Elevations of Creatine Kinase Isoenzyme CK\textsubscript{i} in Patients with Exposure Induced Hypothermia*

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ABSTRACT

Three cases of exposure induced hypothermia have been detailed. In each case, an elevation in total creatine kinase was observed as well as the development of the brain isoenzyme of creatine kinase, a condition not previously reported. The presence of the brain isoenzyme is assumed to be due to the localized hypoxia which develops as a secondary condition of hypothermia. It appears that conditions do exist wherein the presence of the brain isoenzyme of creatine kinase should not be a totally unexpected or unexplainable situation.

Introduction

Creatine kinase (CK) (EC 2.7.3.2) exists in three isoenzyme forms, each composed of two peptide subunits. The isoenzymes have been classified as CK\textsubscript{i} (brain type), CK\textsubscript{a} (cardiac type) and CK\textsubscript{m} (muscle type) based on their electrophoretic mobility. With electrophoretic separation and fluorescent detection, CK\textsubscript{i} is usually the only CK isoenzyme seen in the sera of healthy human subjects.

The occurrence of CK\textsubscript{i} was once thought to be quite rare and was observed only in a few conditions such as malignant hyperpyrexia, Reye's syndrome, acute brain injury and after neurosurgical intervention. More recently, however, CK\textsubscript{i} has been seen under a number of circumstances including prostatic cancer, gastric cancer, oat cell carcinoma, in renal disease, during labor, during coronary bypass surgery and after cardiac arrest and resuscitation. The isoenzyme has also been studied as a tumor associated marker and has been demonstrated in spinal fluid as a marker of a variety of disease processes. It is becom-
ing increasingly obvious that the occurrence of CK₁ is not as rare as was once believed. Most recently, with increased analytical precision, normal values for serum activity of CK₁ have been determined in both children and adults. The presence of CK₁ is reported here in the sera of three patients with exposure induced hypothermia, a condition in which CK₁ elevations have not been previously reported.

**Methods**

Routine laboratory testing was performed.* All CK isoenzyme studies were carried out by electrophoresis on agarose gel using the Corning ACI system† using the manufacturer's supplies and recommended procedure. Fluorescence owing to non-CK sources was ruled out by viewing the plates under long wave ultraviolet light before treating with enzyme substrate. Fluorescence owing to myokinase was ruled out by treating electrophoresis plates upon which separations of both patient specimens and standards had been performed with an enzyme substrate which did not contain creatine phosphate.† After incubation, these plates showed no fluorescence when viewed under long wave ultraviolet light.

In this study, all electrophoresis plates were viewed under long wave ultraviolet light and results for the various isoenzymes reported as negative to four plus (4+) versus a standard. 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 per 5 ml CK₁, 112 IU per 5 ml CK₂ and 412 IU per 5 ml CK₉ and was reconstituted and used as directed by the manufacturer.

Confirmatory electrophoreses were performed using the Bioware “Biopak” CPK-C method.¶ In this method, the isoenzymes are visualized colorimetrically as a formazan complex.

**Case Histories**

**Case I**

The first patient was a 61-year-old white male who was exposed to a temperature of 5°F for approximately 12 hours. The evening prior to admission he had indulged in intoxicating beverages to excess and subsequently fell in the snow. He was unable to get up and remained there until found by the police the next morning. On arrival at the emergency room, his rectal temperature was below 94°F, respiration 28, blood pressure 140/80 and pulse 140 and irregular. He was confused and oriented as to person only. He was extremely cold to the touch and suffered from frostbite of the nose, ears, hands and feet.

Initially, he had a blood pH of 7.269, pCO₂ of 28.9 mm Hg, pO₂ of 136.4 mm Hg and bicarbonate of 12.8 mEq per liter. His serum sodium was 140 mEq per liter, potassium 4.3 mEq per liter, chloride 94 mEq per liter, CO₂ 17 mEq per liter, BUN 17 mg per 100 ml and glucose 115 mg per 100 ml. His white blood cell count was 12,700 per mm³, red blood cell count 5.16 million per mm³, hemoglobin 16.5 g per 100 ml and hematocrit 48.8 percent. Creatine kinase was 4450 mU per ml (normal 30 to 150 mU per ml), lactate dehydrogenase was 875 mU per ml (normal 100 to 225 mU per ml) and serum glutamic oxaloacetic transaminase was 325 mU per ml (normal 10 to 40 mU per ml). Electrophoretic separation of his CK isoenzymes showed 1+ CK₁, trace CK₂ and 4+ CK₉.

