.3. Biochemical markers

Sixteen heparinised blood samples were taken before, and 0.75, 1.5, 2, 3, 3.5, 4, 6, 8, 12, 18, 24, 30, 36, 42 and 48 h after initiation of thrombolytic therapy. Samples were centrifuged and analysed the same day for plasma creatine kinase (CK) activity, while an aliquot was frozen at −20°C for myoglobin mass determination. Total CK activities were analysed with Boehringer reagents (Cat. No. 1442384) on automated analysers at 37°C, and are reported in units per litre (U/l) or units per ml (U/ml) plasma, with an upper reference limit of 180 U/l. Myoglobin (Mb) was determined at a central site by a fixed-time nephelometric method on a BN 100 nephelometer (Behring Diagnostica, Marburg, Germany). The Mb upper reference limit is 90 ng/ml.

2.4. Continuous vectorcardiographic ECG monitoring

Patients were eligible for enrolment in the GUSTO study if the admission ECG had > 0.1 mV ST-segment elevation in two or more limb leads or > 0.2 mV in two or more contiguous precordial leads [7]. As soon as possible, usually shortly before or after start of thrombolytic treatment, continuous ECG monitoring was started by connecting the patient to a dynamic vector electrocardiographic recording system (MIDA 1000, Ortivus Medical, Täby, Sweden). This system consists of a microprocessor-controlled data acquisition module connected to an IBM-compatible computer. It allows continuous, on-line vectorcardiography, and dynamic analysis of QRS-complex and ST segment changes, with a sensitivity of 5 μV and a sampling rate of 500 per second. Using 8 leads, averaged QRS-T complex templates are calculated from Frank's corrected orthogonal leads X-Y-Z, at 1 min time intervals, with the first template being the reference for the entire recording. All templates were stored on hard disk and subsequently used to calculate QRS-vector difference (QRS-VD) and ST-vector magnitude (ST-VM) [9]. QRS-VD reflects the total areal change compared to the reference QRS-vector at the start of the recording, and is expressed in μV · s. ST-VM is the magnitude of the sum ST-vector measured at the J-point + 60 ms, and is expressed in μV. All averaged QRS-T templates were manually scanned and edited, using a superimposition mode. Templates with artefacts, bundle branch block, detection and marker errors, and postural changes were deleted. Measurements were continued during 24 h, also during coronary angiography with a portable MIDA system, and patients were only disconnected during transport to and from the catheterisation room. ST-VM recovery was defined as a rapid (< 60 min) ST-VM reduction of more than 50%. The time at which the first normalisation started was scored and subsequent 'spikes' or gradual changes were ignored. Times were expressed in hours after the start of first symptoms.

2.5. Calculation of cumulative release of proteins

Cumulative release of CK and Mb per litre of plasma between the onset of symptoms ($t = 0$) and time t, indicated as $Q(t)$, was calculated as described [10] from the expression:

$$Q(t) = C(t) + TER\int_0^t C(\tau)\exp[ERR(\tau - t)]\,d\tau + FCR\int_0^t C(\tau)\,d\tau \quad (1)$$

with $C(t)$ the plasma activity of CK or the plasma concentration of Mb per litre of plasma, TER the fractional

transcapillary escape rate constant, *ERR* the fractional extravascular return rate constant and *FCR* the fractional catabolic rate constant for protein elimination from plasma. Values of $C(t)$ were obtained by subtraction of the normal steady-state plasma concentrations (C_s) from the actually measured activities. If the first protein concentration was measured within 3 h after first symptoms and was below the upper reference values, the obtained value was used for C_s. Otherwise fixed mean values of 75 U/l and 30 ng/ml were used for CK and Mb, respectively. Values used for FCR_{CK} and FCR_{Mb} were $0.20 \cdot h^{-1}$ [10] and $2.6 \cdot h^{-1}$ [11], respectively. For CK, *TER* and *ERR* have been estimated at $0.014 \cdot h^{-1}$ and $0.018 \cdot h^{-1}$ [10]. For Mb, capillary permeability is about 20 times higher than for CK and the ratio of extravascular to intravascular pool (i.e., the ratio *TER / ERR*) is about 4 times larger [12]. Therefore values of $TER = 0.3 \cdot h^{-1}$ and $ERR = 0.1 \cdot h^{-1}$ were used for this protein.

