Spurious Brain Creatine Kinase in Serum from Patients with Renal Disease

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Creatine kinase isoenzyme I (BB) is generally not detectable in normal serum, and its occurrence in serum has been documented in only a few disease states. In particular, increased activity of this isoenzyme has been reported in association with chronic renal failure, hemodialysis, and renal transplantation. The present study demonstrates that the apparent creatine kinase observed in the serum of such renal patients is an artifact, observed as a result of measuring creatine kinase isoenzymes by fluorescence. Our observations resemble those of McKenzie et al. [Clin. Chim. Acta 70, 333 (1976)] concerning an artifact in the fluorometric determination of lactate dehydrogenase isoenzymes in the sera of patients with end-stage renal failure. The artifact binds to albumin, is not a protein, and occurs in some normal sera at very low concentrations. This artifact can be mistakenly identified as isoenzyme I in renal-disease patients if CK isoenzymes are determined fluorometrically.

Additional Keyphrases: analytical error · renal failure fluorometry of creatine kinase · lactate dehydrogenase

The need to separate creatine kinase (EC 2.7.3.2; CK) isoenzymes in the diagnosis of muscular dystrophy and myocardial infarction is well established (1). Creatine kinase is a dimer that occurs in three isoenzyme forms: CK I, CK II, and CK III. Elevations of serum CK III and CK II are used as criteria in the diagnosis of muscle trauma or myocardial infarction, respectively. CK I (also called "BB" or "brain-type CK") is present in many other tissues and especially in smooth muscle (2). CK I is generally not detectable in normal serum, and its presence in serum has been documented in only a few disease states. In particular, increased serum CK I activity has been reported in association with chronic renal failure, hemodialysis, and renal transplantation1 (3, 4).

Here, we demonstrate that the apparent CK I observed in the serum of such renal patients is an artifact, which is observed as a result of measurement of creatine kinase isoenzymes by fluorescence. Our observations resemble those of McKenzie and Henderson (5) concerning an artifact in the fluorometric determination of lactate dehydrogenase (EC 1.1.1.27; LDH) isoenzymes in the sera of patients with end-stage renal failure. Thus far, our characterization has established the artifact as being non-protein in nature, bound to albumin, and possibly occurring in normal serum in low concentrations. This artifact can be mistakenly identified as CK I in renal-disease patients if fluorometric detection is used in determining CK isoenzymes.

Materials and Methods

Sera were obtained from patients with end-stage renal failure who were being maintained on hemodialysis or who had recently received a renal transplant. These specimens are referred to as "renal-patients' sera." Samples were stored at −20 °C and assayed for CK within 24 h. Normal sera were also obtained and treated similarly. CK and LDH electrophoretic separations were performed on the Corning/ACI System (Corning ACI, Palo Alto, Calif. 94306) according to the manufacturer's instructions, using controls obtained from Worthington Biochemical, Freehold, N.J. 07728 (cat. no. 7659). Electrophoresis patterns were scanned with a Clifford Densitometer equipped with fluorescence attachment (Corning Scientific Instruments, Medfield, Mass. 02052). For CK identification we used essentially the Rosalki method (6); production of NADH was measured by its fluorescence at 455 nm when excited at 340–375 nm. To prove the existence of the artificial fluorescent species, normal and renal-patient serum samples were analyzed by the CK isoenzyme procedure with and without the CK assay reagents.

For serum protein electrophoresis we used the Beckman Microzone–Ponceau-S System (Beckman Instruments, Fullerton, Calif. 92634). Protein electrophoresis patterns were photographed under visible light. To locate the point of migration of the fluorescent species, the protein stain was omitted and the plate was photographed under long-wavelength ultraviolet light.

We also fractionated serum CK by ion-exchange column chromatography (Worthington). CK activities were measured with the Beckman System TR (Beckman Instruments), with Beckman reagents.

Sera from normal persons and renal patients were scanned for endogeneous fluorescence with a spectrofluorometer (Model 204-A; Perkin-Elmer Corp., Norwalk, Conn. 06856) after dilution with 0.10 mol/liter NaCl.

Results

A typical CK isoenzyme pattern for renal patient sera is shown in Figure 1A. The pattern for the identical sample carried through the same procedure but without the reagents

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CK ISOENZYME III II I ANODE

A WITH CK SUBSTRATE OVERLAY

B WITHOUT CK SUBSTRATE OVERLAY

Fig. 1. (A) Fluorescent scan of the CK isoenzyme pattern obtained from the serum of a patient on maintenance hemodialysis for end-stage renal disease.

Pattern B, obtained from the same sample, but without substrate overlay, indicates a "blank" fluorescent band in the CK I position.

Fig. 2. Fluorescence emission spectrum of normal serum (---), and serum from a patient being maintained by renal dialysis (--). Both sera diluted 30-fold with water; excitation wavelength, 366 nm.

(substrate) to develop the CK bands is shown in Figure 1B. Note that the fluorescent band in the renal serum remains at the CK I position. These findings were observed for more than 50 sera from renal patients. Analogous experiments in which the LDH fractionation method was used demonstrated the renal sera giving rise to an additional band, anodic to LDH-1.

