Five Creatine Kinase Isoenzymes in Serum of a Patient with Severe Heart Disease

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Abnormal creatine kinase (CK) isoenzyme patterns were observed in the serum of a 64-year-old woman with severe heart disease. Agarose electrophoresis revealed the presence of all the usual CK isoenzymes (MM, MB, and BB) plus an extra band between MM and MB. Total serum CK activity was within the normal range. Within 2 h after the patient suffered cardiorespiratory arrest, a fifth CK isoenzyme appeared, cathodal to MM. After cardiac valve replacement, the patient's serum showed a high activity of CK, but the isoenzyme pattern showed only MM and, transiently, an MB band. With return of the serum CK activity to normal, the CK isoenzyme pattern also became normal, virtually ruling out genetic variant(s). The abnormal CK isoenzyme patterns might have been the consequence of severe hypoxemia in the patient, thus such patterns may represent an ominous prognostic sign. The association of the abnormal pattern upon admission with rapid deterioration of the condition of the patient suggests prompt attention for the prevention of complications.

The isoenzymes of creatine kinase (CK; adenosine triphosphate:creatine N-phosphotransferase, EC 2.7.3.2) can conveniently be separated by electrophoresis. Normal human serum usually contains only the MM isoenzyme. By electrophoretic separation, "atypical" CK isoenzymes have been observed in the sera of some patients (1-5). Here, we report a case of severe cardiac disease in which the serum, on agarose gel electrophoresis, exhibited four CK isoenzyme bands with normal total CK activity on admission to the hospital, and five bands when the serum total CK activity was elevated after cardiorespiratory arrest.

Case History

A 64-year-old white woman with rheumatic and coronary arteriosclerotic heart disease was admitted for planned cardiac surgery. The patient had had acute rheumatic fever and scarlet fever at the age of 10. She did well until the age of 57. At that time she was hospitalized for atrial fibrillation and substernal chest pain and mitral stenosis was diagnosed. At age 63, she had increasing symptoms of angina and congestive heart failure. Cardiac catheterization revealed mitral stenosis and three-vessel coronary artery disease.

Triple coronary artery bypass grafts and a closed mitral commissurotomy produced only in a transient improvement in her clinical status. Seven months after her initial operation, routine postoperative cardiac catheterization revealed mitral regurgitation and occlusion of all her coronary bypass grafts. Over the ensuing months she had increasing biventricular failure.

At the time of her current admission for operation she had marked dyspnea on minimal exertion, three-pillow orthopnea, paroxysmal nocturnal dyspnea, peripheral edema, and abdominal swelling. She denied having any chest pain. An electrocardiogram showed atrial fibrillation with nonspecific ST-T wave changes. Her serum potassium concentration was low, so she was given oral liquid potassium supplementation.

On day 3 of her hospitalization she had a witnessed cardiorespiratory arrest. Resuscitation was immediately instituted and the patient was intubated. After adequate ventilation the patient recovered to an idioventricular rhythm. She was given sodium bicarbonate and returned to atrial fibrillation with a good blood pressure. The potassium concentration in a specimen drawn during the arrest was high (9.2 mmol/L), the glucose concentration remarkably low (120 mg/L).

The patient was considered to be critically ill and it was thought she would not survive this insult without intervention. So three hours after the arrest she was taken to the operating room, where under cardiopulmonary bypass her mitral and tricuspid valves were both replaced by porcine (Hancock) valves.

Her postoperative course was initially complicated by a low cardiac output, but by the fifth postoperative day she was off all intravenous medications and was maintaining an adequate cardiac index. She diuresed with dyazide and lasix to her preoperative weight by the 11th postoperative day and was discharged on her 17th postoperative day.

Materials and Methods

The serum was separated from the clotted blood by centrifugation, kept at 4 °C, and analyzed on the same day. A 20-test chemistry panel—including CK, lactate dehydrogenase (LD; EC 1.1.1.27), aspartate aminotransferase (AST; EC 2.6.1.1), and alanine aminotransferase (ALT; EC 2.6.1.2)—was run on the sera by the continuous-flow method (SMAC; Technicon Instruments Corp., Tarrytown, NY 10591). Our reference intervals for the above enzymes were defined in a study of 205 healthy blood-bank donors by the 2.5 and 97.5 percentiles as follows: CK (females), 21–147 U/L; LD, 133–248 U/L; AST, 8–31 U/L; and ALT, 3–44 U/L. CK isoenzymes were separated by a standard electrophoretic procedure on agarose gel and were quantitated fluorometrically (Corning Medical, Medfield, MA 02052). We routinely assess the presence and the approximate distribution of CK isoenzymes visually by placing the agarose films into a fluorescent view box. For specificity studies, we used reagents of another commercial kit (Bio-Dynamics/bmc, Div. of Boehringer-Mannheim, Indianapolis, IN 46250), with and without cre-

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atine phosphate, to identify CK activity in Cornings's agarose electrophoretic system. LD isoenzymes were determined colorimetrically after electrophoresis in agar gel as described previously (6). The reference interval with this method for LD isoenzymes has been reported elsewhere (7).

