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RADIOIMMUNOASSAY OF CARDIAC MYOSIN LIGHT CHAIN II IN THE SERUM FOLLOWING EXPERIMENTAL MYOCARDIAL INFARCTION

R. Nagai, M.D., S. Ueda, M.D. and Y. Yazaki, M.D.

Cardiovascular Research Unit,

The Third Department of Internal Medicine,

Faculty of Medicine, University of Tokyo, Tokyo, Japan

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Summary

A sensitive radioimmunoassay of cardiac myosin light chain II (LC II) was developed, and changes of serum LC II levels were studied in experimental myocardial infarction in dogs. In our assay, 0.2-5 ng of LC II were effectively measurable. The time-course curves of serum LC II level after coronary occlusion had two characteristics which had not been observed in serum enzyme studies; (1) LC II level rose rapidly and stayed up during a long period, (2) changes of serum LC II levels were biphasic in some cases.

INTRODUCTION

Many kinds of enzymatic methods such as CPK, GOT and LDH or the radioimmunoassay of myoglobulin have now been widely used for diagnosis of myocardial infarction. However, most of them are not necessarily sufficient in sensitivity and specificity. In this study we attempted to develop a sensitive radioimmunoassay of a smaller subunit of cardiac myosin molecule, light chain II (LC II), which is a specific protein to the cardiac muscle with molecular weight of 20,000.

With this radioimmunoassay, LC II levels in serum of dogs with coronary occlusion were determined serially until 7 days after operation. We also studied the disappearance rate of LC II from the blood stream when injected intravenously.

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MATERIALS AND METHODS

PURIFICATION OF LIGHT CHAIN II Cardiac myosin was extracted from canine left ventricles and interventricular septa by the dilution technique described previously (1), and purified by chromatography on DEAE Sephadex A-25. Light chains were dissociated from myosin molecule by guanidine denaturation. The two species of light chains with molecular weights of 27,000 (light chain I, LC I) and 20,000 (light chain II) were fractionated by preparative disc electrophoresis in the presence of sodium dodesyl sulfate (2).

PREPARATION OF ANTISERA Antisera to LC II were prepared by immunizing adult white rabbits with total light chains. Total light chains (5 μg) was dissolved in 0.75 ml of 0.1 M potassium phosphate buffer, pH 7.4, and the solution was emulsified with an equal volume of complete Freund's adjuvant and injected into multiple subcutaneous sites at weekly intervals.

RADIOLABELING OF LIGHT CHAIN II LC II was iodinated with [^{125}I] using the chloramine T method (3). [^{125}I]LC II was separated from inorganic [^{125}I] by gel filtration on Sephadex G-100 column (50 x 1.0 cm). The specific activity of the iodinated LC II was of the order of 30 μCi/μg.

RADIOIMMUNOASSAY PROCEDURE The radioimmunoassay procedure employed phosphate buffer saline (0.05 M potassium phosphate buffer, pH 7.4, 0.15 M NaCl) containing 1% bovine serum albumin as buffer solution for all dilutions. Reagents were added into the 10 x 75 mm glass tubes in the following order; 1) 150 μl diluting buffer, 2) 50 μl 0.1 M EDTA (Na salt), pH 7.4, 3) a 100 μl sample to be assayed (suitably diluted serum or standard LC II solution), 4) 100 μl of antiserum, diluted 1:10,000 to give a final dilution of 1:50,000, and,5) 100 μl of [^{125}I]LC II, about 10,000 cpm. All determinations were performed in duplicate. Separation of free from antibody-bound LC II was accomplished by the double antibody technique. Using [^{125}I]LC II alone, with no added unlabeled LC II, and with the method described, 30% of added [^{125}I] was recovered in the precipitate. With excess antibody (1:50 final dilution of anti-LC II antiserum), 90% of the [^{125}I] was found in the precipitate. Protein concentration was determined by the Lowry method (4), using bovine serum albumin as a standard.

ANIMAL PREPARATION AND SERUM SAMPLES Mongrel dogs weighing 7-10 kg were anesthetized with pentobarbital sodium. A left thoracotomy was performed, the pericardium opened, and the left anterior descending coronary artery was dissected free from adjacent tissue and occluded by a silk suture in 10 dogs. In 2 sham-operated dogs, the left anterior descending coronary artery was dissected, but not occluded.

Blood samples were obtained at 0,2,4,6 and 12 hours, 1,2,3,5 and 7 days after operation for serial LC II determinations.

RESULTS

ANTI-LC II ANTISERUM The antiserum obtained after immunization of a rabbit with total light chains gave a single precipitine line against either purified LC II or against partially purified cardiac myosin, and the lines showed a reaction-of-identity with each other.

STANDARD CURVE Fig.1 shows the displacement of [^{125}I]LC II from antibody by increasing amounts of unlabeled pure LC I, LC II

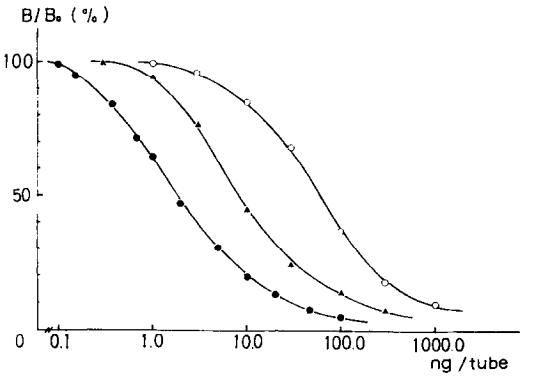

Fig.1. Displacement of [^{125}I]LC II from antibody by LC II(●), LC I(○) and total light chains of dog skeletal muscle (▲).

and total light chains of dog skeletal muscle. Amounts of 0.2 ng of LC II were effectively measurable. Cross-reactivity between LC II and total light chains of dog skeletal muscle was calculated as 20%. Cross-reactivity between LC I and LC II was 4%. Serial dilutions of serum of one dog with coronary occlusion gave results indistinguishable from the standard curve.