2.6. Model function for sudden interstitial protein release

If reperfusion at time t_s results in sudden and complete liberation of cellular proteins into interstitial space, the protein release into plasma would start immediately at its maximal rate (f_{max}). Assuming also that a particular protein leaks into plasma with a fractional washout rate constant *FWR*, the following simple input function $f(t)$ for the rate of protein release into plasma is obtained:

$$f(t) = f_{max} \exp[-FWR(t - t_s)] \text{ for } t \geq t_s. \quad (2)$$

It is assumed that there is no release before time t_s, while at time t_s the release starts at its maximal rate f_{max} and then decreases exponentially. Integrating this expression with respect to time, one obtains for the cumulative release $Q(t)$:

$$Q(t) = Q_{tot}\{1 - \exp[-FWR(t - t_s)]\} \text{ for } t \geq t_s, \quad (3)$$

Table 1
Baseline data

Patient	Age (y)	Sex	Infarct-related artery	Treatment *	Treatment delay (h) †	First TIMI-score	First angio delay (h) †	Second TIMI-score	Second angio delay (days) †	Maximal pre-cath. ST-VM (μV)
1 §	72	F	LAD	3	2.8	0 → (3)‡	4.3	n.d.	n.d.	267
2	44	M	LCX	4	2.6	2	3.8	3	8	427
3	44	F	RCA	1	2.8	3	5.8	n.d.	n.d.	461
4	40	M	LAD	3	2.8	3	4.3	3	6	398
5	66	M	RCA	4	3.5	3	5.2	3	4	315
6	81	M	LAD	3	2.2	3	3.0	3	3	627
7	58	M	LAD	1	3.3	2	4.7	3	7	524
8	78	M	RCA	3	3.5	2	4.8	3	7	410
9	71	M	LAD	3	5.6	2	7.0	3	7	220
10	48	M	LAD	4	1.8	3	3.0	3	5	200
11	64	M	RCA	3	1.5	3	2.5	0	6	341
12	70	F	LAD	3	1.8	3	3.8	1	7	167
13	47	M	LAD	1	2.1	0 → (3) ‡	3.8	n.d.	n.d.	147
14	60	M	LAD	3	1.9	3	2.8	3	8	307
15	60	M	RCA	1	3.3	3	4.8	3	6	135
16	68	M	LAD	2	2.4	2	3.8	3	3	76
17	49	M	LCX	2	2.6	3	4.1	3	1	194
18	43	M	LAD	3	1.8	1	3.2	3	1	489
19	82	F	LCX	2	2.8	0	4.7	n.d	n.d.	142
20	42	F	LCX	2	0.8	1	2.3	2	7	391
21	56	M	LAD	4	2.9	0	4.4	3	7	272
22	70	M	LAD	2	1.8	0	3.3	3	6	240
23	62	M	RCA	1	3.3	1	4.5	0	5	141
24	61	M	LAD	3	2.3	1	4.2	3	6	197
25	72	M	LCX	4	5.0	0	5.7	0	6	212
26	68	M	graft	3	1.8	0	3.5	3	6	223
Mean	61				2.7	1.8	4.1	2.5	5.5	289
s.d.	13				1.0	1.2	1.1	1.1	2.0	140

* 1 = SK + subcutaneous heparin; 2 = SK + intravenous heparin; 3 = front-loaded tPA + intravenous heparin; 4 = SK + tPA + intravenous heparin.

† Times are expressed in hours or days after first symptoms.

‡ TIMI score before and after PTCA.

§ Order of patients adapted to Table 2.

LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery; ST-VM = ST-vector magnitude; n.d. = no data available.

with Q_{tot} the total amount of protein released ($Q_{tot} = f_{max}/FWR$). A small protein like Mb will have a higher value of *FWR* than CK.

Values of $Q(t)$, as calculated from Eq. (1), were fitted to Eq. (3) with a standard least-squares procedure (Function Solver of Microsoft Excel, Frontline Systems Inc.). In this way, individual values of the three parameters Q_{tot}, *FWR* and t_s were obtained. In order to promote equal influence of data points, fits on Mb curves were restricted to 24 h after first symptoms, during which time release has mostly been completed. The quality of the obtained fit was expressed as the residue (res) (i.e., the mean root square deviation per data point) expressed as a percentage of mean $Q(t)$ value.

