The Detection of Creatine Kinase Isoenzyme CK₁ in Serum

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ABSTRACT

The brain isoenzyme of creatine kinase (CK) (EC 2.7.3.2) has been identified in the serum of 21 of 950 patients tested. Samples were received over a period of about 20 months. Analysis was performed by electrophoresis on agarose gel with isoenzyme bands visualized by fluorescence after enzyme coupled reactions producing NADH. Specialized substrate preparations were used to rule out the possibility that observed fluorescence was due to enzymatic activity other than that of CK isoenzymes. Patients who were hypothermic and/or had undergone cardiopulmonary resuscitation accounted for 19 of the 21 patients showing the brain isoenzyme. The inciting mechanism for the release of CK₁ into the circulation is not clearly understood.

Introduction

Creatine kinase (CK) (EC 2.7.3.2) exists in three isoenzyme forms. Each isoenzyme is composed of dimers of two peptide subunits, M and B. The isoenzymes have been classified as BB or CK₁ (brain), MB or CK₂ (cardiac) and MM or CK₃ (muscle). Using an electrophoretic technique, only CK₃ is usually seen in the serum of healthy human subjects.

Pattern abnormalities of CK₂ and CK₃ isoenzymes have been well investigated and correlated with a number of disease states. Much less attention has been directed to CK₁ levels and their relationship to pathological conditions. Until recently, the presence of CK₁ was felt to be a rare occurrence and seen in only a few conditions such as malignant hyperpyrexia, Reye's Syndrome, acute brain injury and after neurosurgical intervention. Abnormal levels of CK₁ are now being noted in an increasing variety of pathological states and greater interest has been stimulated in the relationship between this isoenzyme and disease. CK isoenzymes have been analyzed in our laboratory over a period of about 20 months. During this time, 21

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patients were found to have $CK_1$ isoenzyme present in their sera. It is the purpose of this paper to report on our experience with $CK$ isoenzyme analysis, with special reference being made to those cases in which $CK_1$ was isolated.

**Materials and Methods**

The patients used in this study came primarily from the critical care or medicine units of the St. Louis City Hospital. A small number came from the University Hospital at Little Rock. A total of 950 patients had one or more blood samples checked for the presence of $CK$ isoenzymes. Normally, patients admitted for coronary care had serial samples drawn during the first 72 hours of hospitalization.

All $CK$ isoenzymes were determined by electrophoresis on agarose gel using the Corning ACI electrophoresis system. Electrophoretic procedures were all carried out by the manufacturer’s suggested methods using supplies available from the manufacturer. Substrate without creatine phosphate was kindly supplied.*

All electrophoresis plates were checked for fluorescence before the addition of any substrate preparation. Completed electrophoresis plates were read under long wave ultraviolet light and reported as negative through four plus (4 +) versus a standard.

**TABLE I. CREATINE KINASE ISOENZYME PATTERNS**

<table>
<thead>
<tr>
<th>ISOENZYME(S)</th>
<th>NUMBER OF PATIENTS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CK_3$</td>
<td>786</td>
<td>82.7</td>
</tr>
<tr>
<td>$CK_3, CK_2$</td>
<td>122</td>
<td>12.8</td>
</tr>
<tr>
<td>$CK_3, CK_2, CK_1$</td>
<td>20</td>
<td>2.1</td>
</tr>
<tr>
<td>$CK_3, CK_1$</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>None</td>
<td>21</td>
<td>2.2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>950</td>
<td>*99.9</td>
</tr>
</tbody>
</table>

* Totals less than 100 due to mathematical rounding off.

CPK/LDH Isoenzyme Control† Lot number 1575131 was used as a comparison standard on all electrophoreses. This standard contains a mean concentration of 100 IU/5 ml $CK_1$, 112 IU/5 ml $CK_2$, and 412 IU/5 ml $CK_3$ and was reconstituted and used as directed by the manufacturer.

Confirmatory electrophoreses on some $CK_1$ positive samples were performed by the clinical chemistry laboratory‡ using the Bioware “Biopak” CPK-C method.§

In this method the isoenzymes are visualized colorimetrically as a formazan complex.

**Results**

During this study, 950 patients had one or more isoenzyme analyses performed. The isoenzyme patterns and their frequencies are listed in table I. Precisely 97.8 percent exhibited at least one of the isoenzymes. As expected, the predominant isoenzyme was $CK_3$ which was present alone in 82.7 percent of the patients. $CK_2$ was present in only 14.9 percent of the patients, a rather low percentage considering that suspicion of myocardial infarction was the most frequent reason the analysis was ordered. $CK_1$ was found in 2.2 percent of the subjects.

