Creatine kinase isoform analysis in the detection and assessment of thrombolysis in man

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ABSTRACT Recent demonstrations of the efficacy of intravenous thrombolytic therapy in acute myocardial infarction have emphasized the need for a noninvasive index of successful reperfusion. The tissue form of MM creatine kinase (MM3) is known to undergo posttranslational conversion to modified forms MM2 and MM1 after release into the plasma following acute infarction. Since this conversion is rapid, sustained elevation of plasma MM3 may be a marker of the prolonged creatine kinase release characteristic of nonreperfused infarction. Therefore, we investigated the rate of decline of plasma MM3 in a consecutive series of patients undergoing thrombolytic therapy of acute myocardial infarction, all of whom underwent acute angiography to assess treatment success, as well as in 30 conventionally treated patients. Among 55 patients with angiographically documented successful reperfusion (group I A), the rate of decline of MM3 was 4.18 ± 1.25%/hr (mean ± SD); in contrast, the rate of decline was 2.37 ± 1.11%/hr in 39 patients with angiographically documented unsuccessful reperfusion (group IB) and 1.77 ± 1.46%/hr among the 30 patients receiving conventional treatment (group II) (p < .001 for groups IB and II vs group IA). A cutoff value of 3.1%/hr minimized the overlap between the groups; 48/55 (87%) patients with successful reperfusion had a rate of decline of MM3 of 3.1%/hr or more, while 29 of 39 (74%) patients in whom thrombolysis was unsuccessful and 27 of 30 (90%) patients receiving conventional treatment had a rate of decline less than 3.1%/hr (p < .001 for groups IB and II vs group IA). In contrast, the time from onset of symptoms to peak MB–creatine kinase, a commonly used marker of reperfusion, exhibited substantial overlap between groups. We conclude that creatine kinase isoform analysis may provide an early noninvasive index of successful reperfusion.


RECENT STUDIES suggest thrombolytic therapy in patients with acute myocardial infarction (AMI) is likely to result in salvage of ischemic myocardium1-5 and reduction in mortality.4,5 If treatment is initiated within 4 hr of onset of symptoms,1-4,6 Intravenous therapy, which has now been shown to be effective,7,8 eliminates the unavoidable delay associated with cardiac catheterization and has rapidly become the preferred route over intracoronary drug administration. However, early documentation of vessel patency remains an important consideration, in part to define subsets of patients who may be at risk for reocclusion and therefore considered for further therapy.9 A number of noninvasive markers have been shown to be crude indicators of thrombolysis, including arrhythmias, relief of pain, resolution of ST segment elevation, and time from onset of symptoms to peak plasma MB–creatine kinase (CK) (EC 2.7.3.2).10 However, none of these have been demonstrated to reliably reflect successful thrombolysis.

In our studies on enzymatic assessment of reperfusion, it became apparent that the newly described plasma isoforms of the MM-CK isoenzyme should offer an advantage over analysis of plasma total CK or MB-CK activity. The existence of the three isoforms of the MM-CK isoenzyme in the plasma of patients after AMI was first noted by Wevers et al.11 The same investigators also demonstrated that the isofrom present in tissue, referred to as MM3, is the predominant form in plasma in the early hours after AMI.12 We subsequently purified the separate isoforms of MM-CK and showed that MM3, the only isoform present in tissue,
is sequentially converted to MM2 and MM1 in plasma after release from necrotic tissue, and that a similar conversion is observed after exposure of purified tissue MM-CK to plasma in vitro. We also showed the conversion to be mediated by the sequential cleavage of the amino acid lysine from the carboxyterminus of each subunit of the MM isoenzyme by the plasma enzyme carboxypeptidase-N (CP-N). These findings have been confirmed by others. Taking advantage of the earlier release and disappearance of the tissue isoform of MM-CK with successful early reperfusion compared with its release and disappearance with absent or late reperfusion, we explored this as a possible noninvasive means to detect early thrombolysis. A simple convenient technique was developed for detection and assessment of the plasma isoforms based on agarose electrophoresis. Analyses were performed on serial plasma specimens from 103 patients with AMI undergoing thrombolytic therapy in whom coronary angiography was performed before and after initiating therapy, and on plasma samples from 30 patients with AMI treated conventionally.