He was treated with intravenous fluids, thermal blankets and warm water enemas. He responded well to this therapy with rapid warming of his temperature and spontaneous conversion of his atrial fibrillations to a sinus rhythm.

The day after admission his CK was 6000 mU per ml, LDH 900 mU per ml and SGOT 725 mU per ml. CK isoenzymes were trace CK₁, trace CK₂ and 4+ CK₉. Five days after admission his CK had dropped to 120 mU per ml, LDH to 85 mU per ml and SGOT to 220 mU per ml. His CK isoenzyme pattern was normal, showing only a trace of CK₉.

**Case II**

The second patient was an 85-year-old black male who was exposed to a temperature of 0°F with a wind chill factor of -30°F. It was not known how long he was exposed to the weather, but it was at least two hours. He was ill clothed for the weather conditions. There was neither history nor evidence of alcohol ingestion. He was noticed to be in distress by police

* Snodgras Laboratory, Saint Louis City Hospital, Saint Louis, MO.
† Coming Medical Works, Medfield, MA.
‡ Kindly supplied by Coming Medical Works.
§ Helena Laboratories, Beaumont, TX.
¶ Clinical Chemistry Laboratory, Barnes Hospital, Saint Louis, MO.
§§ Bioware Products, Whippany, NJ.
officers and brought to the emergency room for treatment.

On arrival, his rectal temperature was below 94°F, respiration 20, blood pressure 98/60 and pulse 90 and regular. He was well oriented but in moderate distress with marked shivering. His skin was very cold, but he had only minor frostbite of both pinnae.

Initially, his serum sodium was 142 mEq per liter, potassium 4.3 mEq per liter, chloride 114 mEq per liter, CO₂ 21 mEq per liter, BUN 33 mg per 100 ml and glucose 90 mg per 100 ml. His white blood cell count was 16,000 per mm³, red blood cell count 4.21 million per mm³, hemoglobin 12.9 g per 100 ml and hematocrit 39.1 percent. His creatine kinase was 1010 mU per ml, lactate dehydrogenase 245 mU per ml and serum glutamic oxaloacetic transaminase 78 mU per ml. His creatine kinase isoenzymes were 2+ CK₁, trace CK₂ and trace CK₃.

He was treated with intravenous fluids and warm water baths (100°F). He responded quite well to this warming therapy.

The day following admission his enzyme concentrations had all increased with CK reaching 5300 mU per ml, LDH reaching 500 mU per ml and SGOT reaching 250 mU per ml. His CK isoenzymes were now trace CK₁, trace CK₂ and 4+ CK₃. Two days after admission his CK had decreased to 2900 mU per ml with the other enzymes following suit. The patient had progressed well by this time and no further blood tests were performed. The patient was followed in the clinic two weeks after the incident and all enzyme activities had returned to normal values.

CASE III

The third patient was a 55-year-old white male exposed to a temperature of 17°F for an indeterminate period of time. He had been seen twice in the emergency room with an impression of alcohol intoxication each time. Eighteen hours after the last visit he was found lying in the snow and was admitted to the hospital. His only recollection was of being intoxicated and of having fallen numerous times, some with injury. He apparently lost consciousness and was lying on the ground out of doors for at least six hours before being found by police officers who took him to the emergency room.

On arrival at the emergency room, his rectal temperature was 85°F, respiration 26, blood pressure 127/80 and pulse 130 and regular. He was cold to the touch, shivering and arousable but not oriented as to time, place or person. He had multiple contusions of the right eye, elbows, knees and ankles. There was no frostbite.