In table II are listed the patients with $CK_1$ present, their isoenzyme patterns and their diagnosis. They are divided into six main categories, although there is some overlapping of patients within the groups. Thirteen of the patients experienced cardiac arrest and underwent cardiopulmonary resuscitation. In seven of

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* Coming Medical Works, Medfield, MA.
† Helena Laboratories, Beaumont, TX.
‡ Barnes Hospital, St. Louis, MO.
§ Bioware, Inc., Wichita, KS.
TABLE II
PATIENTS EXHIBITING CK\textsubscript{1} ISOENZYME

<table>
<thead>
<tr>
<th>NUMBER OF PATIENTS</th>
<th>ISOENZYMES PRESENT</th>
<th>CATEGORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>CK\textsubscript{3}, CK\textsubscript{2}, CK\textsubscript{1}</td>
<td>Post cardiopulmonary resuscitation</td>
</tr>
<tr>
<td>3</td>
<td>CK\textsubscript{3}, CK\textsubscript{2}, CK\textsubscript{1}</td>
<td>Hypothermia</td>
</tr>
<tr>
<td>3</td>
<td>CK\textsubscript{3}, CK\textsubscript{2}, CK\textsubscript{1}</td>
<td>Open heart surgery</td>
</tr>
<tr>
<td>1</td>
<td>CK\textsubscript{3}, CK\textsubscript{2}, CK\textsubscript{1}</td>
<td>Post CPR &amp; Hypothermia</td>
</tr>
<tr>
<td>1</td>
<td>CK\textsubscript{3}, CK\textsubscript{1}</td>
<td>Metastatic G. l. Adenocarcinoma</td>
</tr>
<tr>
<td>1</td>
<td>CK\textsubscript{3}, CK\textsubscript{2}, CK\textsubscript{1}</td>
<td>Subendocardial Myocardial infarction</td>
</tr>
</tbody>
</table>

these cases, there was concurrent myocardial infarction. Five patients arrested from presumed cardiac arrhythmias but without definitive proof of myocardial infarction. Hypothermia was considered a second category. All three patients in this group were admitted to the hospital after suffering exposure induced hypothermia. One of these patients had severe frostbite, one minor tissue damage and the last had no visible tissue damage at all. The last patient had an admitting temperature of 84°F. The other two patients had temperatures of less than 94°F. The precise temperature was unknown since only a routine thermometer was used during their admission and warming therapy. One patient had both hypothermia and cardiac arrest. It is not possible to determine the exact category of her CK\textsubscript{1} elevation. Hypothermia also complicated the situation in those patients who underwent open heart surgery. These were three infants who had surgery under deep hypothermic conditions. The CK\textsubscript{1} could have been increased by the hypothermia or could be due to other unknown factors.

One patient had metastatic gastrointestinal tract adenocarcinoma, primary unknown. It was not possible to obtain tissue for analysis and it can only be suspected that the tumor represented the source of the serum CK\textsubscript{1} or led to its release by localized damage to tissues rich in CK\textsubscript{1}.

The last patient is a source of confusion. She suffered a subendocardial myocardial infarction but did not arrest and showed no evidence of shock. She does not fit any of the other classifications and no clear cause of her CK\textsubscript{1} could be elucidated.

Discussion

Special precautions were taken in this study in an effort to affirm that what was interpreted as CK\textsubscript{1} on electrophoresis was, in fact, the brain type isoenzyme. All electrophoretic separations were carefully examined before their incubation with substrate to rule out the presence of non-specific fluorescence. Since myokinase can react with some of the components of the substrate preparation to produce the same fluorescent end product (NADH) as the CK isoenzymes, a myokinase inhibitor, adenosine monophosphate (AMP), was added to the commercially prepared substrate. To rule out the possibility that the AMP might not be adequate or that there might be other interfering fluorescent compounds on the plates, a substrate preparation lacking creatine phosphate was used on electrophoresis plates on which samples and controls previously shown to contain the CK isoenzymes by normal methods had been separated. None of these plates showed any fluorescence, indicating that what was observed on the plates was indeed the CK isoenzymes.

It has been our experience that clinicians order CK isoenzymes in only a limited number of situations. CK isoenzymes are most frequently requested for the evaluation of myocardial and skeletal muscle diseases. Seldom are they ordered for other disease states. This may in part
explain why elevations of serum CK$_{1}$ have previously been considered to be a rare event. That it is not as uncommon as originally thought is borne out by more recent studies.

Serum CK$_{1}$ was noted in about half of a group of renal failure patients undergoing dialysis; Smith found it in a patient with an acute renal failure following staphylococcal pneumonia.$^{5,14}$ CK$_{1}$ has been found in patients with oat cell carcinoma, in a case of metastatic gastric adenocarcinoma and in patients with stage D carcinoma of the prostate.$^{3,4,10}$ It has been seen in patients who have undergone cardiopulmonary resuscitation and in patients undergoing aortocoronary bypass surgery.$^{5,14,17}$ Its occurrence in hypothermia has been previously noted in our laboratory.$^{11}$ It is extremely likely that as more investigators regularly search for the presence of CK$_{1}$ in a greater variety of diseases, more examples will be discovered.

At this point, the diagnostic value of CK$_{1}$ is quite speculative. In fact, the source of serum CK$_{1}$ is still speculative, as is the mechanism by which it enters the circulation. Although 19 of our 21 cases involved hypothermia and cardiac arrest, both of these conditions cause multiple type insults to the various organ systems; it is not clear which of these insults represent the inciting mechanism for the release of CK$_{1}$ into the circulation. More careful studies will be necessary before this can be properly understood.

References

11. MCDANIEL, R. C. and DEVINE, J. E.: Elevations of creatine kinase isoenzyme CK$_{1}$ in patients with exposure induced hypothermia. (Submitted for publication.)