Effects of Therapeutic Coronary Reperfusion on Aspartate Aminotransferase Isoenzymes in Sera of Patients with Acute Myocardial Infarction

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We examined the kinetics of the catalytic activities of aspartate aminotransferase (AST, EC 2.6.1.1) isoenzymes in serum of 28 patients with myocardial infarction who were to receive either intracoronary urokinase—reperfusion angiographically proved—or conventional therapy (control group). Cytosolic (soluble) AST (s-AST) activity in serum increased rapidly immediately after recanalization, reaching a maximum 12 h after the onset of infarction. In the control group, this peak was reached 28 h after the onset (P <0.001). Peak s-AST activity was similar in the two groups. Peak activity and peak time for mitochondrial AST (m-AST) were the same for the two groups of patients; intervention that affects myocardial perfusion caused only a slight additional increase in m-AST activity in the early post-infarct period. There may be advantages to measuring m-AST, which is briefly influenced by reperfusion, instead of the usual cytosolic enzymes for assessment of myocardial damage in patients with myocardial infarction treated with thrombolytic therapy.

Additional Keyphrases: urokinase · enzyme activity

There is general agreement as to the beneficial effects of thrombolytic therapy for acute myocardial infarction (AMI) (1). Intracoronary thrombi can be lysed in the early stages of AMI and myocardial function in the ischemic zone may be improved by promptly re-establishing blood flow. Extensive studies have reported the effect of infusing thrombolytic agents such as streptokinase, urokinase, or tissue plasminogen activator on the release of the so-called cardiac enzymes from myocardium after an infarct (2–7). In particular, the reperfusion leads to rapid release of these enzymes, possibly as a result of a "washout" effect. This enzyme washout is useful clinically as a noninvasive marker for successful coronary recanalization (8–11).

Aspartate aminotransferase (AST; EC 2.6.1.1) is present in two isoenzyme forms, one associated with cytoplasm (s-AST), the other with mitochondria (m-AST) (12). In a previous study (13), we characterized in detail the time course of the appearance of AST isoenzymes in serum after AMI: m-AST correlated poorly with the enzymes commonly used in infarct diagnosis [creatinine kinase (CK; EC 2.7.3.2) and its MB isoenzyme; lactate dehydrogenase (EC 1.1.1.27); and α-hydroxybutyrate dehydrogenase (EC unassigned)], and apparently provides different biological information. Therefore, we wanted to investigate the effects of therapeutic thrombolytic procedures on the appearance of AST isoenzymes in serum. In particular, the present study was designed to compare the pattern of m-AST release in patients receiving successful thrombolytic therapy with that of a similar group of AMI patients who received conventional treatment.

Materials and Methods

Patients and Clinical Protocol

Fourteen patients with evolving AMI, admitted to the coronary-care unit of our Cardiological Division, were successfully completely reperfused as a result of intracoronary application of urokinase (plasminogen activator, EC 3.4.21.31). The following criteria were used for patient selection: (a) acute chest pain, persisting for at least 20 min and unrelied by intravenous nitroglycerin; (b) electrocardiographic changes consistent with myocardial ischemia (ST-segment elevation or depression >0.1 mV or T-wave inversion in two or more leads); (c) no increase of serum CK values above the upper reference limit; and (d) no specific contraindications to thrombolytic therapy (14). The method of fibrinolytic therapy has been previously described (5). Complete re-establishment of antegrade flow in the occluded coronary artery was proved angiographically, both at the time of lysis and three weeks after AMI.

In addition, 14 conservatively treated patients with documented transmural AMI (15) served as a control group to define the temporal course of AST isoenzyme activities from the time of admission through discharge from the hospital. In keeping with the suggestions of Muller et al. (16), we did not use cardiac catheterization in this group, because this represented an ethically unjustified risk. Hence, the method used in the controls to exclude that there has been spontaneous reperfusion must be indirect (e.g., persistence of chest pain and of electrocardiographic changes, absence of reperfusion arrhythmias, slow and late appearance of myocardial enzymes in the serum) (17). Table 1 gives details on the two groups of patients. No patients had any other illness known to increase AST activity.

Peripheral venous blood was sampled for determination of AST isoenzymes in the serum of both groups: immediately after admission to the hospital, before any intervention; every 2 h for the first day, every 6 h for the next 48 h, then every 12 h for the next two days. Within 2 h of collection, the nonhemolyzed sera were frozen at −20 °C in hermetically sealed plastic containers and analyzed within 24 h.

