IgA–CK-BB Complex with CK-MB Electrophoretic Mobility Can Lead to Erroneous Diagnosis of Acute Myocardial Infarction

Rafael Venta,1 Sofía A. Geijo,1 Aquilino C. Sánchez,1 Constantino G. Bae,1.2 Luis A. Bartolome,2 Gerardo Casares,2 Carlos Lopez-Otin,3 and Francisco V. Alvarez1,3

This patient, on admission, presented with a tentative diagnosis of myocardial infarction: the electrocardiogram showed a nonspecific ST-segment and T-wave abnormalities, and total creatine kinase (CK; EC 2.7.3.2) activity was slightly increased (238 U/L). However, a high electrophoretic value for CK-MB (50% of total CK activity) and the electrophoretic pattern of lactate dehydrogenase (EC 1.1.1.27) isoenzymes ruled out myocardial infarction. The isoenzyme migrating as CK-MB was found later to contain no immunologically normal CK-M subunits, and it was bound to IgA. A mixture of the patient’s serum and a human serum control containing all CK isoenzymes showed altered electrophoretic mobility only for CK-BB, indicating that the patient’s serum contained antibodies to the B unit of CK. Elution from a Sephadex G-200 column showed that the peak at which most of the anodic CK was eluted corresponded to a molecular mass of approximately 200 kDa. Evidently this atypical isoenzyme was an IgA–CK-BB complex. Because this macro CK type 1 can mimic CK-MB, it may therefore be a source of confusion.

The importance of the isoenzymes of creatine kinase (CK; EC 2.7.3.2) as a diagnostic tool in patients suspected of having acute myocardial infarction has long been known (1–5).

Previous reports (1–9) have described variants of CK presenting with atypical electrophoretic mobility, appearing between the CK-MM and CK-BB regions. These variants have been termed “atypical CK” or “macro CK type 1.” Macro CK type 1 usually consists of a complex of CK-BB isoenzyme with an immunoglobulin in serum, most often IgG (3–5, 10) but sometimes CK-MM (6).

The isoenzyme of CK that migrates cathodic to CK-MM on agarose gel electrophoresis, termed “macro CK type 2” (MCK-2) or “mitochondrial CK” (CK-mit), was first described by Jacobs et al. (11). Some investigators have tried to make chemical use of this isoenzyme, finding MCK-2 in the sera of patients with acute myocardial infarction and severe shock (12, 13) and in the serum of some patients with cancer (14–16). We have also described the presence of a MCK-2 in a healthy person (16).

The presence of CK-MB (10–15% of total CK activity) was recently described in patients not suffering acute myocardial infarction (17, 18).

So far, most of the cases described with atypical CK isoenzymes were detected in patients who had increased serum CK activity, but this increase was brief.

Here we describe studies of such a CK isoenzyme, detected in a patient who had a slightly high value for total serum CK activity on admission to the hospital. The atypical isoenzyme migrated to the same position as CK-MB isoenzyme on electrophoresis, but instead of being “true” CK-MB it turned out to be an immunoglobulin A–CK-BB complex. We describe the characterization of the complex as well as its persistence independent of the total CK activity in serum.

Case History

A 59-year-old man was admitted to the hospital complaining of moderately severe chest pain after physical effort. He had been slightly hypertensive for the past five years, and had been a heavy smoker and drinker until a few years before. An electrocardiogram taken at that time revealed a nonspecific ST-segment and T-wave abnormalities. Neither electrocardiographic changes nor chest pain was observed during his stay at the hospital.

Four weeks after he left the hospital, the results of a physical examination were unremarkable. His family history and his biochemical test results at that time were also unremarkable.

Results of laboratory tests were within normal limits at the time of admission, except for total CK activity, 238 U/L (reference interval 25–195 U/L). This value remained above normal limits until 48 h after admission (445 U/L), an increase explainable by release of skeletal-muscle isoenzyme (CK-MM). On the fourth hospital day, total CK activity decreased to within normal limits. No further changes have been observed subsequently.

Electrophoresis of CK isoenzymes revealed two bands: the most anodic band (CK-MB?) accounted for 42% (99.7 U/L) of the total CK activity (238 U/L). This high percentage of apparent CK-MB, together with an unclear biochemical pattern, suggested making further investigations before diagnosing a myocardial infarction.

Analysis for total serum lactate dehydrogenase (LD; EC 1.1.1.27) activity and agarose gel electrophoresis of LD isoenzymes gave no abnormal results. The LD1/LD2 ratio was <1 (i.e., was not “flipped”), which suggested that myocardial infarction was unlikely.

The patient was subsequently discharged without any further complication. Nevertheless, his CK isoenzyme pattern was unchanged from that seen at the time of admission.

