Activities of Some Enzymes in Serum after Therapy with Intracoronary Streptokinase in Acute Myocardial Infarction

Tai C. Kwong, Patricia G. Fitzpatrick, and Robert L. Rothbard

Enzyme kinetics for creatine kinase (CK), CK-MB, aspartate aminotransferase (AST), and lactate dehydrogenase (LD) in serum were followed in 14 patients who had suffered acute myocardial infarction and who were given intracoronary streptokinase shortly (mean 4.9 h, SD 2.6 h) after onset of symptoms. In the 10 patients for whom thrombolysis was successful, CK activity peaked earlier (12.8 vs 21.6 h) and at higher values (3548 vs 2436 U/L) than in the four patients for whom the treatment was unsuccessful. The mean maximum rate of increase in CK was threefold greater in the former group (574 vs 169 U/L per hour), but the total amount of CK released into the circulation and the fractional disappearance rates were similar for both groups. The profiles for AST and CK-MB for successfully treated patients closely resembled those for CK. LD, however, peaked significantly later than CK (25.7 vs 12.8 h). Early peaking of CK or CK-MB after nonsurgical reperfusion can be potentially useful as a noninvasive in vitro index to the success of therapy of myocardial infarction with thrombolytic agents.

Additional Keyphrases: creatine kinase • aspartate aminotransferase • lactate dehydrogenase • isoenzymes • monitoring therapy

Extensive ischemic injury to the myocardium is a major cause of mortality in patients who have suffered acute myocardial infarction. Because the prognosis after infarction appears to be related to infarct size and to remaining ventricular function (1), various methods for reducing the extent and severity of myocardial damage have been tried, including attempts to decrease myocardial oxygen demand, increase oxygen supply by emergency bypass surgery, and (most recently) attain reperfusion (i.e., patency) of the occluded artery by infusing thrombolytic agents such as streptokinase or urokinase. Recent coronaryangiographic evidence has shown that nearly 90% of patients with acute transmural infarction who were evaluated within 4 h of the onset of symptoms had a total occlusion of the infarct-related vessel (2). Salvage of the ischemic myocardium depends critically on the duration of coronary artery occlusion, so early nonsurgical reperfusion by intracoronary infusion of streptokinase to promptly restore blood flow reportedly (3–6) results in improvement of left ventricular function, a rapid decline of elevated ST segments in the electrocardiogram, relief of pain, and a rapid release of creatine kinase (CK). In these studies successful reperfusion was documented by angiography.

Results of recent randomized trials for intravenous infusion of streptokinase have suggested that such therapy significantly decreases mortality (7); this is less invasive, less expensive, and easier to perform than intracoronary administration. Unlike intracoronary infusion procedures, however, evidence for reperfusion after intravenous administration of the lytic agent cannot be based on coronary angiography, and assessment of myocardial reperfusion by noninvasive techniques is necessary. In anticipation of a need for such an indicator, we followed the activities of some serum enzymes in 14 patients who were given intracoronary streptokinase, to see whether the early peak in enzyme activity after thrombolytic therapy could serve as an indicator of the success of thrombolysis.

Patients and Methods

Patients who were evaluated and agreed to participate in the study had to fulfill the following criteria: streptokinase therapy begun within 8 h of onset of chest pain; clinical and electrocardiographic evidence of acute transmural myocardial infarction; angiographic evidence of thrombotic occlusion of a major coronary artery or a major branch of one; and no substantial contraindication of lytic therapy such as recent major surgery or cerebral vascular accident, severe uncontrolled hypertension, or significant gastrointestinal bleeding.

Once informed consent was obtained, the patients were taken to the cardiac catheterization laboratory. After an occluded artery was evident radiologically, patients were randomized to receive either the standard intracoronary dose (10 000 U bolus followed by a 4000 U/min drip) or a low dose (1000–4000 U bolus and a 300–1000 U/min drip) of streptokinase. Serial angiograms were done every 15 min after this therapy was started. At the end of 45 min, if there was no evidence of thrombolysis, low-dose failures were switched to standard-dose therapy, and the infusion was continued for as long as 45 min. Patients randomized to standard therapy received streptokinase for at least 45 min, whereupon therapy was discontinued if there was no evidence of thrombolysis.

