Chromatographic and Electrophoretic Separation of Creatine Kinase Isoenzymes Compared

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We compared two techniques for separating and evaluating serum creatine kinase isoenzymes—fluorometric agarose electrophoresis and Sephadex chromatography—in 50 patients, 25 of whom had confirmed acute myocardial infarction. In every case isoenzyme MB (heart isoenzyme) was detected with equal sensitivity by either procedure. Evidently, only the presence or absence of MB is clinically significant; none of the 25 patients without infarction had detectable MB activity in their serum. Columns connected to a continuous-flow sample line for analyses of the eluting stream without further modification produced satisfactory results.

Additional Keyphrases: myocardial infarction • diagnostic aids • enzyme activity

Recently much clinical interest has focused on the usefulness of creatine kinase (ATP:creatinine N-phosphotransferase; CK; EC 2.7.3.2) isoenzymes in the diagnosis of acute myocardial infarction. The MB isoenzyme of CK is virtually specific for the myocardium. We compared two techniques for separating CK isoenzymes: Elevitch's fluorometric agarose electrophoresis (1) and Mercer's Sephadex chromatography (2).

Materials and Methods

Sample Preparation

Human tissue was obtained at autopsy. Brain, small intestine, heart, and skeletal muscle were extracted for 5 min with tris(hydroxymethyl)aminomethane buffer (pH 7.0, 50 mmol/liter, containing 100 mmol of NaCl and 0.1 mmol of dithiothreitol per liter) with use of a household-type blender. The homogenates were centrifuged (4 °C, 2500 × g, 1 h) and the supernates were used for electrophoretic and chromatographic studies.

Sera from patients were either analyzed within 24 h or stored at -20 °C and analyzed within three days.

Analytical Methods

Agarose electrophoresis. ACI cell, power supply, and agarose plates (Corning, 490 San Antonio Rd., Palo Alto, Calif. 94306), were used. Sample volume applied was 1 µl. The enzyme detection system used was that described by Elevitch (1), in which creatine phosphate is converted to creatine and ATP, the latter reacted with hexokinase and glucose-6-phosphate dehydrogenase, and the resulting NAD+ to NADH conversion followed fluorometrically. Patterns were scanned with a Model 111 fluorometer, equipped with an automatic scanner (Model 2; G. K. Turner Associates, Palo Alto, Calif. 94303).

Column chromatography. The minicolumns were standard 12.5-cm disposable Pasteur pipets, plugged at the bottom with glass wool and filled with Sephadex A-50. Eluting buffers were tris(hydroxymethyl)aminomethane, 50 mmol/liter, with increasing NaCl concentrations of 100, 200, and 300 mmol/liter, the first two being at pH 8.0 and the third at pH 7.0 (2). Serum, 1 ml, was applied to the column, allowed to penetrate the Sephadex, and the first effluent was collected. Three 1-ml portions of the first eluting buffer (100 mmol/liter NaCl) were introduced, and three 1-ml eluates successively collected. To effect a complete separation between the MM and MB isoenzymes, we introduced a fourth elution of 1 ml of the first buffer. Similarly, three 1-ml portions of the sec-
ond eluting buffer (200 mmol/liter NaCl) were applied and three 1-ml eluates collected. If one is interested in the BB fraction, a third set of three 1-ml portions of the third eluting buffer (300 mmol/liter NaCl) may be applied.

Activity Measurements

Total activity. Total CK activities in patients' sera and in eluates of chromatographed sera were measured by continuous flow (3). The reaction is: creatine phosphate + ADP + α ATP + creatine + ATP. The creatine is measured colorimetrically by using α-naphthol and diacetyl. One unit is defined as the activity producing 1 nmol of creatine per minute at 37 °C. A tenth milliliter of sample is incubated for 25 min at 37 °C, and the reaction is linearly related to activity to 300 U/ml. For normal sera we obtain values up to 85 U/ml; sera with values higher than 1000 are appropriately diluted with the first eluting buffer before the sample is applied to the column.