2.7. Statistical analysis

Statistical analysis was performed with BMDP (Statistical Software Inc., Los Angeles, 1990). The significance of differences was tested two-tailed according to Mann-Whitney, using BMDP routine 3S. The significance of relations between variables was tested by linear regression, using BMDP routine 1R.

Table 2
Parameters obtained from fits on protein release

Patient	Results for myoglobin (Mb)				Results for creatine kinase (CK)				Start of ST-recovery (h)
	t_s * (h)	*FWR* † (h^{-1})	Q_{tot} ‡ (mg/l)	Res § (%)	t_s * (h)	*FWR* † (h^{-1})	Q_{tot} ‡ (U/ml)	Res § (%)	
Group A: Patients with 90-min TIMI score 3 or 2, and ST-VM recovery									
1	5.1	0.36	21.8	2.5	4.4	0.077	9.2	6.8	5.1
2	3.9	0.49	26.7	1.7	4.0	0.087	19.1	1.8	3.3
3	3.7	0.64	13.9	1.9	3.4	0.105	8.2	4.7	4.0
4	2.7	0.78	13.6	1.4	2.2	0.113	18.1	5.3	3.2
5	4.1	0.28	12.9	0.6	4.5	0.099	5.0	2.5	< 4.2 ¶
6	2.9	0.19	26.8	0.7	2.7	0.059	6.6	2.6	2.8
7	3.9	0.65	27.0	1.8	3.5	0.069	20.6	4.0	4.0
8	3.5	0.35	18.4	1.4	3.0	0.079	11.3	4.3	< 3.9
9	5.1	0.46	48.3	2.6	5.0	0.114	24.3	7.9	6.0
10	2.0	0.55	14.2	2.0	2.1	0.140	78.2	6.9	2.6
11	2.0	0.32	2.6	3.4	3.2	0.061	5.0	3.8	< 1.8
12	2.5	0.56	3.1	2.2	3.4	0.057	4.0	3.1	2.2
13	4.0	0.38	10.9	2.3	3.8	0.094	7.3	5.1	3.9
14	2.1	0.24	5.5	2.1	3.2	0.045	4.4	3.3	< 2.3
mean	3.3	0.45	17.2	1.9	3.4	0.086	16.3	4.3	3.5
SD	1.0	0.18	12.5	0.7	0.8	0.028	19.9	1.7	1.2
Group B: Patients with first TIMI score 3 or 2, without ST-VM recovery									
15	4.8	0.33	7.6	9.8	4.9	0.052	7	7.1	–
16	3.6	0.29	42.7	4.6	2.6	0.081	18.4	7.6	–
17	2.3	0.48	13.9	1.9	2.5	0.116	11.3	6.2	–
Group C: Patients with first TIMI-score 0 or 1, and later ST-VM recovery									
18	1.6	0.23	4.4	4.2	2.1	0.047	2.8	6.8	1.8
19	5.2	0.44	51.1	3.2	5.3	0.108	16.4	7.2	5.4
Group D: Patients with first TIMI score 0 or 1, and without ST-VM recovery									
20	3.4	0.04	13.3	7.2	7.1	0.019	11.9	5.3	–
21	2.5	0.31	17.7	3.8	2.5	0.098	5.4	6.3	–
22	3.6	0.36	45.0	6.0	3.2	0.072	33.0	5.8	–
23	5.5	0.37	10.6	11.7	5.0	0.064	7.2	8.0	–
24	2.4	0.38	14.9	4.4	2.8	0.094	5.2	5.5	–
25	4.7	0.01	8114.4	9.2	7.2	0.041	20.3	9.2	–
26	3.1	0.26	10.1	3.7	3.5	0.052	7.2	1.6	–
Mean	3.6	–	–	6.6	4.5	0.063	12.9	6.0	–
s.d.	1.1	–	–	3.0	2.0	0.028	10.3	2.4	–

* t_s = start of protein release in hours after first symptoms.
† *FWR* = fractional washout rate constant for transport from interstitium to plasma.
‡ Q_{tot} = total (cumulative) protein release.
§ Res = mean square root deviation per data point, expressed as percentage of the mean.
¶ Possible overestimation, because of ST-recovery from the start of recording.