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Table 1. Clinical Characteristics of the Two Groups of Patients Studied

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Thrombolysis group (n = 14)</th>
<th>Control group (n = 14)</th>
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<tbody>
<tr>
<td>Age, y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56 (43-61)</td>
<td>56 (41-82)</td>
</tr>
<tr>
<td>Male sex</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Killip class&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.42</td>
<td>1.3 ± 0.50</td>
</tr>
<tr>
<td>Infarct location&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Inferior</td>
<td>Anterior</td>
</tr>
<tr>
<td>Time from onset of symptoms to admission, min&lt;sup&gt;d&lt;/sup&gt;</td>
<td>95 (20-180)</td>
<td>120 (60-180)</td>
</tr>
<tr>
<td>Admission signs&lt;sup&gt;e&lt;/sup&gt;:</td>
<td>Blood pressure, mm Hg&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>135 (100-180)</td>
<td>145 (100-190)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80 (60-100)</td>
<td>80 (70-120)</td>
</tr>
<tr>
<td>Heart rate, no./min&lt;sup&gt;g&lt;/sup&gt;</td>
<td>75 (60-100)</td>
<td>80 (45-115)</td>
</tr>
<tr>
<td>Serum CK, U/L&lt;sup&gt;h&lt;/sup&gt;</td>
<td>80 (26-122)</td>
<td>74 (45-119)</td>
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<sup>a</sup> Median value (and range).  <sup>b</sup> Mean ± SD.  <sup>c</sup> Number (and percent).  <sup>d</sup> On arrival at emergency ward.

Assay of AST and AST isoenzymes

Total AST activity was measured by the method recommended by the Scandinavian Committee on Enzymes (18). AST isoenzymes were determined by an immunoprecipitation method with antibody specific for s-AST, as previously described (19). After precipitation and separation of s-AST, we measured the residual activity (m-AST) in the same way described above for total AST activity; s-AST activity was estimated by difference. Enzyme activities for each sample were determined in duplicate with a Cobas Bio centrifugal analyzer (F. Hoffmann-La Roche and Co., Ltd., Basle, Switzerland) at 37 °C. Results were expressed as U (μmol·min<sup>-1</sup>). Time–activity curves were constructed for each patient. From these curves we derived peak activity and time required to reach the peak value for AST isoenzymes (20). The clearance rate (fractional disappearance rate, K<sub>d</sub>) for each isoenzyme activity was calculated by linear regression analysis from the linear portion of the declining slope of the time–activity curves, plotted semilogarithmically. The points used in calculations were the corrected isoenzyme activities, that is, the measured activities minus the base values of 18 U/L for s-AST and 1.8 U/L for m-AST. These base values were chosen to be close to the median values of the reference interval (23). At least three measurements were used to determine K<sub>d</sub> for each patient.

Statistical Analysis

Differences between medians of two groups of data were tested with the Wilcoxon rank-sum test. Differences with P values >0.05 (two-sided) were considered not significant. Chi-square analysis was performed for data in 2×2 contingency tables.

Results

Baseline characteristics were similar in both groups of patients (Table 1). There were no significant differences in clinical findings between patients assigned to the thrombolysis group and those assigned to the control group. There were more men in the urokinase group, but the difference was not significant (chi-square = 2.15).

Table 2 gives the characteristics of AST isoenzymes measured in patients: maximal activity value; time of maximal value, measured from the onset of chest pain; and K<sub>d</sub>. Serum s-AST activity increased rapidly immediately after recanalization, reaching an early peak 12 h after the onset of infarction (Figure 1). In the control group, the peak was reached 28 h after the onset of AMI (P <0.001). The peak s-AST activity was similar in the two groups, although the average activity was lower in the control group. Figure 1 (right) depicts the composite m-AST time–activity curves for the two groups. The difference between the times of peak activities for m-AST in the urokinase-reperfused and the conventionally treated patients was not statistically significant, in contrast to what was observed for s-AST. Again, there was no difference in peak activity between the patients with reperfusion and those with persistent occlusion (Table 2). As compared with conventional treatment, intervention that affects myocardial perfusion seemed to cause a slight but significant additional increase in m-AST activity in the early post-AMI period. As compared with the control group, the reperfused patients exhibited significantly higher m-AST values 10 (P <0.001) and 16 h (P <0.05) after the onset of AMI (Figure 1). In two patients this early increase represented the highest values for m-AST activity in the post-infarction period. Finally, the K<sub>d</sub> values of AST isoenzymes from the circulation were not statistically different between the two groups of patients studied, thus showing no significant treatment-related differences in the isoenzyme elimination.