Baseline laboratory evaluation included a chest roentgenogram, a 12-lead electrocardiogram, standard continuous-flow serum analysis (SMA 6 and SMA 12), CK and CK-MB activities, complete blood-cell count and platelet count, prothrombin time, thrombin time, activated partial thromboplastin time, and blood fibrinogen concentration. Measurement of thrombin time and fibrinogen concentration were repeated every 15 min during the streptokinase infusion. CK, CK-MB, AST, and LD assays were repeated every 2 h during the first 24 h, then every 12 h for as long as 96 h after the start of the infarction episode.

About 24 h after therapy with streptokinase, patients were returned to the catheterization laboratory for coronary angiography, to confirm continued patency of the previously occluded artery, and ventriculography, for comparison with pre-treatment studies.

CK activity in serum was assayed with a kit involving N-
acetylcysteine activation (6) (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250). LD and AST activities were measured with kits purchased from Worthington Diagnostics (Freehold, NJ 07728). Enzyme assays were performed at 37 °C with a centrifugal analyzer (Multistat III; Instrumentation Laboratory, Lexington, MA 02173) according to the instrument settings recommended by the kit manufacturers. For electrophoresis of CK and LD isoenzymes we used cellulose acetate strips (Helena Laboratories, Beaumont, TX 77704). After densitometric scanning, isoenzyme activities were expressed as percentages of total CK or LD activity. The upper limits of the reference intervals for CK are: 59 (women), 142 (men); for AST, 36; and for LD, 225 U/L. For the proportion of CK-MB it is 4%.

We calculated the rates of increase in serum CK and CK-MB activities by linear regression analysis for each patient from the linear portion of the upward slope of the time–activity curves.

We calculated the clearance rates (fractional disappearance rates, $K_d$'s) for CK and CK-MB for each patient from the declining slopes of the time–activity curves by linear regression analysis, using a logarithmic scale for serum CK and CK-MB activity (9).

The "integrated appearance functions" of CK and CK-MB are approximations of the total amounts of enzyme released into 1 L of serum. To calculate these functions, we used the formula of Shell et al. (10): $E_t = K_d \times E_0$, where $E_t$ is the CK or CK-MB activity at time $t$ after onset of symptoms and $K_d$ is the fractional disappearance rate for CK or CK-MB.

Results

Sixteen patients met the above-mentioned criteria for intracoronary therapy with streptokinase. Two were excluded because of incomplete sample collection. Of the remaining 14 (13 men and one woman), 10 achieved reperfusion and four did not. Table 1 lists the characteristics of these 14 patients. Four of the 10 patients for whom reperfusion was attained and one of the three patients for whom it was not had above-normal activity of serum CK at the time of admission, but for none of these did the value at the time of admission exceed threefold the upper limit of the normal reference interval. Table 1 also lists the values at admission for CK, CK-MB, AST, and LD.

Serum CK activities of patients in the reperfused group were highest $12.8 \pm 5.3$ h (mean ± SD) after the onset of symptoms—9 h earlier than the $21.6 \pm 3.9$ h for those patients who did not reperfuse (Table 2). The reported (11) time for peak CK activity in serum of myocardial infarction patients treated conservatively is 20 to 30 h after onset of symptoms, similar to the mean of 21.6 h for those patients in this study for whom thrombolysis was not achieved.

The mean peak activity of CK was significantly greater in the reperfused group than in the nonreperfused patients (3548 vs 2536 U/L; $p < 0.05$). The mean maximum rate of increase of CK in the reperfused group was more than threefold that in the nonreperfused group (574 vs 169 U/L per hour; $p < 0.005$). Together, these two variables produced a CK curve for reperfused patients that resembled a spike rather than the gentle rise commonly seen in such curves for conservatively treated patients. Disappearance of CK activity from the circulation, however, appeared to be similar for both groups of patients, because the fractional disappearance rates ($K_d$'s) were not statistically different ($−0.029$ vs $−0.027$ h$^{-1}$; $p < 0.6$). The total amounts of CK released into the circulation as represented by the plateau values of the integrated appearance functions were also not significantly different (9979 vs 8653 U/L; $p < 0.5$).

The data for CK-MB activity were similar to those for CK; the isoenzyme increased at a greater rate and peaked earlier in the reperfused group, but the total amounts released and the rates of elimination were not significantly different for reperfused and nonreperfused patients (Table 2). Figure 1 shows representative CK and CK-MB curves for a reperfused patient and a nonreperfused patient.

The other enzymes followed the same trend as CK.