3. Results

Data were obtained from 37 patients. Of these patients, 8 patients were excluded because of gaps exceeding 60 min in the first 6 h of ECG recording, mostly because the patients were not promptly reconnected to the MIDA recorder after return from catheterisation to the CCU. One patient was excluded because of missing early plasma samples and 2 more patients because of a secondary rise in plasma protein levels. Table 1 presents baseline data on the remaining 26 patients. Treatment delay and delays until angiographic assessment of TIMI scores are indicated. Although scheduled at 90 min, the initial TIMI perfusion scores were obtained between 42 (minimum) and 180 (maximum) min after initiation of therapy.

ST-VM recovery occurred in 14 out of 17 patients with a 90-min TIMI score of 3 or 2 (see Tables 1 and 2). In these 14 patients, ST-VM recovery occurred consistently either before or during catheterisation. In the other 3 patients (15, 16 and 17) with 90-min TIMI scores 3 or 2, no subsequent ST-VM recovery was observed, which suggests that recanalisation was achieved before continuous ECG recording was started. Indeed, in patients 16 and 17 the ECG recording taken at entry to hospital showed considerably higher ST-elevations than the subsequent MIDA recordings (500 vs. 76 μV and 800 vs. 194 μV, respectively), while patient 15 had only limited ST-segment elevation in both ECG recordings (200 vs. 135 μV).

The temporal relations between ECG parameters and protein release are illustrated in Fig. 1 for patient 3 from Table 1. This patient had average residues for the fits on Mb and CK in group A, as shown in Table 2. Rapid ST-VM recovery, starting 4 h after first symptoms, coincided with QRS-VD development and the onset of protein release. Application of the model fit to this patient revealed onset of Mb and CK release at 3.7 and 3.4 h, respectively (see Table 2). Release of CK proceeded relatively slowly, with $FWR = 0.105 \cdot h^{-1}$, corresponding to a half-life time of washout $t_{1/2} = (\ln 2)/FWR = 6.6$ h (see Table 2). Release of Mb was more rapid, with $t_{1/2} = (\ln 2)/0.64 = 1.1$ h, and lagged only 1–2 h behind QRS-VD increase.

Results of the model fits are presented in Table 2. Patients were divided into 4 groups, according to decreasing probability of reperfusion. Group A consisted of 14 patients with angiographic and electrocardiographic evidence of early reperfusion (i.e., a 90-min TIMI score of 3 or 2 and previous or simultaneous ST-VM recovery). Group B also had 90-min TIMI scores 3 or 2, but showed no ST-VM recovery. In these patients reperfusion probably occurred prior to the start of continuous ECG recording, as discussed. Group C consisted of 2 patients with ST-VM recovery who had 90-min TIMI scores of 0 or 1. One

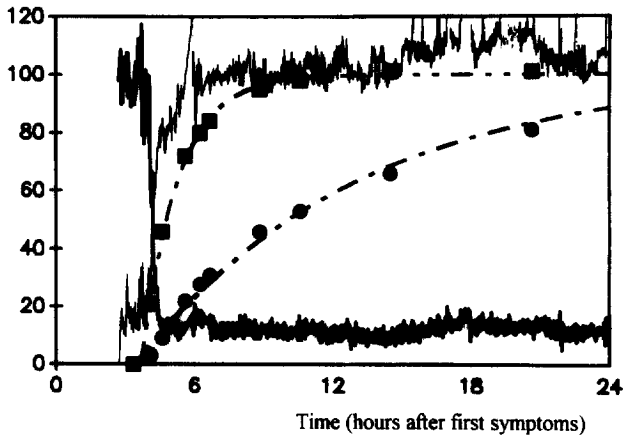


Fig. 1. ST-VM normalization (decreasing bold signal) and QRS-VD increase (increasing signal) in patient 3. The start of ST-VM normalisation, at 4 h after first symptoms, coincides with the onset of release of Mb (■) and CK (●). Broken lines through these symbols present the results of fitting. Values are expressed as percentage of reference values: i.e., the ST-VM value at the start of normalisation (410 μV), the QRS-VD value after 12 h (2300 μV·s), and the total release values Q_{tot} obtained from fitting (13.9 mg/l for Mb and 8.2 U/ml for CK).