Materials and methods

Population. The study population consisted of 103 consecutive patients with AMI who underwent acute coronary angiography for thrombolytic therapy at either The Methodist Hospital, Ben Taub General Hospital, or the Veterans Administration Medical Center in Houston (group I) and 30 patients with AMI who were treated conventionally (group II). Diagnosis of AMI was based on a history of recent chest pain lasting at least 30 min together with the presence of a characteristic rise and fall of plasma total and MB-CK. All patients in group I underwent angiography and initiation of treatment no later than 7 hr after the onset of symptoms. Angiography was repeated 90 min after the start of drug infusion or immediately after percutaneous transluminal coronary angioplasty (PTCA) to document success of therapy. Treatments used in group I included intravenous streptokinase (15 patients), intracoronary streptokinase (21 patients), intravenous tissue plasminogen activator (47 patients), and PTCA used alone (12 patients) and in combination with a thrombolytic agent (16 patients). Eight patients in group I were found to have an incompletely occluded infarct-related artery and did not receive thrombolytic therapy. Plasma samples were drawn every 4 hr for 48 hr for total CK, MB-CK, and MM-CK isoform analysis.

Patients were excluded from analysis if they had three or more episodes of ventricular fibrillation requiring cardioversion (seven patients), or if adequate samples for CK analysis were not obtained within the first 18 hr after the onset of symptoms (two patients).

Sample collection and storage. Plasma CK samples were collected in vacuum tubes pretreated with EGTA to give a final concentration of approximately 50 mM. EGTA inhibits CP-N, thus preventing conversion of the isoforms in vitro. Samples were then centrifuged for 5 min at 1500 rpm and β-mercaptoethanol (BME) (5 mM) was added to the plasma. Samples were stored at −20°C.

Total CK and MB-CK assay. Plasma total CK activity was spectrophotometrically assayed at 37°C according to the technique of Rosalki with an Electroneuclones microcentrifugal analyzer and Calbiochem Behring fluorometric reagents. Plasma MB-CK determinations were performed by the batch adsorption technique of Henry et al.

MM-CK isoform analysis. For MM-CK isoform analysis, plasma samples were diluted to a total CK activity of 150 IU/liter with 10 mM Tris HCl buffer containing 2 mM BME, 50 mM EGTA, and 0.2% bovine serum albumin (pH 7.4). One microliter of sample was then applied to each well of a Corning thin-layer 1% agarose gel. Electrophoresis buffer was constituted consisting of 97% (vol/vol) 50 mM Tris barbital buffer, pH 9.15, and 3% (vol/vol) Polybuffer 96 (Pharmacia). Electrophoresis was performed for 90 min at 150 V and 4°C. Gels were then overlaid with 500 μl of Corning Cardiotrac-CK reagent, incubated for 5 min at 37°C and dried for 15 min at 60°C. Dried gels were scanned on a Corning 710 Fluorometer/densitometer for MM3, MM2, and MM1 fluorescence and peak integration was edited manually. Isoform activity was expressed as a relative percent of total MM-CK activity. Only gels that produced scans with minimal background fluorescence were used for analysis.