### Table 2. Data for Serum Enzymes

<table>
<thead>
<tr>
<th></th>
<th>Reperfusion group</th>
<th>Non-reperfusion group</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time between onset of symptoms and peak, h</td>
<td>12.8 (5.3)</td>
<td>21.6 (3.9)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CK</td>
<td>12.9 (5.3)</td>
<td>21.8 (4.9)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CK-MB</td>
<td>15.3 (7.3)</td>
<td>23.3 (4.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AST</td>
<td>25.7 (13.2)</td>
<td>42.4 (12.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LD</td>
<td>3548 (902)</td>
<td>2436 (609)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Peak acty, U/L</td>
<td>543 (170)</td>
<td>374 (131)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CK</td>
<td>574 (354)</td>
<td>169 (58)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>CK-MB</td>
<td>89 (47)</td>
<td>27 (12)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fractional disappearance rate, $K_d$, per h</td>
<td>$−0.029$ (0.011)</td>
<td>$−0.027$ (0.004)</td>
<td>NS</td>
</tr>
<tr>
<td>CK</td>
<td>$−0.039$ (0.009)</td>
<td>$−0.033$ (0.001)</td>
<td>NS</td>
</tr>
<tr>
<td>Total amount of enzyme released, U/L*</td>
<td>9979 (4152)</td>
<td>8653 (3300)</td>
<td>NS</td>
</tr>
<tr>
<td>CK-MB</td>
<td>1713 (800)</td>
<td>1352 (894)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The mean plateau value of the integrated appearance function (10).

NS, not significant.
although LD peaked significantly ($p < 0.05$) later than CK, regardless of the outcome of therapy (Table 2). AST and LD in patients who reperfused peaked earlier than in those who did not. The latter group of patients had results that agreed with values reported (11) for conservatively treated patients (20 to 30 h and 30 to 60 h to peak, respectively).

**Discussion**

The effectiveness of thrombolytic therapy to limit myocardial damage by early restoration of coronary blood flow is being intensely investigated. Coronary angiography is used in selecting for intracoronary therapy with streptokinase patients who have complete coronary occlusion, and in demonstrating the success or failure of the treatment. In assessing the efficacy of *intra venous* administration of thrombolytic agent, Schroder et al. (12) relied also on angiographic data. If intravenous therapy with streptokinase proves to be successful, the benefit of thrombolytic therapy can be extended from the small proportion of patients currently being treated with intracoronary streptokinase at specialized centers to a much larger patient base in smaller hospitals, where facilities for coronary catherization and angiography are not routinely available. Easily accessible noninvasive indexes to reperfusion will be needed; the early peaking of CK activity could serve as such a marker.

The enzyme data obtained from the 14 patients in this study demonstrated early peaking of CK in patients for whom the treatment was successful; moreover, their rates of CK increase and their peak CK values were greater. Thus, these patients demonstrated the enzyme wash-out phenomenon, in basic agreement with experimental studies on dogs (13) and reports of intracoronary streptokinase therapy in patients with transmural myocardial infarction (4, 5).

The CK peaked later in the four patients who did not reperfuse, the mean interval being similar to that reported in the literature for patients being treated in coronary care units. Our study was not a randomized trial; all patients with transmural myocardial infarction who met the criteria specified above were given streptokinase therapy. Thus no control patients were available and we used reported values for comparison. However, the means for the reperfusion group and the nonreperfusion group are clearly different, 12.8 and 21.6 h, respectively.

In some conservatively treated patients, CK peaks as early as 8 h after onset of symptoms (14), which is similar to the early CK peaks in reperfused patients after streptokinase treatment. This early peaking of CK may reflect small infarcts. In such cases the values for peak CK will be much lower than those seen in patients successfully treated with streptokinase. Alternatively, the early peak may result from spontaneous recanalization, leading to reperfusion. Ong et al. (14), in their series of 52 patients with transmural myocardial infarction, showed that 24 patients had early enzyme release (CK peaked at an average of 8.8 h) and an improved ventricular function. This lends further support to earlier angiographic evidence (2) that the proportion of patients with total coronary artery occlusion declined with time. Apparently, patients with earlier CK peaks are those who reperfuse, either by action of a thrombolytic agent or by spontaneous recanalization.

The mean rate of increase in serum CK in reperfused patients was higher than in those who did not reperfuse, which is consistent with the idea of a wash-out phenomenon. The fractional disappearance rate, as calculated from the monoexponential part of the decay curve according to the method of Norris et al. (9), showed that CK was eliminated at about the same rate in both groups of patients, which agrees with other reports (15, 16). This suggests that the different enzyme kinetics of the two groups of patients can be attributed to differences in the rates at which CK enters the circulation rather than to separate mechanisms of clearance from the circulation.