1 Servicio de Análisis Clínicos (address for correspondence) and 2 Sección de Cardiología, Hospital San Agustín, Avilés, Asturias, Spain.
3 Departamento de Biología Funcional, Facultad de Medicina, Universidad de Oviedo, Asturias, Spain.
4 Nonstandard abbreviations: CK-MM, CK-MB, and CK-BB: skeletal muscle, heart, and brain types of cytoplasmic CK isoenzymes; and LD1 and LD2, isoenzymes of lactate dehydrogenase (LD).

Received January 26, 1989; accepted May 10, 1989.

CLINICAL CHEMISTRY, Vol. 35, No. 9, 1989 2003
Materials and Methods

Total CK activity in serum was measured according to the optimized standard method described by Szaasz et al. (19), with use of kit reagents from Boehringer Mannheim, Mannheim, F.R.G. The assay was done at 37 °C in a Hitachi-717 analyzer (Boehringer Mannheim). Our CK reference interval for healthy men is 25–196 U/L. To measure the low activities in eluates and supernates, we increased the amount of the sample in the assay to 20 μL; other conditions were unaltered.

Lactate dehydrogenase assays and other biochemical tests were also performed with the Hitachi-717 analyzer, all according to the manufacturer’s instructions.

The CK isoenzymes were separated by electrophoresis on agarose gel as described elsewhere (20). The reagents, controls, and apparatus for electrophoresis, including the Model 720 fluorometer, were purchased from Corning Medical Ltd., Halstead, U.K. The LD isoenzymes were separated by use of the same technique and apparatus.

To identify the CK isoenzyme bands that contained M subunits (CK-MM or CK-MB), we treated the serum sample with antibodies to subunit M for 5 min at room temperature, then precipitated the M subunit–antibody complex with a second antiserum for another 5 min at room temperature (these antisera were from Roche Diagnostics, Nutley, NJ 07110). We then electrophoresed, as above, the supernate remaining after precipitation. The absence of an initial band indicates the presence of M subunits; the presence of other bands indicates the absence of M subunits in them.

To determine which type of immunoglobulin was bound to the CK isoenzyme, we mixed 100 μL of serum with 100 μL of rabbit anti-sera (Atlantic Antibodies, Scarborough, ME 04074) to human IgA, IgM, or IgG; incubated at room temperature for 15 min; centrifuged (9600 × g, 15 min); and then electrophoresed on agarose. The absence from any tube of material corresponding to the band that migrates in CK-MB would enable the identification of an IgA-, IgG-, or IgM–CK type 1 complex.

To estimate the molecular size of the complex, we fractionated 1 mL of serum on a 40 × 1 cm column of Sephadex G-200 (particle size range 40–200 μm; Pharmacia, Upsala, Sweden), equilibrated, and eluted at room temperature with Tris buffer (50 mmol/L, pH 7.4) at the rate of 12 mL/h. We collected 0.5-mL fractions with a fraction collector. The void volume of the column (fraction 21, 10.5 mL) was determined with Blue Dextran (Pharmacia Fine Chemicals, Upsala, Sweden).

We could measure the CK activity of the different fractions by increasing the amount of the sample in the assay. Of the total CK activity applied to the column, 92% was accounted for.

The standards we used to estimate molecular mass were albumin, IgA, and IgG.

Results

The values for total CK in the patient’s serum samples that were collected at the time of admission, on the second day, and three and seven months later were 298, 445, 159, and 190 U/L, respectively. Electrophoresis of different samples and a control revealed two bands in all sample lanes, moving in the same position as CK-MM and CK-MB, and three bands in the control, including CK-BB (Figure 1). The percentage of the most anodic band of CK activity (CK-MB?) was about 25% of the highest value of the total CK activity and 65% of the lowest value of total CK activity of the patient. This indicated an atypical CK activity of 80–110 U/L (mean 95 U/L). Neither of the bands was visible when the same sample was incubated with the CK-isoenzyme substrate without creatine phosphate after electrophoresis; therefore, the bands did indeed represent creatine kinase activity.

Figure 2 shows the CK activity of the electrophoretic bands of samples taken at different times from the patient. The band migrating as CK-MB maintained a reasonably constant value for CK activity, independent of the total CK activity of the samples. The CK activity belonging to the most anodic band was too high and persistent (at present, longer than seven months) to be CK-MB. Therefore, we electrophoresed LD isoenzymes, but saw no LD1/LD2 >1.0 (the classical ‘flipped ratio’).