On admission, his blood pH was 7.117, pCO₂ was 34.7 mm Hg, pO₂ was 71.0 mm Hg and bicarbonate was 10.7 mEq per liter. His serum sodium was 144 mEq per liter, potassium 5.0 mEq per liter, chloride 100 mEq per liter, CO₂ 9 mEq per liter, BUN 21 mg per 100 ml and glucose 120 mg per 100 ml. His white blood cell count was 28,600 per mm³, red blood cell count 4.81 million per mm³, hemoglobin 18.0 g per 100 ml and hematocrit 51.3 percent. His creatine kinase was 2000 mU per ml, lactate dehydrogenase was 395 mU per ml and serum glutamic oxaloacetic transaminase was 80 mU per ml. His creatine kinase isoenzymes were 1+ CK₁, 2+ CK₂ and 4+ CK₃.

He was treated with warming blankets, warm water enemas and intravenous fluids. He responded rapidly with therapy.

The morning after admission his CK had increased to 6000 mU per ml, LDH to 775 mU per ml and SGOT to 225 mU per ml. His enzyme concentrations remained high until the third hospital day when CK was 3550 mU per ml, LDH 397 mU per ml and SGOT 226 mU per ml. Further enzyme studies were not carried out during the remainder of his hospitalization. The patient was followed in the clinic two weeks after the incident and all enzyme activities had returned to normal.

Discussion

Elevations of serum enzymes have been well documented in exposure induced hypothermia.2-9,15,20 Significant rises in serum enzymes were seen in each of these studies although no investigations of isoenzymes were made.

The isoenzymes of creatine kinase were studied by Meltzer in specimens obtained from rats which had been exposed to the cold while under restraint.15 The restraint, which prevented physical activity to maintain body temperature, was achieved by wrapping the rats in gauze and securing the wrap with tape. Elevations of as much as 28 fold were observed in total creatine kinase activity with the concomitant development of significant amounts of the brain isoenzyme. The production of the brain isoenzyme was attributed to a possible increase in the permeability of the blood brain barrier as well as increased permeability of brain cells. This claim was based in part on the studies of Angel dealing with the effect of different types of stress on the blood brain barrier of the rat.1 Studies confirming the production of the brain isoenzyme of creatine kinase in rats held under cold restraint have been carried out in our laboratories.

Hypothermia seems to be the only common denominator in our patients. Frostbite was extensive in one, present to a minor degree in another and absent in the third. The third patient did have ex-
tensive tissue damage from numerous falls, but this would only account for a rise in the activity of CK₃, not CK₁. Two of the patients had been intoxicated, but the other had no evidence of recent alcohol ingestion. Intoxication appears to be only a contributing factor to the hypothermia. Although all of these patients had CKₑ present in their serum, there was no electrocardiographic evidence that any had suffered a myocardial infarction.

The exact source of the CKᵢ in these patients is not known. It appears, however, that a systemic insult is the cause as all three of the creatine kinase isoenzymes were present in these patients. Bristow and coworkers¹⁹ have suggested that the total rise in creatine kinase owing to hypothermia is from muscle and feel that hypothermia serves to protect the brain from damage owing to hypoxia. This hypothesis, however, does not explain the presence of CKᵢ or CKₑ in the serum of hypothermia victims which has been observed by us.

It is our opinion that the presence of CKᵢ in the serum of these patients is due to a hypoxic condition which results from hypothermia. This theory is supported by the findings of Meberg and coworkers¹⁴ and Kjekshus and Vaagenes¹⁶ who have demonstrated the presence of the brain isoenzyme of creatine kinase in the spinal fluid of both newborn infants and adults who have been hypoxic. This is borne out by observations made in our hospitals.⁴ CKᵢ has been shown by us to be present in the serum of patients undergoing open heart surgery where intentionnal hypothermia is used as a protective measure as well as in patients who have suffered cardiopulmonary arrest and been resuscitated. Arrest does produce localized hypoxia and it is suggested by us that this is the major contributing factor to the production of CKᵢ as well as total creatine kinase owing to increased cellular permeability.

Summary

Three cases of exposure induced hypothermia have been followed. It has been shown that the brain isoenzyme of creatine kinase (CKᵢ) has been released into the serum in each case. It is the feeling of the present authors that the appearance of this isoenzyme is due to the localized hypoxia which occurs secondary to the hypothermia. It appears that the brain isoenzyme of creatine kinase may not be the rare occurrence that it was once assumed to be.

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References