Discussion

Early coronary reperfusion with thrombolytic agents is now widely used in AMI patients who reach the coronary-care unit shortly after the onset of pain, to recanalize the obstructed coronary artery and, consequently, to limit the infarcted area (1). In patients who are successfully treated with thrombolytic agents, cardiac enzymes appear significantly faster in the circulating blood (2–6). Consequently, the fibrinolytic dissolution of the coronary clot leads to characteristic time–activity profiles of myocardial enzymes, including immediate appearance, higher rate of enzymatic activity increase, and earlier peak activity (5). In this study we examined AST isoenzyme patterns in urokinase-induced reperfused patients and in nonreperfused patients after AMI, to determine the effect of therapeutic coronary reperfusion on these isoenzymes.

Table 2. Serum AST Isoenzymes in the Two Groups of Patients Studied

<table>
<thead>
<tr>
<th>Time of peak, h after onset</th>
<th>Control group</th>
<th>Thrombolysis group</th>
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<tbody>
<tr>
<td>s-AST</td>
<td>28.1 (17.9–36.7)</td>
<td>12.0 (8.6–34.3)*</td>
</tr>
<tr>
<td>m-AST</td>
<td>49.6 (30.8–62.7)</td>
<td>44.6 (4.9–72.0)</td>
</tr>
<tr>
<td>Peak activity, U/L</td>
<td>s-AST</td>
<td>189 (88–413)</td>
</tr>
<tr>
<td>m-AST</td>
<td>14.2 (6.6–53.5)</td>
<td>14.7 (6.6–37.9)</td>
</tr>
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<sup>a</sup> Significantly different from control group at P <0.001.
The data from our control group agree well with those of previous published studies (13, 21). After fibrinolytic therapy, serum s-AST immediately begins to increase at the time of reflow, the release rate being several fold that for the control group, and the release ending earlier. The time–activity curves for s-AST are very similar to those seen for total AST in previous works (4, 5, 8). In contrast, the washout phenomenon seems to influence the release of m-AST in the circulation. In particular, the course of m-AST activity in thrombolysis patients is biphasic, with an early increase of serum activity of about 50% of maximal enzyme activity in the serum, and a late main peak, 45 h after onset. The delayed increase in m-AST activity in serum, present in both groups of patients, indicates that most of this isoenzyme is retained intracellularly, in the mitochondrial structures of the tissue damaged, for several hours after AMI, and its release is constant and late, even if the coronary flow is re-established early.

In view of these and other properties of m-AST—especially its slow elimination from plasma, which permits cumulative release to be calculated accurately from a few samples—we conclude that the use of this isoenzyme in estimating the extent of myocardial damage offers several advantages. In fact, differences in serum enzyme kinetics as a result of reperfusion should lead to an alteration of the known relation between pathological infarct size and enzyme release and may invalidate comparison between thrombolytic agents and other treatments (7, 22). Vatner et al. (23) first demonstrated experimentally, in dogs, that estimates of infarct size based on CK data after reflow overestimated infarct size by threefold. With the development of thrombolytic therapy, similar observations have been made in the clinical setting. Data derived from studies by Blanke et al. (3) and Tamaki et al. (24) suggested that, for infarcts of equal size, CK or CK-MB release is greater per equivalent infarct volume in the patients treated with recanalization than in the conventionally treated group. Roberts and Ishikawa (25) showed that the relationships between accumulated CK release and anatomical infarct size differed under unperfused AMI and during AMI followed by early reperfusion. The usefulness of an enzyme like m-AST, which is briefly influenced by reperfusion, for assessment of myocardial damage in thrombolysis-treated patients could obviate these shortcomings.

m-AST activity in serum reportedly reflects necrotic injury at the subcellular level (13, 26, 27). If so, then in our patients we cannot conclude that the total amount of necrotic tissue is diminished after treatment with urokinase, given the similarity of peak m-AST activity in the two groups of patients. A potential drawback in using m-AST to evaluate the area of necrosis is the presence of this isoenzyme in liver and skeletal muscle; for example, the release of liver AST as a result of poor hepatic perfusion or passive liver congestion—well-recognized complications of AMI—may diminish the specificity of this marker and overestimate the myocardial damage. To establish the clinical value of m-AST as an enzymatic index to the necrotic area in AMI patients will require further prospective randomized investigations.

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References

Fig. 1. Time course of (left) s-AST and (right) m-AST (median values) in serum of patients with AMI, according to the presence (continuous line) or absence (dashed line) of early coronary reperfusion. n = 14 in each group.