Creatine Kinase Isoenzyme Variants in Human Serum

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In serum from about 800 patients, total creatine kinase and its subunit B activities were determined by the recommended Scandinavian creatine kinase method in the absence and presence of a creatine kinase M subunit inhibitory antibody. Eight patients had supranormal subunit B activities, but normal or near-normal values for total creatine kinase activity. Electrophoresis of sera from these eight patients showed, in addition to the normally migrating isoenzyme MM, one or two abnormally migrating creatine kinase isoenzymes, located between normally migrating isoenzymes MM and MB. Experimental data suggest that these abnormal bands may be isoenzyme BB with changed electrophoretic mobility. The eight patients had no particular disorder in common.

Additional Keyphrases: CK BB in human serum - CK electrophoresis on agarose - atypical CK isoenzyme - myocardial infarction - Ischemic heart disease

Occurrence of atypically migrating bands on CK1 isoenzyme electrophoresis has been reported. Lim (1) observed two atypical bands in serum, besides the three regular CK isoenzyme bands. The most anodic extra band, designated CK I-B, migrated between the MB and BB isoenzymes and was found in some neurology patients. The most cathodic extra band, designated CK II-B, migrated between MM and MB isoenzymes and was found in some patients suffering from severe angina pectoris. Sax et al. (2) and Fiolet et al. (3) noticed atypically migrating serum CK fractions located between MM and MB. These extra bands were found in sera of some patients with abnormally high CK MB activities as measured by an ion-exchange column-chromatographic method. Leroux et al. (4) demonstrated and described some physical properties of an atypical serum band, CK Z, migrating on electrophoresis between regular CK MM and CK MB.

During routine determination of total serum CK and S-CK B activities as described elsewhere (5,6) in about 800 patients we found eight patients who showed one or two abnormally migrating CK bands. S-CK B was above normal and total serum CK was normal or near-normal in all eight. Similar findings of increased S-CK B activity despite normal total serum CK activity as measured with the Merckotest CK MB immunoinhibition method have been reported by Lang et al. (Klin. Wochenschr., in press).

Materials and Methods

In about 800 patients, S-CK and S-CK B activities were determined in one or several samples. About 600 of these patients were admitted on suspicion of acute myocardial infarction. The remaining patients suffered from other conditions, such as cerebrovascular disease, hepatobiliary-tract disease, thyroid dysfunction, gastrointestinal malignancy, muscular disease, and renal failure. Furthermore, 30 patients undergoing heart catheterization, coronary angiography, or direct current countershock were included. Of these 800 patients, eight showed the combination of a normal, or slightly increased, total serum CK value and a CK B value increased above our acute myocardial infarction discrimination limit of 15 U/liter (6). We studied these cases further.

We measured total serum CK by an officially recommended method (7). On the basis of an evaluation of total serum CK activities in 90 healthy blood donors the upper reference limit was set to 250 and 150 U/liter for males and females, respectively. As previously described (6), the discrimination limit for total serum CK activity in acute myocardial infarction was 350 U/liter.

S-CK B subunit dependent activity was determined as residual CK activity after immunoinhibition of CK M subunit-dependent activity by a CK M subunit-inhibiting antibody (5). This antibody (anti-M) had been developed by E. Merck, Darmstadt (8-10), and was generously put at the authors' disposal. The immunoinhibition procedure measures activity associated with CK B subunit, but it does not discriminate between isoenzymes BB and MB. Consequently, the results are expressed as S-CK B activity. They are not multiplied by two to express the results as a calculated MB value. The acute myocardial infarction discrimination limit for S-CK B activity was set at 15 U/liter, as elsewhere described (6). Within-series imprecision of S-CK B measurement (n = 10 samples) was: X, 56.4 U/liter; SD 1.8 U/liter; and CV, 3%.

We electrophoretically separated CK isoenzymes on agarose gel, 20-μl samples of serum being applied to the gel (12 g/liter of 75 mmol/liter barbital buffer, pH 8.6). During the electrophoresis, buffer was continuously circulated and water-cooled at 10 °C.