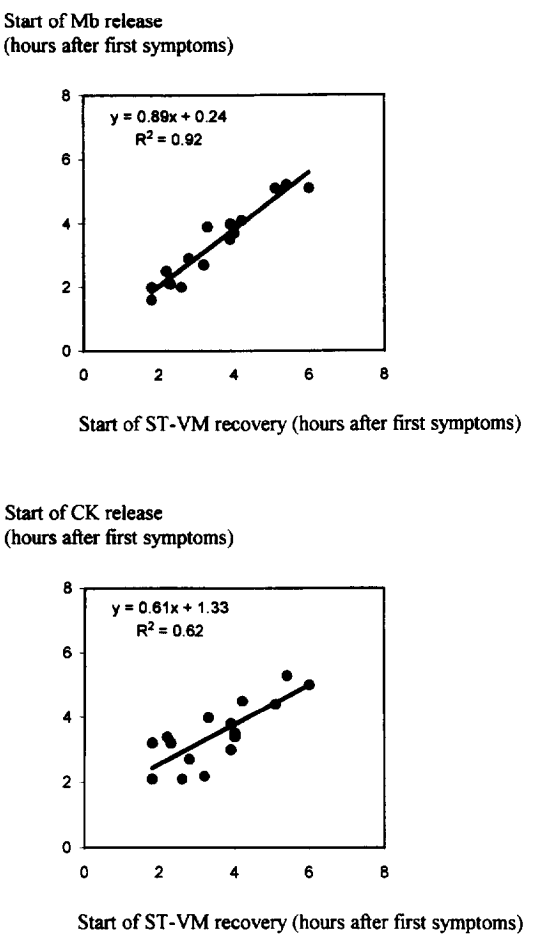
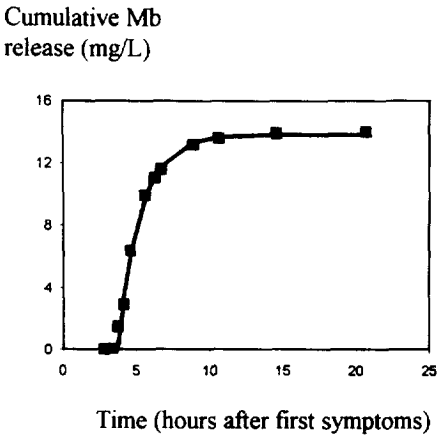


Fig. 2. Correlations between the start of protein release into plasma and the start of ST-VM recovery in 16 patients with ST-VM recovery (groups A and C). Linear regression equations and squared correlation coefficients are also shown.

patient showed ST-VM recovery and sudden protein release at the start of thrombolytic therapy, while he had an occluded artery at 84 min after initiation of therapy, probably indicating re-occlusion after early reperfusion. The other patient had ST-VM recovery shortly after angiography, probably reflecting late reperfusion. Group D had no ST-VM recovery and 90-min TIMI scores of 0 or 1, so recanalisation did probably not occur in these patients within 24 h. However, only 2 of these patients (nos. 23 and 25) also had an occluded artery at second angiography.

All patients with ST-VM recovery (groups A and C) had good model fits with residues of less than 5% for Mb and less than 8% for CK. The somewhat larger residues for CK are probably related to the longer period of fitting (48 instead of 24 h for Mb). This longer time interval was required because of the slow washout of CK, as shown in Figs. 1 and 5, but also increased the risk of error in calculated cumulative release due to changing parameter values. In these 16 patients, mean times of onset of protein release ($t_s = 3.4$ h for Mb and $t_s = 3.5$ h for CK) agreed well with the mean time of 3.5 h for the onset of ST-VM normalisation. Moreover, in individual patients there was a good correlation between these data, as shown in Fig. 2



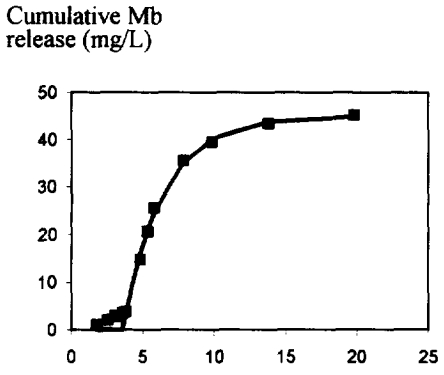


Fig. 3. Cumulative Mb release (symbols) and obtained fits (continuous lines) for two representative patients with residues approximating the average residues in the reperfused group (patient 3 from group A; upper panel) respectively in the non-reperfused group (patient 22 from group D; lower panel).

