Macro Creatine Kinase in a Case of Carnitine Palmitoyltransferase Deficiency

Hitoshi Deguchi,1 Naruji Sugiyama,2 Hiroshi Kawamura,3 Taizo Uemura,1 Akira Shimizu,4 and Masahiro Yamamoto1

A stout man was admitted to the hospital with acute rhabdomyolysis associated with macro creatine kinase (macro-CK, EC 2.7.3.2). This anomaly of CK was detected by gel electrophoresis as an atypical band between CK-MB and CK-MM, classified according to Stein's criteria (Clin Chem 1982; 28:19–24) as type 1, and identified by immunofixation electrophoresis as containing CK isoenzymes MM and MB and immunoglobulin A. Muscle biopsy showed that the etiology of rhabdomyolysis in this case was deficiency of carnitine palmitoyltransferase (CPT, EC 2.3.1.21) in the muscle. We report the first observation of macro-CK in a case of CPT deficiency; its presence may result from recurrent rhabdomyolytic attacks owing to CPT deficiency, and may suggest underlying enzymic abnormality in muscle.

The presence of macro creatine kinase (macro-CK, EC 2.7.3.2) has been reported in various conditions, e.g., malignancy (1), ischemic heart disease (2), and even in a relatively healthy person (3). However, it has not been reported in carnitine palmitoyltransferase (CPT, EC 2.3.1.21) deficiency, although nearly 40 patients with CPT deficiency have been described since 1973 (for a review, see ref. 4). Type-1 macro-CK contains immunoglobulin and may result from recurrent myolytic attacks in various diseases. We report here the first observation of macro-CK in a patient with CPT deficiency, who developed rhabdomyolysis with renal damage.

Case History

A 34-year-old man (178 cm tall and weighing 80 kg) complaining of broth-like dark urine was admitted to our hospital. Manager of a department store, he had joined "Hell Camp," a renowned Japanese management school for more aggressive salesmen and managers. After the usual strenuous exercise, he advanced to a more intensive course, in which he did more than 60 push-ups while fasting. He felt myalgia, heat, and swelling in his upper extremities and anterior chest wall, without muscle cramps; the next day he noticed dark urine. Physical examination revealed normal vital signs and nonpitting edema on the swollen muscles. Neurological examination revealed no abnormality. Laboratory examinations showed increased concentrations of aspartate aminotransferase (EC 2.6.1.1) (626 U/L; normal, 11–34 U/L), and alanine aminotransferase (EC 2.6.1.2) (469 U/L; normal, 7–33 U/L), and extremely high concentrations of CK (131.6 kU/L; normal, 45–174 U/L) with atypical CK and oliguria, which suggest acute rhabdomyolysis and renal damage, respectively. Six months previously, as well as 18 years ago, the patient had also noticed dark urine.

We initiated and maintained saline diuresis, during which time the concentration of CK increased. Fourteen days after onset his concentration of myoglobin in plasma returned to normal, renal function was restored, and his swollen extremities and weak grip were alleviated without hemodialysis. Electromyography revealed myopathic motor unit potentials in the swollen upper extremities, with normal conduction velocity. Eight days after onset, cardiac scintigraphy (99mTc-pyrophosphate) clearly highlighted the swollen, painful bilateral upper extremities and pectoral muscles, and swollen renal contours. Muscle biopsy showed a few regenerating fibrils and scattered necrotic muscle fibers that were phagocytic, suggestive of acute rhabdomyolysis. The specimen showed many intact muscle fibers by the ATPase reaction, but thorough decrease in both type-1 and -2 fibers by NADH reductase reaction with tetrazolium. Oil Red stain for neutral fat showed no excess lipid. An atypical CK band between CK-MB and CK-MM was identified by gel electrophoresis as CK-bound immunoglobulin, which was identified by immunofixation electrophoresis as immunoglobulin A (IgA) (κ,λ). Several months after the onset of symptoms, an ischemic forearm test showed a normal increase in lactate concentrations in plasma. Muscle biopsy was repeated for further analysis.

Materials and Methods

We determined values for total CK and other biochemical tests with a Dri-chem 5000 Analyzer and Fuji kit reagents (Fuji Photo Film, Saitama, Japan), according to the manufacturer's instructions. Our reference interval for total CK in healthy men was 45–174 U/L. We separated the CK isoenzymes by electrophoresis on a cellulose acetate membrane (Titan III; Helena Laboratories, Beaumont, TX), as described elsewhere (5). The reagents and staining material (Cardiotrac-CPK) were from Ciba-Corning Diagnostics, Medfield, MA; controls and apparatus for electrophoresis, including densitometry (Cliniscan EDC-type), were also from Helena Laboratories. To estimate the molecular mass of the atypical CK, we used thin-layer (1 mm) gel-filtration chromatography, with Sephadex G-200 superfine gel (particle size range, 10–40 μm; Pharmacia, Uppsala, Sweden), as described (6). The standards used to estimate molecular mass were albumin, IgG, and IgM. We determined by immunofixation which type of immunoglobulin was bound to the CK isoenzyme, according to the standard method described by Ritchie and Smith (7). Antibodies to immunoglobulin antisera (e.g., anti-κ, -λ, -IgM, -IgA, and

1 Third Department of Internal Medicine, Nissei Hospital, affiliated with Nippon Life Insurance Co., Osaka, Japan.
2 Department of Pediatrics, Nagoya City University Medical School, Nagoya, Japan.
3 First Department of Internal Medicine, Osaka Medical College, Takatsuki, Japan.
4 Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan.