In a preliminary effort to classify the spurious fluorescence, normal and renal patients' sera were subjected to routine protein electrophoresis. The fluorescence was located by omitting the protein stain (Ponceau-S) and photographing the electrophoretogram under ultraviolet light. The fluorescence in sera from renal patients was associated with the albumin fraction. In normal serum, a slight fluorescence in the albumin region was also observed.

We did several studies to positively distinguish the material responsible for the fluorescence in the renal-disease sera from NADH. Figure 2 shows the fluorescence of a typical renal patient's serum compared to normal serum. The serum was diluted 30-fold with distilled water (pH approximately 7.0) and fluorescence emission spectra were recorded. Renal patient samples exhibited an emission peak at 440 nm when excited at 366 nm. In another experiment, CK substrate was added to a renal patient's serum, and serial fluorescence spectra were recorded during 60 min. The initial emission spectrum (Figure 3A, 1 min) essentially represents the endogenous fluorescence in the renal-patient serum. As the CK reaction proceeded, NADH was produced, altering the emission spectrum until, at 60 min, the spectrum resembled that of NADH. When this reaction mixture (from Figure 3, spectrum D) was further diluted (900-fold with distilled water), two emission maxima could be detected, at 449 and 425 nm.

Fig. 3. Fluorescence spectra of a mixture of 0.1 ml serum from a patient on renal dialysis, 1.0 ml of the CK assay solution, and 1.9 ml water.

Recorded at 1 min (A), 10 min (B), 25 min (C), and 60 min (D). Emission wavelength maximum shifts with time. Excitation wavelength, 366 nm.

Fig. 4. Fluorescence spectrum of a portion of the solution from spectrum (D) of Figure 3, which was further diluted to 900-fold with water.

Excitation wavelength, 366 nm.
(Figure 4). Presumably, the 449-nm maximum was primarily due to NADH and the 425-nm shoulder was ascribable to the fluorescence of the renal-disease sera.

Renal-patients’ sera were also treated with trichloroacetic acid (200 g/liter solution), to precipitate serum proteins. After centrifuging, the supernatant fluid was decanted and its fluorescence emission spectrum was recorded. Most of the fluorescence was present in the supernate. Attempts by ion-exchange column chromatography to separate the CK isoenzymes were inconclusive. The endogenous fluorescence in renal serum samples eluted with the CK I fraction; however, some eluted with the CK II fraction or remained on the column.

Discussion

Using fluorometric detection methods, Galen reported abnormally high CK I in the serum of 19 patients with renal failure who were undergoing dialysis (3). Wesley and Byrnes (7) similarly observed that significant CK I was present in the serum of patients in different stages of chronic renal failure. In contrast to an earlier report, our results indicate that CK I is not increased in these patients; rather, there is an increase in a substance that acts as a fluorescent interference in the determination of CK I by electrophoresis and fluorescent densitometry. The substance is found in some sera, but in much lower concentrations; it is not-protein in nature. Since the preliminary report of this work, Aleyassine et al. have confirmed the artificial nature of this fluorescence in a similar patient population, by a different fluorometric CK separation method (12).

Before concluding that this fluorescence was spurious, we made several measurements of “CK I” in the serum from four patients who received renal transplants. The data indicated that the fluorescence quantitatively correlated with the clinical status of the transplant; that is, the artificial fluorescence returned to the intensities found in normal serum after one month if the graft was successful, perhaps as a result of improved renal clearance.

Several of our renal samples were extracted into acidified chloroform and subjected to mass spectral analysis after separation by gas chromatography. However, only increased fatty acids were identified in these samples—evidently none of the gas-chromatographic peaks identified by mass spectroscopy corresponds to the fluorescent substance. Various other solvent systems were tried, unsuccessfully. In several respects our observations confirm the report of McKenzie and Henderson (5) who reported an artificial fluorescent band for the fractionation of LDH isoenzymes in a simular population of patients:

1) The observed excitation and emission spectra in both reports are similar.

2) The fluorescence decreases after successful kidney transplant.

3) The artificial fluorescence coincides electrophoretically with albumin.

We suspect that the endogeneous fluorescent compound may be the result of vitamin B₆ (pyridoxine) supplementation in the diet of renal patients. As a class the vitamin B₆ compounds (and metabolites) fluoresce in the same spectral region as the artifact. But McKenzie and Henderson concluded from in vivo and in vitro evidence that the vitamin B group was not responsible for the effect. However, addition of pyridoxine to normal serum produces a fluorescence spectrum different from the one produced when it is added to renal-patient’s serum. Coursin and Brown have shown that 30 mg of pyridoxine HCl, given orally every 4 h for one day, did produce fluorescence in the blood (8). Further, pyridoxine is rapidly converted to pyridoxal phosphate in vivo, and there are marked differences in the extent to which each B₆ compound is bound to albumin (9). Anderson also found that pyridoxine added to plasma did not bind to protein; however, pyridoxal phosphate was substantially bound along with some pyridoxal (9). The actual clearance rate of B₆ compounds from serum is not well-defined; however, Snell reported that only 15–20% of small amounts of [³H]pyridoxine administered to human subjects was recovered in the urine the first day, and that the remainder was excreted with a half life of 18–38 days (10).

Increased dietary supplementation of B₆ compounds lead to increased tissue levels of pyridoxal phosphate, which may account for prolonged increases in serum fluorescence, even after supplements are discontinued (11).

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References


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