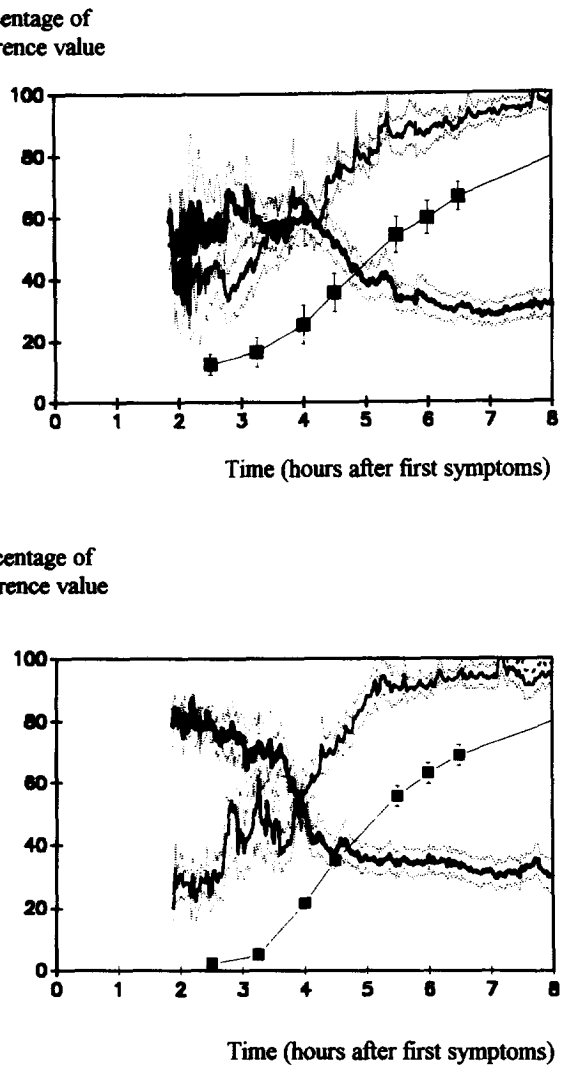


Fig. 4. Effect of synchronising data to the start of Mb release. Upper panel shows average ST-VM (decreasing bold signal), QRS-VD (increasing signal) and cumulative release of Mb (■), for 16 patients (groups A and C). Lower panel shows the same averages after a time shift in each individual patient such that the moments of onset of Mb release coincide. Values were expressed as percentage of reference values as in Fig. 1. Standard errors of the mean are also indicated.

(Mb, $r = 0.96$; CK, $r = 0.79$). An example of the fit obtained for Mb in this group, for patient 3 who had average residues, is shown in the upper part of Fig. 3.

Patients from group D, with persistent occlusion, had poor fits for Mb with residues significantly larger ($P = 0.0003$) than the residues of group A, and unrealistic, outlying, parameter values for patients 20 and 25. Moreover, as shown in the lower part of Fig. 3 for patient 22, who had an average residue for this group, the release rate of Mb initially deviated systematically from the fitted curve because it increased gradually. Apparently, the basic assumption of sudden and complete interstitial release was not valid.