**Results**

On admission, the patient's serum total CK, AST, and ALT were all within normal limits. Total LD activity was slightly higher than normal (Figure 1). In turn, sera obtained from the patient 1, 2, and 3 h after the cardiorespiratory arrest revealed an abrupt and progressive increase in the activity of all four enzymes. Cardiac surgery caused an even greater increment in the enzyme activities. Nine days postoperatively, values for CK, AST, and ALT were all again within normal limits, while LD returned to the slightly above-normal pre-operative activity two weeks after cardiac surgery (Figure 1). A similar enzyme profile was seen three months after this hospital admission, with normal values for serum CK, AST, and ALT and with borderline-increased total LD (257 U/L).

Although total CK activity was normal (74 U/L), the serum specimen obtained on the first day of hospitalization displayed a grossly abnormal pattern of CK isoenzyme distribution (Figure 2A). It showed the presence not only of MM (72%), MB (7%), and BB (6%), but also an extra band (X1; 15%) between the position of MB and MM. One hour after the cardiorespiratory arrest, the increasing total CK activity was associated with increased activities of MM and MB. At the same time, no apparent change could be observed in the activity of BB (Figure 2B). Two hours after the arrest, however, a new band (X2) appeared, cathodal to MM, and increased the number of detectable CK isoenzymes to five (Figure 2C). This aberrant band (X2) was also present in the 3-h serum specimen.

Analysis of serum samples with very high total CK activities after cardiac surgery revealed a CK isoenzyme pattern consisting of strong MM (95%), MB (5%), and a weak BB band (trace) (Figure 2D). Dilution of the sera for analysis in the linear range could not fully account for the disappearance of the extra bands, because undiluted sera also showed barely visible activity at the expected position of the extra bands. The decline in total CK activity was paralleled by a decrease in MB activity, and the strong MB band had disappeared by the third postoperative day. The return of serum total CK activity to normal values was accompanied by an isoenzyme distribution such that practically 100% of the activity was in the MM band and only traces in BB, MB, and X1 (Figure 2E). A normal pattern with 100% of MM was seen, together with normal total CK activity (31 U/L) in a serum specimen obtained three months after this hospital admission.

We emphasize that none of these bands appeared in the absence of the specific substrate, creatine phosphate.

Serial determination of LD isoenzymes is shown in Figure 3. Pattern A was obtained for a serum sample obtained on admission; it is essentially a normal pattern. Patterns B and C, obtained for serum sampled 1 and 2 h after the cardiorespiratory arrest, show increased density of LD5 associated with increased total LD activity. Cardiac surgery did not alter this pattern (Figure 3D) and the increased LD5 persisted for a week after the operation. Interestingly, the reversal of the LD1/LD2 ratio, which characteristically occurs after myocardial infarction, was not observed. Recovery of the patient from her operation was associated with a decline in the serum total LD activity to admission values and with the development of a normal LD isoenzyme distribution (Figure 3E). Three months after this hospitalization, the patient continued to have borderline-increased serum total LD (257 U/L) with a normal LD isoenzyme pattern.

**Discussion**

Variant CK isoenzymes as determined by electrophoresis and exhibiting electrophoretic mobilities different from normal have been previously reported (1-5). These abnormal CK isoenzyme patterns were frequently seen on serum samples with increased total CK activity (2-5) and in general only one aberrant isoenzyme was found in addition to one or, rarely, two normal isoenzymes.

The patient described here exhibited several unique features in her CK isoenzyme distribution on agarose electrophoresis. First, was the abnormal CK isoenzyme pattern in the presence of normal total CK activity at the time of admission. Second, an aberrant band (X1) was present between MM and MB in addition to the simultaneous occurrence of all three of the usual isoenzymes—MM, MB, and BB—in the admission serum specimen. Third, after cardiorespiratory arrest, parallel with the increase in total CK activity, a second
aberrant isoenzyme band (X2) appeared, cathodal to MM, increasing the number of recognizable CK bands in the isoenzyme pattern to five.

The CK isoenzyme distribution on agarose electrophoresis with four bands at normal total serum CK activity and with five bands accompanying increased serum CK may represent the most abnormal CK isoenzyme patterns reported thus far. These bands were dependent on the presence of creatine phosphate in the incubation mixture. From their electrophoretic mobility it can only be inferred that X1 may be a "macro" CK (a complex of BB and immunoglobulins) (3, 4) while X2 could be of mitochondrial origin (2, 5). Because X1 was present on admission and greatly diminished in activity after the patient's heart condition was improved by surgery, a causal relationship to the long-persisting congestive heart failure (chronic hypoxemia) is possible. The other "extra" band, X2, appeared after the cardiorespiratory arrest which, in turn, might have precipitated mitochondrial damage through severe acute hypoxemia. The presence of a normal CK isoenzyme distribution in the patient's serum three months after the finding of grossly abnormal patterns virtually rules out any genetic variant(s).

The simultaneous presence of well-detectable activities of all three normal CK isoenzymes in the peripheral blood is rare. The finding of four CK isoenzymes in the serum of our patient with a normal total CK activity upon admission was associated with rapid deterioration of her clinical condition. Such an observation suggests impending complications, such as the cardiorespiratory arrest that eventually occurred in this patient. Although our patient had a long history of coronary artery disease, she never was documented as having had a myocardial infarction by either electrocardiographic and LD isoenzyme criteria.

The changes in total activity of LD, CK, and AST in serum, as well as the CK and LD isoenzyme patterns after cardiac surgery, are in accord with previous findings on patients undergoing open-heart surgery (9–13). The increased activity of ALT was an unexpected finding and points to significant liver damage that developed primarily as the consequence of the cardiorespiratory arrest in the patient.

References