To determine if this anodic CK fraction contained M or B subunits, we incubated serum from the patient with antibodies to CK-M and determined the isoenzymes in the supernate. The same experiment was performed with a
control containing CK-MM, CK-MB, and CK-BB. Figure 3 shows the results. After immunoprecipitation of the control with anti-M antibodies, CK-MM and CK-MB were immuno-
precipitated; but CK-BB was unaffected. Precipitation of the patient's CK activity with anti-M antibodies had no effect on the electrophoretic mobility of the anodic band that migrated on CK-MB, whereas the CK-MM isoenzyme was completely eliminated.

Evidently this anodic band contained no M subunits. Therefore, if it was not CK-MB it could be a complex of CK-BB isoenzyme with another constituent of serum, as previously described (7, 10, 21).

After precipitation with CK-M antibody, the CK activity of the supernate was 79 U/L (total CK activity 120 U/L), which corresponded exactly with the activity of the most anodic band.

Figure 4 illustrates the results obtained in the experiment done with the patient's and normal serum, both mixed with control serum. It shows the affinity of CK-BB from the control with the anodic band of the patient's serum, thus confirming the presence of a CK-BB complex. In contrast, electrophoresis of the normal serum plus control did not result in a shift in any band.

Figure 5 shows the elution profile of the patient's serum on chromatography on a Sephadex G-200 column. The normal-size dimeric CK with a mean activity corresponding to 50% of the total CK activity was eluted in fractions 32–43 (peak fraction, 38). The other peak, which corresponds to an immunoglobulin–CK-BB complex, was eluted in fractions 19–31 (peak fraction, 25). We estimated the molecular mass of these peaks of CK activity by size-exclusion chromatography, using protein standards with known molecular mass. The slowest peak (the second) corresponded to an average molecular mass of 87 kDa, and it should contain CK-MM (theoretical Mr, 80 kDa). The first peak corresponds to an average Mr of about 191 kDa. To characterize the nature of the immunoglobulin–CK-BB complex, we measured the concentration of IgA, IgG, and IgM in the different fractions from the column. We incubated material corresponding to this peak and the patient's serum with antibodies to IgA, IgG, and IgM, using a normal serum as control. The peak that corresponded to IgA showed a molecular mass of about 164 kDa (theoretical Mr, 160 kDa).

We measured CK activity of fractions from the column, increasing the volume of the sample to 20 μL, the maximum permitted in the Hitachi-717 analyzer. Before elec-

trophoresing the eluted peaks with CK activity, we concentrated fractions 24–28 and 36–40 in a Minicon concentrator (Amicon Corp., Lexington, MA 02173). The peak that was eluted closer to the void volume (fractions 24 to 28) showed a single band, migrating in the agarose gel identical to CK-MB, which correspond to the immunoglobulin–CK-BB complex. The other concentrated peak also showed a single band, but its mobility was similar to that of CK-MM (Figure 6).

After treating with antibodies to IgA, IgG, and IgM the patient's serum and the material corresponding to the peaks eluted from the Sephadex G-200 column, we observed that the CK activity of the peak corresponding to material with the highest molecular mass was inhibited, totally or partly, by the presence of anti-IgA antibodies and that the rest of the anti-immunoglobulins had no effect. When we treated the patient's serum with anti-IgA, we obtained the same results: the most anodic electrophoretic peak disappeared (Figure 7). This indicates that the IgA–CK-BB complex is responsible for the band that migrates like CK-MB in electrophoresis and is eluted in the peak corresponding to material with an Mr of 191 kDa. This
molecular mass does not equal the sum of IgA plus CK-BB, but it can be explained, because the IgA–CK-BB complex appears close to the void volume of the column.

Finally, Figure 8 shows a summary of the different CK isoenzymes in the electrophoresis.

Discussion

The most common cause of increased CK-MB is acute myocardial infarction, but increased CK-MB does not necessarily indicate acute myocardial infarction, because this isoenzyme is also present in other tissues (17, 18, 22, 23).

Determination of LD isoenzymes has become routine in assessing myocardial damage and confirming a diagnosis of acute myocardial infarction (24-25); such determination is even more important if the CK-MB activity is very high and persistent.

Our finding of what was thought to be a high CK-MB activity in a serum sample with a total CK activity that only slightly exceeded the reference interval, as well as an LD1/LD2 ratio of <1 (not finding the classical “flipped” LD ratio), prompted us to undertake its characterization as a macro CK type 1.

Binding of an enzyme to an immunoglobulin has already been described for other enzymes, including amylase (26) and lactate dehydrogenase (27).

Type 1 CK complexes have been identified as CK-BB bound to IgG (28), IgA, or IgM (29), CK-MM bound to IgA (30), and CK-MB bound to IgG (27, 31). Although the electrophoretic mobility of these fractions is usually between that of CK-MM and CK-MB or CK-MB and CK-BB (8), an IgA–CK-BB complex that migrated as CK-MB was reported in an elderly woman with chest pain (7). The atypical CK complex we describe here migrated as a CK-MB and constituted a high percentage of the CK activity in the serum sample, independent of the total activity (Figure 2).