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-IgG) were purchased from Dako, Glostrup, Denmark, and the Separex membrane (2 x 30 mm) from Fuji Photo Film.

To isolate the immunoglobulin fraction in serum, we added one aliquot of 500 mmol/L glycine-HCl buffer (pH 3.4), followed by two aliquots of 4 mol/L ammonium sulfate solution, and then centrifuged the mixture. We mixed and washed the precipitate with 1.34 mol/L ammonium sulfate solution, centrifuged, redissolved the precipitate with isotonic saline, and precipitated the protein with an equal volume of 4 mol/L ammonium sulfate solution; this cycle was repeated three times. The resulting precipitate was dissolved with isotonic saline, dialyzed against 67 mmol/L sodium phosphate buffer, and finally concentrated to one-third of the original serum volume. The immunoglobulin fraction of the patient's serum was mixed with CK isoenzyme control sera (rich in CK-MM, -MB, and -BB); after incubation at 4 °C for 12 h, we electrophoresed this mixture on agarose, with staining as described previously. We determined CPT activity in muscle by the isotope-exchange method (8).

**Results**

The concentration of CK in the patient's serum on admission had increased to 131 kU/L and showed an abnormal band between CK-MB and CK-MM. We identified this abnormal band by thin-layer gel-filtration electrophoresis (Figure 1) as macro-CK (relative molecular mass, ~750, 000, based on its relative mobility). Macro-CK was still detected after 10 days, but not four months later. Renal failure, often associated with acute rhabdomyolysis, was slight and was alleviated two weeks after treatment was begun (Figure 2). Immunofixation electrophoresis showed that this macro-CK was composed of IgA (κ,λ) and CK (Figure 3). The immunoglobulin fraction of the patient's serum was reactive with CK-MM and CK-MB, but not CK-BB, which produced tailing of the CK-MM and -MB bands and decreased the original CK-MM and -MB bands (Figure 4). The muscle biopsy specimen revealed increased free and total carnitine (275% and 191% of that of an unaffected control, respectively), although the concentration of esterified carnitine remained unchanged. The CPT activity in muscle was only 40 pmol/min per milligram of protein (16% of control).

**Discussion**

We report the first case of acute rhabdomyolysis with macro-CK in a patient with CPT deficiency. Since the report of two brothers with recurrent myoglobinuria in 1973 (9), more than 40 patients with CPT deficiency and muscular presentation have been identified. In our case, CPT activity decreased to ~16% of that in the normal control, which led to insufficient energy and served as the etiologic factor in recurrent rhabdomyolysis. In such a patient, the great demand for energy in severe illness (10), prolonged hard exercise, or fasting may exceed the available supply and result in rhabdomyolysis, often associated with fatal renal failure (11). Although macro-CK has been reported in association with many diseases, e.g., malignancy and ischemic heart disease, and even in a relatively healthy person, its presence may suggest underlying disorders.

IgA from our patient showed reactivity with CK isoenzymes MM and MB, but not BB. We conclude from clinical findings that the CK component in this macro-CK is probably CK-MM, although CK-MB is also possible. The origin of the macro-CK is unknown, but recurrent rhabdomyolytic attacks may have induced antibody production against denatured CK (M-subunit), which perhaps created an intravascular catabolic pathway for CK degradation (12). The rarity of both conditions makes chance association unlikely, but the only way to prove a relationship would be to study a large number of patients. In the past two years, at least two cases of macro-CK in rhabdomyol...
Analysis have been reported in Japan (personal communications).

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Fig. 4. Reconstruction of macro-CK: Ig fraction of patient’s serum was mixed with CK-isoenzyme control sera, electrophoresed, and stained with formazan; (left) stained gel and (right) diagram

Reconstituted macro-CK (tailing) identified shows Ig activity with CK-M subunits, but not with B subunit. Lane 1, CK-MM-rich serum; lane 2, sample in lane 1 mixed with patient’s Ig; lane 3, CK-MB-rich serum; lane 4, sample in lane 3 mixed with patient’s Ig; lane 5, CK-BB-rich serum; lane 6, sample in lane 5 mixed with patient’s Ig; lane 7, patient’s Ig; lane 8, patient’s serum; and lane 9, normal control.

References

In Japanese.