The peak values for CK were higher after successful thrombolysis, probably owing to a wash-out of enzymes after thrombolysis and reperfusion. The mean total amount of CK released into the serum (integrated appearance function) was greater among the reperfused group, but the difference is not significant ($p < 0.5$). This is in agreement with reports on patients given intracoronary streptokinase (16) and patients who reperfused spontaneously (14), but is at variance with the finding of Schwarz et al. (17). Failure to demonstrate a difference among our patients could be in part because of large variation in infarct sizes. In studies on dogs, however, the amount of CK in serum was twice as high per gram of infarcted myocardium after reperfusion, as compared with values in permanent occlusion (13). Only some 15% (18, 19) of the CK activity depleted from the myocardium appears in the circulation, owing to inactivation during transport in the lymph. The increased rate of enzyme transit into the systemic circulation during a "wash-out" could have reduced the extent of enzyme inactivation.

The ratio of the amount of CK depleted from the myocardium to the amount that appears in serum differs for reperfused and nonreperfused dogs (13); thus, the use of serial CK values and the formula developed by Sobel and associates (1, 10) for calculating infarct sizes for comparison between the two groups of patients is not valid. Calculation of infarct size in reperfused patients is also complicated by the possibilities that patients might have less than total coronary occlusion and that clot formation and lysis may be a dynamic process.

The kinetics of CK-MB and the circulation after treatment with streptokinase followed those of CK; the enzyme peak occurred earlier in reperfused patients than in conservatively treated patients. Nevertheless, the use of CK-MB rather than CK to monitor patients on thrombolytic therapy would be more specific because the problem of the contribution of nonmyocardial CK is minimized. Quantification of CK-MB activity, however, is analytically more demanding, so this test is not as readily available as CK.

The profiles of AST and LD are similar to those of CK. Thus, enzyme wash-out appears to be a general phenomenon for cardiac enzymes. CK, however, is the preferred enzyme marker. The release of liver AST, the result of poor hepatic perfusion after myocardial infarction, makes it a less specific marker. LD is similarly nonspecific. Furthermore, the later peaking of LD (25 h) is also a disadvantage.

Our data suggest that the early peaking of enzymes in reperfused patients may be sufficiently distinct from that of nonreperfused patients that it can be used as a marker for thrombolysis. In intravenous thrombolytic therapy, a similar early enzyme peak could serve as a noninvasive alternative to post-treatment angiography for monitoring success of therapy.

**References**


**Corrections**

**Vol. 29**

p 642: In column two, sixth line from the bottom, change "25 000" to "54 000." Insert an addition (italicized here) to the next to last line in that column so that it reads ". . . , then dialyzed overnight against the same buffer at 4°C, then divided . . .

p 843: In line six under Results and Discussion, change "mg" to "μg".

p 1537: In revision of this paper a paragraph of introduction was inadvertently omitted (which cites refs. 1–5), as were the first two paragraphs under Materials and Methods, which should read as follows:

Human placentas were obtained from the obstetrical department within 12 h of delivery. Fresh human abdominal muscle was obtained within 12 h of autopsy. All tissues were stored at 4°C and processed, as described below, within 6 h.

Placenta and abdominal muscle were stripped free of membranes and blood vessels, cut into small pieces, and extensively washed at 4°C with cold saline (NaCl, 9 g/L). Muscle CK-MM and placental CK-BB were purified according to the method of Armstrong et al. (6), with the following modifications: we used dithioerythritol (Sigma Chemical Co., St. Louis, MO 63178), 15 mmol/L, instead of 2-mercaptoethanol, omitted the final (NH4)2SO4 precipitation and final Sephadex G-100 fractionation, and used DE-52 (Whatman Ltd., Maidstone, Kent, U.K.) instead of DEAE-cellulose (Cellex-D; Bio-Rad Laboratories, Richmond, CA 94804).

p 2026: First paragraph, lines nine and 16: delete "500" (the Dextran used in the cited reference was a 50 000-dalton preparation, not the 500 000 Da Pharmacia preparation).

p 2029: In line 10 of the first full paragraph in column two, change "1.171" to "0.117."

p 2125: First two columns and Table 1: All lipase results expressed as kU/L should be divided by 3600.

**Vol. 30**

p 170: The next-to-last paragraph was inadvertently omitted in layout and should read as follows:

If the sample was first incubated at 37°C until the serum became clear (i.e., until the cryoprecipitate dissolved) and the enzyme activity immediately determined at 30°C (Figure 2, curves 1 and 2), normal activity was observed.