Isoenzyme activity. After the electrophoretic patterns were scanned, the percentage of MB isoenzyme activity was found by calculating the total peak areas (one-half the base times the height) and dividing the area calculated for MB by the sum of all areas.

In the case of column chromatography, absorbances of the eluates were similarly used.

Results

Sera from 50 patients were studied by both techniques. Of these patients, 25 had acute, clinically confirmed myocardial infarctions; MB was present in all (Table 1). The t value (2.34) is barely significant at the 95% level of certainty and not significant at the 98% level. The correlation coefficient was 0.71 for results by the two techniques. In the sera from 25 patients without myocardial infarction, MB was not detected by either technique.

A problem with separating serum isoenzymes in chromatographic columns is the phenomenon of “overflow.” A small amount of MB overflows into the BB region, as illustrated in Figure 1, in which CK activity is detected in eluates 8 and 9, which are supposed to contain BB, but electrophoresis of these eluates indicates that the isoenzyme is MB and not BB. However, in all cases, whether with patients' sera or with tissue homogenates, MM was definitively separated from MB, as confirmed by electrophoresis of all eluates. Table 2 illustrates both this separation and the overflow phenomenon.
We did an isoenzyme separation by "continuous-flow elution" by directly connecting the column to the sample line of the AutoAnalyzer without further modification (Figure 2). Separation was effected in 15–20 min. Volumes of serum and eluting solutions were half those used by Mercer (2).

We tried a column with a larger reservoir than the Pasteur pipet, the "Curtis T4" column (Curtis Nuclear Corp., Los Angeles, Calif. 90058). Results were comparable to those from the column as specified by Mercer (2), but the relative positions of the fractions were slightly different.

Discussion

In the 25 cases of acute myocardial infarction, we found electrophoresis and column chromatography to be equally sensitive in detecting MB. The relative percentages of MB as assessed by both techniques were fairly well correlated (r = 0.71). Clinically, only the absence or presence of MB is significant, and the diagnosis of myocardial infarction can be made with this information. This agrees with other investigators (4, 5). For this purpose, ultraviolet visualization of the electrophoretic pattern is sufficient. In all cases of confirmed myocardial infarction, MB was detected. Where ultraviolet visualization was ambiguous, densitometric scanning of the pattern established the presence or absence of trace MB activity. None of the specimens with trace amounts of MB activity was from a patient with acute myocardial infarction.

Table 2. Results of Electrophoresis of Separate Eluates of Homogenate of Heart

<table>
<thead>
<tr>
<th>Eluate no.</th>
<th>MM</th>
<th>MB</th>
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<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>−</td>
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<tr>
<td>2</td>
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<td>−</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Presence (+) or absence (−), as confirmed by electrophoresis. BB was detected in none.*

Samples with MB activity as low as 2.2 U/liter were measured.

Recent investigations (7–9) indicate that with chromatography and electrophoresis it is possible to detect MB in normal individuals and in patients without myocardial infarction.

Either electrophoresis or chromatography is suitable for the separation of CK isoenzymes. Chromatography has the advantage of low cost, ready availability of materials and equipment, and the use of any existing CK procedure. When more than five specimens are to be analyzed, electrophoresis is more
convenient. The agarose plate can handle eight specimens and does not require dilutions. However, the fluorometer and scanning equipment are costly and not part of a clinical laboratory’s usual equipment.

Chromatography has the problem of overflow of MB into the BB region but, because BB is hardly ever found in serum (6), the problem is academic—MM and MB are well separated in every case, and consequently the last three elutions for BB may be omitted. When tissue homogenates containing all three isoenzymes are chromatographed on Sephadex columns, MM is always separated from MB, but not MB from BB. Electrophoresis indicated the presence of both MB and BB in those eluates that are supposed to contain only BB.

“Continuous-flow elution” can effectively separate the three isoenzymes. This method requires total elution and analysis time of about 20 min and an instrument dedicated to CK with expensive substrates pumped continuously. Automation of sample application combined with gradient elution may reduce the time involved, but requires further study.

The Curtis column holds larger volumes of serum and eluting buffers. In this column MB isoenzyme eluted in a later fraction than in the Pasteur pipet.

References