Validation of the isoform assay. MM-CK was purified from human skeletal muscle as previously described. Tissue MM-CK was shown by chromatofocusing to consist only of MM3 by absence of any contaminating bands on agarose electrophoresis and presence of a single MM-CK peak with pI = 7.58. One thousand international units of purified MM3 was then completely converted to MM1 by incubation with 5 U of carboxypeptidase-B (CP-B) at 37°C for 180 min. Completion of conversion was documented by the absence of MM3 or MM2 bands on agarose electrophoresis and presence of a single MM-CK peak with pI = 7.30 by chromatofocusing. Purified MM3 and MM1, having activities ranging from 0 to 280 IU/liter, were then combined in known ratios in the presence of 50 mM EGTA and agarose electrophoresis and densitometry performed immediately as described above. A separate set of isoform assays was performed to determine whether conversion of MM3 to MM2 and MM1 in the presence of 5 U CP-B was completely inhibited by 50 mM EGTA. Enzymatically detected bands on agarose gels were shown to be due to CK activity by omission of phosphocreatine from the incubation reagents.

Plasma kinetics of MM-CK isoforms. The relative percent of MM-CK present as the MM3 isoform over time after the onset of symptoms was determined and plotted for each patient. The rate of decline in the percent MM3 (the slope of the %MM3 vs time plot) was calculated based on the sample exhibiting the peak percent MM3 value (usually the first or second sample) and all subsequent samples up to 18 hr after the onset of symptoms.

Angiography. All group I patients underwent coronary angiography by the Judkins or the Sones technique both before the initiation of thrombolytic therapy and immediately after treatment (at 90 min after the start of infusion or immediately after PTCA). Coronary angiograms were interpreted by independent observers blinded to the results of isoform analysis. The patency of the infarct-related artery was classified by use of a four-point scale with a grade of 0 representing complete occlusion; grade 1, a subtotal occlusion with incomplete anastomotic opacification of the distal vessel; grade 2, complete opacification of the vessel proximally and distally but delayed clearance of dye distal to the occlusion relative to the unobstructed vessels; and grade 3, normal antegrade flow. Successful reperfusion was defined as a change from grade 0 or 1 to grade 2 or 3 after intervention. All other outcomes were regarded as unsuccessful. Patients in group I were subdivided into those in whom there was angiographically documented successful reperfusion (group IA) and those in whom reperfusion was unsuccessful (group IB).

Statistical analysis. The rate of decline of percent MM3
(slope) was derived for each patient from the best-fit line to the plasma percent MM3-CK values obtained within 18 hr of the onset of symptoms as outlined above by linear regression analysis with the least squares method. Intergroup comparisons of MM3 slope and time to peak MB-CK were made by a two-tailed t test. Statistical significance of differences in the number of patients in each group whose values fell above or below a slope of 3.1%/hr was determined by chi square analysis.

**Results**

**Isoform assay.** Four hundred six determinations of MM1 and MM3 were made; densitometric values for MM1 and MM3 correlated closely with known ratios. As shown in figure 1, the linear regression line for MM3 was $y = 0.99x + 0.002$ ($r = .99$; SEE = 0.05). The corresponding plot for MM1 also closely approximated the line of identity: $y = 0.99x + 0.003$ ($r = .99$; SEE = 0.05). Interobserver and intraobserver error for manual editing of isofrom peaks were 0.76 ± 0.49% and 0.59 ± 0.68% (mean ± SD), respectively (80 determinations each).

EGTA completely inhibited the conversion of MM3 to MM2 and MM1 by CP-B.

**MM3 slope and reperfusion.** Agarose gels and graphs of the change in percent MM3 after the onset of chest pain in typical patients with and without reperfusion are shown in figures 2 and 3. The rate of decline in MM3 and appearance of the modified isofroms were very gradual in the patient in group IA. In contrast, plasma samples from the patient in group IA exhibit a rapid decline in MM3, as reflected by a slope of −5.0%/hr.

The decline in %MM3 was more rapid in group IA than in groups IB or II. The rate of decline of %MM3 in the first 18 hr after the onset of symptoms was

FIGURE 1. Validation of densitometric analysis. Purified MM1 and MM3 having CK activities ranging from 0 to 280 IU/liter were combined in varying ratios so that MM3 activity made up 0%, 12.5%, 25%, 33%, 40%, 50%, 60%, 67%, 75%, 87.5%, or 100% of the total MM-CK activity. Electrophoresis and densitometric scanning were then performed as described in the Methods section. Mean and SD values obtained by densitometry for each known ratio are shown. The experimental line closely approximates the line of identity.