After electrophoresis, a filter paper soaked with the CK reagent was placed on the agarose slide, incubated in a humid chamber for 45 min at 37 °C, and the filter paper removed. Fresh, dry filter papers were placed on the wet agarose slide, to soak up the NADPH produced. These filter papers were then dried at about 50-60 °C with a hair dryer and viewed in ultraviolet light. The fluorescence of NADPH formed at the sites of the different CK bands on the gel appeared at corresponding positions on the paper, but considerably intensified as compared to the fluorescence seen on a wet agarose slide. The sensitivity of the electrophoretic method was estimated at 15-20 U/liter by means of calibration experiments with dilutions of a pure human CK MB preparation of known activity.

The electrophoretic method was combined with immunoinhibition experiments. After electrophoresis, the agarose gel was overlaid with a filter paper that had been soaked in anti-M dissolved in imidazole buffer (100 mmol/liter, pH 6.7
Fig. 1. CK electrophoretic patterns of sera from eight patients with abnormally migrating CK isoenzyme bands

Serum from a patient with acute myocardial infarction, with additional human BB, was used as an electrophoretic marker. Rm values of CK MM, CK MB, CK BB, and of the abnormally migrating bands are shown. Total S-CK and S-CK B activities and the ratio of S-CK B to total S-CK are given

at 25 °C. The concentration of antibody was the same as the routine concentration used for CK B determinations (5). After paper and gel had been in contact for 20 min, the filter paper was replaced by a new filter paper, soaked with CK reagent with anti-M in the routine concentration. The slide was incubated at 37 °C and fluorescence developed as described above. Control experiments demonstrated that this procedure inhibited intense MM bands to only trace fluorescence, CK MB bands were distinctly decreased, and CK BB fluorescence was not visibly changed.

Results

Figure 1 shows the serum electrophoretic patterns for the eight patients with abnormally migrating isoenzyme bands. All eight sera were run on the same slide. The figure is a print of the original filter paper on which the fluorescence bands have been outlined and filled in with a pencil. Thus, while the positions and shapes of the bands are correctly reproduced, the relative fluorescence intensities are not shown. Serum from a patient with acute myocardial infarction, supplemented with human BB, was used as an electrophoretic marker.

As shown, total CK activities in serum were within the reference range in cases 1–7, slightly above it in case 8. In contrast, S-CK B activities exceeded our infarct discrimination limit of 15 U/liter in all cases. The highest value for serum CK B was found in case 8, for whom the CK B activity was 10-fold the discrimination limit. The fractions S-CK B/total serum CK varied from 17 to 87%.

On isoenzyme electrophoresis, the CK bands were identified according to their relative electrophoretic mobilities (Rm). Normally migrating BB and MM bands were assigned Rm values of 1.00 and 0.00, respectively. Consequently, normally migrating MB had an Rm value of 0.50.

Serum from all eight patients exhibited a more or less intense CK band in the MB MM band with an Rm value of 0.17. Two patients, cases 2 and 8, presented an additional aberrant band with an Rm value of 0.35. All bands were present in several samples and were not associated with any particular acute disease known to us. Control experiments without creatine phosphate in the CK reagent showed that none of the bands resulted from adenylate kinase activity.

On immunoinhibition of the CK bands in the agarose gel with the CK M subunit-inhibiting antibody as described above, the isoenzyme with the mobility of normal CK MM was completely inhibited in all patients' sera and control sera. The normally migrating MB of the control serum was distinctly, but not completely, inhibited. In contrast, the fluorescence intensities of the bands with Rm values 0.17 and 0.35, respectively, were not visibly decreased in any case.

The case histories of the eight patients were as follows (numbers refer to Figure 1).

Case 1: A 26-year-old woman with a systolic heart murmur since birth. Cardiac investigation, including heart catheterization, did not show evidence of organic heart disease.

Case 2: A 62-year-old woman suffering from diffuse muscle pain and headache. The clinicians' diagnosis was temporal arteritis. No signs of cardiac disease were found.

Case 3: A 79-year-old woman admitted because of chest pain considered as angina pectoris. There was, however, no evidence of acute myocardial infarction as evaluated by the combination of enzymatic and electrocardiographic criteria (6) and other clinical data.

Case 4: A 29-year-old woman suffering from thyrotoxicosis, being treated with carbimazol. No evidence of cardiac disease. A consistent electrophoretic pattern was observed in three different samples obtained during five months.

Case 5: A 77-year-old woman with known ischemic heart disease. She was admitted with chest pain considered as angina pectoris. No evidence of acute myocardial infarction from other enzyme tests and electrocardiogram.