Due to different times of recanalisation in different patients, the sudden resulting change in ST-VM, QRS-VD

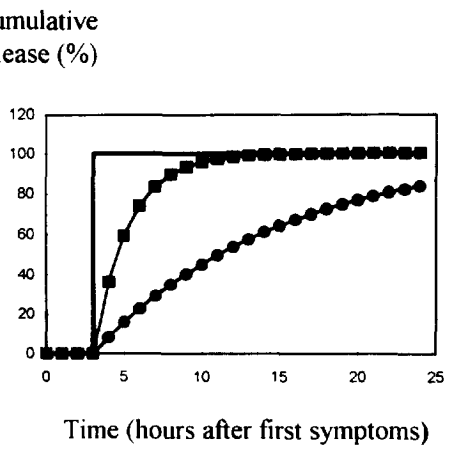


Fig. 5. Model functions for cumulative protein release, expressed as a percentage of total release. The continuous curve shows instantaneous and complete *interstitial* protein release, assumed to occur 3 h after first symptoms. The resulting cumulative release *into plasma* of Mb and CK, indicated by squares and circles respectively, lags behind.

will be generally obscured in averaged plots. This is illustrated in Fig. 4. The upper panel shows the result for simple averaging of all 16 patients from groups A and C. The lower panel shows the results when, before averaging, individual data were shifted in time such that the moment of onset of Mb release (t_s in Table 2) coincided at the mean value of 3.4 h. The upper panel in Fig. 4 shows a seemingly gradual onset of Mb release, ST-VM recovery and QRS-VD development, during more than 3 h. The lower panel is compatible with observations in individual patients, with a sharp start of Mb release and ST- and QRS-vector changes completed within 1.5–2 h.

Longer reperfusion delays could increase the amount of protein liberated from the cardiomyocytes and thus cause higher initial release rates into plasma. In order to test this hypothesis, the relation between t_s and the product $FWR \cdot Q_{tot}$ was studied for group A in Table 2 ($FWR \cdot Q_{tot}$ equals the initial protein release rate as can be verified by time-differentiation of Eq. (3) from Section 2). Indeed, a significant ($P = 0.028$) correlation with $r = 0.59$ was found for Mb, but not for CK.

Time shifts between interstitial protein release and release into plasma are shown in Fig. 5 for patients of group A. Using the mean *FWR* values for group A from Table 2, an arbitrary value of $t_s = 3$ h, and $Q_{tot} = 100\%$, cumulative release was calculated from Eq. (3) in Section 2. Complete and instantaneous interstitial release at $t = 3$ h is followed by relatively rapid release into plasma of Mb, completed within 10–12 h. In contrast, the release of CK, although starting at the same time as the release of Mb, still continues after 24 h.

4. Discussion

It has been demonstrated in large, randomised trials that recanalisation of the infarct-related artery after AMI is associated with more rapid ST-VM recovery, faster Q-wave evolution, smaller infarct size and accelerated cardiac enzyme release [13,14]. Originally it was assumed that such accelerated release reflected enhanced washout of enzymes from the heart, as a result of restoration of blood flow to the ischemic area. After experimental demonstration of 'reperfusion injury', however, it was appreciated that it could also reflect acute, reperfusion-induced, cellular release of proteins [6,15]. As it is important to understand the possible role of reperfusion injury in the clinical setting of recanalisation therapy after AMI, the present study aimed to shed more light on this controversy.

Patency of the infarct-related artery after attempted thrombolysis was documented by 90 min coronary angiography, the universally accepted gold standard. The exact moment of reperfusion was estimated from the onset of rapid ST-VM recovery, assessed by continuous vectorcardiography. The working hypothesis was: If reperfusion at time t_s results in acute and massive liberation of cytosolic myocyte proteins into the interstitial space, protein release into plasma should suddenly start at t_s with a maximal release rate f_{max}. A model function describing such behaviour was fitted to observed protein release. Evidence for the validity of this approach is discussed below.

4.1. Temporal relations between ECG, coronary flow and myocardial protein release

A close temporal relation between changes in ST-segment elevation and coronary occlusion or reperfusion has been demonstrated in several earlier studies [16–20]. Recent Holter studies and continuous ECG-recordings have confirmed these results and have demonstrated that phasic changes in ST-elevation were associated with cyclic flow changes [21–23].

In patients with AMI not treated with thrombolytic therapy, a close temporal agreement was reported between the duration of Mb release, completed in 16 ± 7 h (mean $\pm$ s.d.), and QRS-changes as well as ST-VM recovery, completed in 14 ± 5 and 11 ± 5 h, respectively [24]. Much more rapid completion was found after thrombolytic therapy [25]. Mb release was completed in 5.5 ± 3.3 h, QRS-changes in 4.4 ± 2.5 h, and ST-VM recovery in 2.9 ± 2 h. Coronary patency was not documented. Quantitative agreement between infarct size estimated from Mb release and infarct size estimated from ST- as well as QRS-changes was also reported [26].