FIGURE 2. Agarose gels performed on plasma samples drawn 8, 12, 16, and 20 hr after the onset of symptoms in representative patients who underwent angiographically documented successful (A) and unsuccessful (B) reperfusion.

$-4.18 ± 1.25\%$/hr (mean ± SD) in group IA, compared with only $-2.37 ± 1.11\%$/hr in group IB ($p < .001$) and $-1.77 ± 1.46\%$/hr in group II ($p < .001$) (figure 4). A value of $-3.1\%$/hr was selected to mini-
mize overlap between group IA and groups IB and II. By this standard, 48 of 55 patients in group IA were correctly identified to have a slope greater than the cutoff (sensitivity 87%) while 29 of 39 patients in group IB had a slope less than 3.1%/hr (specificity 74%) (p < .001). In addition 27 of 30 (90%) patients in group II exhibited a slope below 3.1 (table 1).

Among patients whose acute angiographic status was documented (group I), 83% (48/58) of the patients with a slope of 3.1%/hr or more had undergone successful thrombolysis, while in 81% (29/39) of the patients with a slope less than 3.1%/hr thrombolysis was unsuccessful (table 1).

Among group I patients comparisons were made between those whose initial angiography revealed complete occlusion of the infarct-related vessel (grade 0) and those whose arteries were incompletely occluded before treatment (grades 1 to 3; table 2). The mean slopes for groups IA and IB patients subgrouped by pretreatment grade of stenosis did not differ. The percent of patients in groups IA and IB with a slope of 3.1%/hr or more (or <3.1%/hr) was also similar in the subgroups of patients in whom initial angiography showed complete and incomplete stenosis. The percent of group I patients with slope of 3.1%/hr or more in whom reperfusion was successful and the percent of group I patients with a slope less than 3.1%/hr in whom it was not likewise was similar in these patients regardless of the baseline vessel patency.

Because group I patients with an initial angiographic grade of 2 or 3 could not, by definition, undergo "successful reperfusion," the data in table 1 were reanalyzed with all such patients (n = 16) excluded. All intergroup comparisons remained significant at the same p values. Although these 16 patients with patent infarct-related arteries at the time of initial angiography may have undergone spontaneous clot lysis, the rate of decline in MM3 was similar in these patients and those with complete occlusion in whom reperfusion therapy was unsuccessful; the mean slope for this subgroup was −2.31 ± 1.19%/hr and 12 of 16 patients had a rate of decline less than 3.1%/hr.

Table 2 also compares patients with Q wave infarction and those with non–Q wave infarction. No differences in mean slope for groups IA and IB, percent of patients in group IA or IB above or below the cutoff, or percent of patients with a slope of 3.1%/hr or more who underwent successful reperfusion were noted in the subgroups with Q wave and non–Q wave infarction.

In contrast to MM-CK isoenzyme analysis, the time from onset of symptoms to peak plasma MB-CK, a commonly used noninvasive index of reperfusion, differed between the two groups but exhibited substantial overlap between group IA and groups IB and II (figure 5). The maximum overall accuracy was obtained with a cutoff time of 17 hr; 45 of 55 successfully reperfused patients had a shorter time to peak MB (sensitivity 82%) and 19 of 39 nonreperfused patients had a longer time to peak MB-CK (specificity 49%). Thus, the overall diagnostic accuracy was 68%.