PRECISION AND REPRODUCIBILITY Serum samples were routinely assayed in duplicate. Within a single assay, duplicate determinations agreed within 5%. When 5 determinations from the same sample were made on different days during 5 months, the coefficient of variation was 8%.

SERUM LC II LEVELS IN DOGS AFTER OPERATION In normal dogs, concentrations of LC II were less than 20 ng/ml. The time-courses of serum LC II after operation are shown in Fig.2. LC II levels in serum began to rise within 6 hours in all cases, reached maximum at 3-5 days in 7 cases and at 2 days in 2 cases. Peak concentrations of LC II ranged 40-320 ng/ml. In 8 out of 10 cases, LC II could be detected at 7 days. In one sham-operated dog, LC II was detected at 2 and 3 days, but its concentrations were less than 30 ng/ml. As

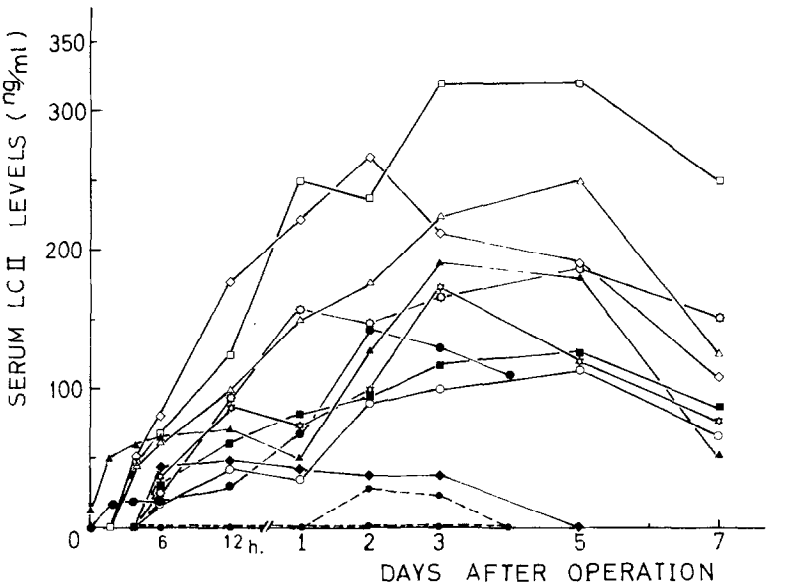

Fig.2. Time-course study following operations. Solid lines represent changes of LC II levels in dogs with coronary artery occlusion. Dotted lines represent changes of LC II in sham-operated dogs.

shown in the time-course figures, 2 peaks appear to come out in 6 out of 10 cases with coronary occlusion. In 4 of these 6 cases, the first peak appeared within 12 hours, and in the other 2 cases it appeared at 24 hours after operation. When LC II was injected into 3 dogs intravenously (0.2 mg/kg), changes of LC II levels after injection showed a biphasic pattern, a rapid initial disappearance phase in the first day and a slower decrease phase thereafter. LC II was almost undetectable at 48 hours after injection.

DISCUSSION

This report describes the development of a radioimmunoassay for circulating LC II in serum after experimental myocardial infarction. This method effectively measures LC II in the amount of as little as 0.2 ng, and has a fairly high degree of reproducibility.

Cross-reactivity between LC II and total light chains of skeletal muscle was 20%. However, LC II levels in sham-operated dogs were

less than 30 ng/ml, and significantly lower than those in dogs with coronary occlusion. Our previous report showed that LC I and LC II had many amino acid residues in common (1). Therefore, it is rather surprising that the cross-reactivity between the two species of light chains was only 4%, reflecting immunochemically dissimilar to each other.

The time-course of LC II in serum after coronary occlusion has some characteristics which have not been observed in serum enzyme studies. In the first, LC II rises rapidly and stays up during a long period after coronary occlusion. From the study of disappearance rate, this long time-course of LC II after coronary occlusion is suggested not due to accumulation of LC II, but mainly due to continuous liberation of LC II into the blood stream. In the second, changes of LC II levels appear to be biphasic in some cases with coronary occlusion. In 6 out of 10 cases, LC II levels decreased once and then increased again. From our study of synthesis rate of light chains, it was suggested that there was a pool of uncombined free light chains in the tissue, especially LC II, not assembled to myosin molecule (5). Thus, when cardiac muscles are injured, the uncombined LC II may be released into the blood stream due to increase in the membrane permiability of myocardial cell, as observed in such enzymes as CPK and GOT. After that, light chains could be continuously liberated by destruction of myofibrillar protein.

This radioimmunoassay is a unique method in sensitivity and specificity for determination of LC II in the serum, and appears to be useful when applied to the diagnosis of myocardial infarction, especially with regard to the specific pattern of time-course of LC II levels in serum after infarction.

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