We demonstrated that this fraction was not the typical CK-MB isoenzyme, because it was not inhibited by the CK-M antibody. Mixing of the serum sample of the patient with a control that contained the three CK isoenzymes, CK-MM, CK-MB, and CK-BB, and electrophoresing demonstrated that the patient had an excess of antibody specific for CK-B in his serum, which altered the electrophoretic

Fig. 6. Electrophoretic fluorometric patterns of the patient's serum CK after elution from a Sephadex G-200 column
A, control; B, patient's serum before elution from the Sephadex G-200 column; C, CK activity of the concentrated fractions of the CK-activity-containing peak closest to the void volume; D, CK activity of the concentrated fractions from the second-eluted peak containing CK activity

Fig. 7. Fluorometric patterns for electrophoretically separated CK isoenzymes in the patient's serum before (A) and after treatment with rabbit antisera to human IgA (B), IgG (C), and IgM (D)

Fig. 8. Summary of CK isoenzyme patterns of control and patient's sera at different stages of investigation (isoenzymes identified in diagram at left)
1, control; 2, patient's serum; 3, patient's serum after immunoprecipitation with anti-IgA antibody; 4, two volumes of patient's serum mixed with one volume of isoenzyme control; 5, two volumes of normal serum mixed with one volume of isoenzyme control; 6, CK activity of the peak closest to void volume from the Sephadex G-200 column; 7, CK activity of the second-eluted peak from the Sephadex G-200 column; 8, patient's serum after treatment with anti-IgA immunoglobulin
mobility only of CK-BB, not CK-MM.

The finding of a peak for CK activity after the elution of the patient's sample serum from a Sephadex G-200 column, a peak corresponding to material with an estimated molecular mass of 200 kDa, suggested the possibility of an immunoglobulin–CK-BB complex migrating as a single band in a position identical to that for CK-MB on agarose gel electrophoresis. The disappearance of this in the presence of anti-IgA antibodies indicated that the atypical CK isoenzyme could be considered to be an IgA–CK-BB complex, especially because the atypical peak was unaffected by antibodies to IgM or IgG.

Most reported CK-type 1 and type 2 isoenzymes were found in cases of malignant tumor (32) or in patients with some other kind of disease (33). As for other enzyme-linked immunoglobulins, there seems to be no clear correlation with specific diseases (34).

Although an atypical CK isoenzyme in a patient's serum may persist for a long time (up to one month and a half), the "in vivo" existence of CK-BB activity within the immunoglobulin–CK-BB complex is usually extremely transient and, in turn, it seems obvious that this is a mechanism for the inactivation of serum CK-BB. Morin (35) showed that creatine kinase activity disappeared in vitro from serum at rates similar to those reported in vivo, and he concluded that CK inactivation must primarily be thermal in nature. Prabhakaran et al. (I) describe a theory based on a catalytic route by which CK-BB activity is eliminated from blood.

In conclusion, we have described an atypical macro CK-type 1 isoenzyme that migrates like CK-MB, and, like the macro CK-type 2 we previously described (16), it was found in serum of a relatively healthy person.

The IgA–CK-BB complex described in this report has substantive differences from others described so far:

(a) It had the highest CK activity yet described when one considers that the patients' total CK activity was within the normal reference interval (25–195 U/L) or only slightly increased the first three days after admission (maximum activity 445 U/L), and this could be explained by previous physical effort.

(b) The atypical IgA–CK-BB complex had a reasonably constant CK activity, 85 to 110 U/L, and it persisted in the serum for a long time.

(c) This case illustrates a disadvantage of using electrophoresis to quantify CK isoenzymes: electrophoretic mobility is nonspecific and therefore fluorescence in the MB region may not be due to CK-MB activity. In those cases in which a high CK isoenzyme activity appears, one should electrophorese the LD isoenzymes, looking for the LD1/LD2 >1 ratio, if one is to be sure of the diagnosis of acute myocardial infarction.

(d) We investigated this patient's immediate family in the same way and found no atypical type 1 isoenzymes.

Hitherto, atypical CK isoenzymes have been described in patients with tumors, and these tumors are probably the source of this isoenzyme. In our case, the patient was clinically healthy, with normal CK activity, but the complex was present in his serum for an unusually long time.

We thank the patient for his collaboration. We also thank all the technicians of the Clinical Biochemistry Section for their collaboration in this work, and Derrellyn Yates for secretarial help.

References
25. Lott JA. Serum enzyme determinations in the diagnosis of