Discussion

In a population of 133 patients, including 103 consecutive patients undergoing reperfusion therapy and 30 randomly selected patients with conservatively treated AMI, our isoenzyme analysis was applicable to 124. Nine patients were excluded: seven because mul-

![FIGURE 4. Scatter diagram of slope values for groups IA, IB, and II. Mean ± 1 SD is also shown for each group. Horizontal line at −3.1%/hr represents the cutoff value that minimized the overlap between groups IA and IB.](http://circ.ahajournals.org/)

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>≧3.1%/hr</th>
<th>&lt;3.1%/hr</th>
<th>Mean slope ± SD</th>
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<tbody>
<tr>
<td>Group IA</td>
<td>48 (87%)</td>
<td>7 (13%)</td>
<td>4.18 ± 1.25</td>
</tr>
<tr>
<td>Group IB</td>
<td>10 (26%)</td>
<td>29 (74%)</td>
<td>2.37 ± 1.11^</td>
</tr>
<tr>
<td>Group II</td>
<td>3 (10%)</td>
<td>27 (90%)</td>
<td>1.77 ± 1.46^</td>
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</tbody>
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^p < .001 vs group IA.
tiple (three or more) defibrillations were associated with prolonged elevations of MM-CK, most of which was probably of skeletal muscle origin, and two patients because only one sample was available from the first 18 hr after the onset of symptoms. In the remaining patients, the mean rate of decline of the unmodified tissue isoform MM3 was significantly greater in patients in whom reperfusion therapy was successful than in the groups in which it was not (as documented by angiography) or that received conventional treatment for AMI. Selecting a rate of decline of MM3 to minimize overlap between the groups provided a sensitivity of 87% for detecting reperfusion and a specificity of 74% among the group I patients. The overall diagnostic accuracy was 82%. In addition, 90% of patients with conventionally treated AMI had a slope less than 3.1%/hr. In contrast, the time to peak MB-CK differed between groups IA and IB, but the segregation achieved was inferior to that seen with isoform analysis: no cutoff time after symptoms could be selected that allowed segregation of group IA from group IB with more than a 68% overall accuracy.

Our rationale in investigating the rate of apparent MM3 decline as an index of reperfusion was based on two considerations. First, it has been well established, both experimentally and clinically, that washout of CK from infarcted tissue into the plasma is completed earlier in reperfused than in nonreperfused infarctions. This is probably the result of several factors, including the abrupt increase in flow to previously unperfused necrotic tissue as well as the accelerated membrane damage thought to occur when irreversibly injured myocardial cells undergo acute early reperfusion. Second, MM3 is rapidly converted to the other isoforms in plasma in vivo by plasma CP-N. After a bolus injection of purified tissue MM3 in the dog, conversion to MM2 occurs at a much faster rate than clearance of any of the MM-CK isoforms from the plasma. Furthermore, plasma conversion of MM3 to the other isoforms in vivo occurs with kinetics very similar to those observed after bolus injection of MM3 in vivo, indicating that the rate of MM3 disappearance is determined primarily by CP-N–mediated conversion rather than by isoform removal from the plasma. Consequently, in early reperfusion, with early cessation of CK release, MM3 as a percent of total MM-CK declines rapidly, in a manner analogous to a bolus injection. In contrast, the prolonged, ongoing release

<table>
<thead>
<tr>
<th></th>
<th>Group IA patients with slope ≥3.1</th>
<th>Group IB patients with slope &lt;3.1</th>
<th>Group II patients with slope &lt;3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>48/55 (87%)</td>
<td>29/39 (74%)</td>
<td>27/30 (90%)</td>
</tr>
<tr>
<td>Patients with total occlusion (grade 0) before treatment</td>
<td>41/48 (85%)</td>
<td>15/21 (71%)</td>
<td>^</td>
</tr>
<tr>
<td>Patients with incomplete occlusion (grade 1, 2, 3) before treatment</td>
<td>7/7 (100%)</td>
<td>14/18 (78%)</td>
<td>^</td>
</tr>
<tr>
<td>Patients with Q wave infarction</td>
<td>45/51 (88%)</td>
<td>22/31 (71%)</td>
<td>18/20 (90%)</td>
</tr>
<tr>
<td>Patients with non-Q wave infarction</td>
<td>3/4 (75%)</td>
<td>7/8 (87%)</td>
<td>9/10 (90%)</td>
</tr>
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</table>

^Acute angiography was not performed in group II patients.