4.2. Transport of muscle cell proteins from heart to plasma

In the dog, as well as in man, proteins released from cardiac myocytes into the interstitial space are mainly transported to the bloodstream by direct intravasation into the microvessels. Only a minor fraction is transported by cardiac lymph [27–29]. Due to a minimal remaining blood flow in the ischemic area, such protein transport is not

flow-limited. A residual collateral flow of only 1% of normal blood flow, for instance, will flush the microvessels within minutes [30]. This explains why proteins infused into ischemic and normally perfused areas of the left ventricular wall appeared equally fast in plasma [31].

The rate-limiting factor in protein intravasation is the vascular permeability barrier [12,27]. A small (18 kDa) protein like Mb passes relatively fast, as confirmed by the half-life time for washout of only 1.1 h found in the present study. This implies that the duration of Mb release will indeed approximately reflect the duration of interstitial protein release. A large (81 kDa) molecule like CK passes much more slowly, as confirmed by the half-life time for CK washout of 6.6 h. So, CK release into plasma will last much longer than interstitial release. For small as well as large proteins, however, the diffusional transport across the vascular wall implies that the rate of protein release into the circulation will at all times directly reflect the interstitial protein concentration.

4.3. Protein release kinetics in non-reperfused patients with AMI

In patients with AMI who were not treated with recanalisation therapy, the release rate of cardiac proteins like CK into plasma (i.e., the function $f(t)$ in Section 2) could be closely approximated by $f(t) = C_0\ t^2.\exp(-2t/t_m)$ with $t_m \approx 20$ h [10]. This is an increasing function with a maximal value at 20 h, which at 3–5 h after first symptoms—the time at which therapeutic recanalisation normally occurs—still has a relatively low value. As discussed in the preceding section, this implies that interstitial protein concentrations must also be maximal after about 20 h and are still low at the time at which therapeutic recanalisation usually occurs. This eliminates the possibility that the maximal release rate observed directly after reperfusion would reflect accumulation of protein during the period before reperfusion.

4.4. Protein release kinetics in reperfused patients with AMI

For all patients with sudden ST-VM recovery (groups A and C from Table 2) the onset of ST-VM recovery and QRS-VD normalisation coincided with abrupt onset of protein release into plasma, with a maximal initial release rate. This finding is shown in Fig. 1 for a representative individual patient and in Fig. 2 for all patients of groups A and C. As discussed in the preceding sections, the rate of protein release into plasma is proportional to interstitial protein concentration and this remains true after reperfusion, although the proportionality constant may have changed [32]. So, the interstitial protein concentration must also have become maximal immediately after reperfusion. Together with the earlier mentioned data, this implies that in the present study, reperfusion resulted in acute and massive interstitial protein release from cardiac myocytes.

Although it has been documented that incomplete perfusion (TIMI 2 flow) is functionally inferior to complete (TIMI 3) flow [33], patients with TIMI 3 ($n = 10$) and TIMI 2 ($n = 4$) scores in group A were pooled since no significant differences were found for the model parameters and for the quality of fits. For the fits on Mb, for instance, mean residues were 1.9% in both groups. This suggests that sudden interstitial protein release after reperfusion occurs for both completely (TIMI 3) and partially (TIMI 2) reperfused patients.

4.5. Limitations of the present study

The time definition obtained in the present study for protein release was determined by the sample frequency of about 45 min between successive initial plasma samples. This implies that onset of release indicated as 'acute' or 'abrupt' may in fact have taken 15 to 30 min to develop.

Abrupt and massive disruption of myocyte membranes after reperfusion does not prove the existence of 'reperfusion injury' in a strict sense. The cells liberating their proteins could already have passed their 'point of no return' before the time of reperfusion. Reperfusion would then only demonstrate damage already done, and no myocytes could be saved by any intervention performed at the time of reperfusion. However, attenuation of acute cellular injury after reperfusion has been claimed for clinical as well as experimental interventions [34,35]. Although, as mentioned in Section 1, even the very existence of reperfusion injury remains controversial, these results offer at least some hope for further improvement of the benefit of reperfusion therapy.

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