Case 6: A 71-year-old man with prostatic carcinoma and ischemic heart disease but no recent acute myocardial infarction.

Case 7: A 67-year-old woman with ischemic heart disease, admitted with symptoms indicative of an acute myocardial infarction during the 3 h before admission. The electrophoretic pattern shown in Figure 1 was that of the sample taken on admission. She developed signs of an acute myocardial infarction with increased serum aspartate aminotransferase (EC 2.6.1.1) activity and diastolic electrocardiographic changes (6).

Total serum CK and S-CK B activities, as well as electrophoretic CK isoenzyme patterns of subsequent samples are illustrated in Figure 2. Total serum CK activity described a curve but did not exceed the acute myocardial infarction discrimination limit. S-CK B activity clearly was already increased at the time of admission. In the subsequent samples, S-CK B activities further increased but finally returned to the value at the time of admission. CK-electrophoresis of serum from the admission sample revealed an aberrant band (Rm 0.17) but no MB band. Electrophoresis of the subsequent samples showed both the Rm 0.17 fraction and the concomitant appearance of a normally migrating CK MB band.

Case 8: A 82-year-old woman suffering from gastric cancer with metastases to multiple organs, including the liver. The patient died; no signs of myocardial injury were found at autopsy.

Discussion

Why our eight patients had an abnormally high S-CK B

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activity has not yet been explained. As described, no common clinical denominator could be found. The abnormal CK B subunit activity may be due to a constitutionally abnormal rate of CK B subunit synthesis or it may be associated with some as yet unidentified chronic process in the patient.

The fact that four of our eight cases suffered from ischemic heart disease, may merely reflect the fact that 75% of our total patient material consisted of patients suspected of having acute myocardial infarction. In contrast, the remaining four patients with abnormal CK bands had no signs of ischemic heart disease. In our opinion, these data do not confirm the suggestion of Lim (1) that such abnormal bands are associated with severe angina pectoris.

As for the nature of the abnormally migrating bands, the following possibilities may be discussed:

1. They are aberrant CK MM bands. This possibility was excluded by our immunoinhibition experiments on the electrophoresis gel.

2. They are aberrant CK MB bands. This appears highly improbable for cases 1, 2, 6 and 8, because their S-CK B activity constituted more than half of the total serum CK activity. Numerous experiments with CK B determinations by the immunoinhibition method on isolated fractions of human CK MB consistently gave between 49 and 51% CK B activity in the MB hybrid. Consequently, the data strongly suggest that the abnormal bands are not MB hybrids.

3. They are aberrantly migrating CK BB bands. This might fit with the quantitative CK B data. Thus, e.g., in case 1 total CK would be distributed on the normal MM band and one abnormal CK BB band, about half on each. In cases 2 and 8, about 70% and 87%, respectively, of the total activity, would be distributed on two aberrant CK BB bands, the remaining 30 and 13%, respectively, on the MM bands.

The possibility of CK BB bands with electrophoretic mobility cathodic to that of CK MB has been experimentally verified (11, 12). We have done similar experiments, but the results were too erratic to warrant any conclusions.

In conclusion, the existence in serum of one or more CK isoenzymes with abnormal electrophoretic mobility, which cause increased CK B subunit activity, must be kept in mind when an immunoinhibition method is used for S-CK B determination in the diagnosis of acute myocardial infarction. Sera with an increased S-CK B activity, despite normal or only slightly increased total serum CK activity, should be particularly suspected to possess this atypical isoenzyme distribution. These cases can be recognized by the present electrophoretic method, but the particular combination of increased S-CK B and normal, or nearly normal, total S-CK activity makes them easily recognizable.

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References


Corrections

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p 900: Insert decimal in figures in Table 1, to read:

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p 2067, left-hand column, line 8: mp is 106-107.5°C

p 2108: The first equation in column two should read

\[ D_C = \frac{^{14}C-cpm}{C_2} \]

p 2109: Under the section on Standard curve, the third sentence has a part missing; it should read "the midrange (Table 2) of the [U-3H]glycocolchicosidic acid (c = 20.6 ± 7) and of the [U-3H]glycocolchic acid. . . .". In the last paragraph of this paper, the correct references are (5-7, 9).

p 2189: In this paper, Figures 3 and 4b should be interchanged.

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The addresses of the authors of the notes beginning on pages 149 and 150 were inadvertently interchanged.