FIGURE 5. Scatter diagram of values for time from onset of symptoms to peak MB-CK value for groups IA, IB, and II. Mean ± 1 SD is also shown for each group. Horizontal line at 17 hr represents the cutoff time that minimized the overlap between groups IA and IB.
associated with nonreperfused infarction results in extended plasma MM3 elevation and an attenuated apparent rate of decline.

The potential influence of CP-N on the rate of conversion has not been defined. In a preliminary report, the plasma level of CP-N activity did not change significantly during the first 24 hr after AMI. However, there was significant individual variation in the plasma levels of CP-N activity. Significant variation in CP-N activity is also suggested by the work of Falter et al. It remains to be determined whether the plasma levels of CP-N will significantly affect the conversion rate of CK isoforms in vivo. Such an effect might explain the scatter in the MM3 slope within each group and the overlap in the MM3 slopes for reperfused and nonreperfused patients. Patients having greater than normal plasma CP-N activity might demonstrate rapid conversion of MM3 in the absence of reperfusion; patients with low CP-N activity could manifest prolonged MM3 elevation despite reperfusion. A refinement in our algorithm, incorporating a correction for differences in baseline CP-N activity, might therefore improve the predictive power of our technique; further research in this area is warranted.

A limitation of the study was the 9 to 12 hr interval between coronary angiography and the completion of isoform analysis. In this intervening period, some patients with occluded vessels may have undergone reperfusion, while others in whom therapy was initially successful may have suffered reocclusion with or without reinfarction. Reocclusion with reinfarction after initially successful reperfusion would be associated with a delayed release of CK, resulting in a decreased slope. Although such a patient would be classified as a "false negative" for this study, the interpretation of early reinfarction as inadequate perfusion would be correct and provide the appropriate implication for further diagnosis and management. In the recent Thrombolysis in Myocardial Infarction study, the overall reinfarction rate was 11% in the first 8 days after thrombolytic therapy, but it is not clear how many of these patients suffered reocclusion in the initial 18 hr. It is of interest that this figure coincides closely with our 13% incidence of false negatives (slope less than 3.1%/hr; vessel reperfused by angiographic criteria).

It is also possible that several of the 10 patients who had patency predicted by isoform analysis, but whose vessels were occluded as documented angiographically at the conclusion of therapy, may have had drug-induced thrombosis shortly after the conclusion of angiography. In addition, three patients who underwent transient reperfusion but had subsequent reocclusion before the completion of angiography exhibited rapid release of CK with a slope of 3.1%/hr or more. Because these patients had a reperfusion grade of less than 2 at the completion of treatment, they were assigned to group IB and therefore accounted for 30% of our false-positive patients. However, in patients in whom clot lysis occurred several hours after completion of angiography, a relatively late release of MM3 would have occurred with prolongation of MM3 elevation, which could lead to an erroneous diagnosis of unsuccessful reperfusion. Although repeat arteriography 18 hr after onset of symptoms would have been optimal, this would be difficult to justify on clinical grounds. Isoform analysis, as presented in this study, while appropriate in assessing groups, may not be adequate for the determination of the reperfusion status of an individual patient. To validate future modifications, particularly those designed to make the method applicable to the individual patient, it may be necessary to perform coronary arteriography 12 to 24 hr after the onset of infarction.

In a recent animal study using isoform analysis, the investigators were able to determine which animals underwent successful reperfusion by using the rate of rise of %MM3 rather than the rate of decline. In the present study, this method could not be applied since in 56% (53/95) of our patients MM3, expressed as a percent of the total CK activity, was already maximal in the first sample obtained after admission. A technique based on MM3 upslope requires several specimens to be obtained between baseline and peak %MM3. This may be difficult since %MM3 is markedly elevated early after onset of symptoms and rapidly rises to its maximal proportion with or without reperfusion. Hashimoto et al. showed significant elevation of the tissue isoform within 1 hr of coronary occlusion in the dog, with peak %MM3 at 4.1 hr. In the same preparation but with early reperfusion, the tissue isoform reached its maximal proportion 30 to 60 min after reperfusion. In patients with acute myocardial infarction without reperfusion, the same group has observed a markedly elevated MM3/MM1 ratio of 14.4:1 (normal, 1.09 ± 0.4) just 3.9 hr from onset of symptoms. Thus, reperfusion in man at 2 to 4 hr after onset of acute myocardial infarction will be associated with the rapid release of MM3 superimposed on an already elevated plasma level of MM3. Consequently, the window for analysis of the upslope of %MM3 in man is small and may lead to significant overlap between reperfused and nonreperfused patients. Nevertheless, in patients in whom reperfusion is implemented very early (at less than 2 hr), assessment of the
MM3 upslope may afford adequate sensitivity and specificity and provide an early diagnosis of reperfusion. Our technique, based on the downslope, requires up to 18 hr after the onset of symptoms before analysis can be completed.

Of particular interest is the independence of the rate of MM3 decline on the completeness of infarct artery occlusion before therapy: successful treatment of patients with subtotal stenosis prior to thrombolytic therapy resulted in an MM3 decline as rapid as that observed in patients with complete occlusion. Conversely, if treatment did not significantly improve flow, there was sustained release of MM3 even if occlusion was incomplete. These findings were contrary to our expectations. In most patients, transmural infarctions are believed to arise from thrombosis at the site of an atherosclerotic plaque, which results in complete occlusion of the vessel. Since the frequency of detecting incomplete occlusions has been noted to increase with increasing time after the onset of infarction, incomplete occlusion has been postulated to result from early spontaneous clot lysis. If this model is correct, the gradual release of MM-CK from such "spontaneously reperfused" infarctions is in contrast to the rapid washout seen in therapeutically induced reperfusion. This might be expected if spontaneous physiologic clot lysis were a more gradual process than drug- or mechanically induced clot lysis, resulting in less rapid myocardial cell membrane breakdown in the infarct zone than occurs in iatrogenic reperfusion. In addition, the no-reperfusion phenomenon may be more pronounced in spontaneous reperfusion, resulting in less perfusion to the irreversibly damaged myocardial layer and thus less of a "surge" of CK release. Alternatively, it is possible that subtotal occlusions observed in the early hours of infarction represent atherosclerotic or thrombotic lesions which were never completely obstructing, but rather advanced such that flow was inadequate, resulting in prolonged ischemia and infarction. This issue remains to be resolved. The sensitivity and specificity of our assay were also unaffected by whether the patient had sustained a Q wave or non-Q wave infarction.

The agarose technique we used did not directly assay CK activity or immunoreactivity, and was thus semiquantitative. However, as shown in figure 1, this method accurately and reliably reproduced the expected MM1/MM3 ratios, as determined by the fully quantitative chromatofocusing assay. An advantage of the gel system we used is that it is already in widespread use in clinical laboratories for detection of the MB isoenzyme, and can be performed rapidly and inexpensively. Other techniques have also been evaluated for the quantitation of the MM-CK isofoms. Although chromatofocusing is useful for the purification and quantitation of the isofoms, it is a cumbersome experimental technique not in use in clinical laboratories. Another technique, isoelectric focusing, has been used, but nonisoform ampholyte artifacts have been a problem. Similarily, Western blot analysis, which requires two electrophoretic steps followed by an immunoassay, is time consuming and costly. Like chromatofocusing, isoelectric focusing and Western blot analysis are experimental rather than clinical techniques.

MM-CK isoform analysis provides sensitivity and specificity superior to other available noninvasive markers of reperfusion. One possible application of isoform analysis would be the early catheterization of patients treated with intravenous therapy whose slope is 3.1%/hr or more since it may be anticipated that PTCA or bypass surgery may be necessary for tight residual stenosis. We are currently evaluating this technique in a prospective study.

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