ASSESSMENT OF MYOCARDIAL INFARCT SIZE BY SERIAL CHANGES IN SERUM CARDIAC MYOSIN LIGHT CHAIN II IN DOGS

RYOZO NAGAI, M.D. AND YOSHIYOSHI YAZAKI, M.D.

The relationship between myocardial infarct size and serum levels of cardiac myosin light chain II (LC II; 20000 daltons) was studied in 24 dogs with left anterior descending coronary artery occlusion. LC II in the serum was measured by the radioimmunoassay which we have recently developed. In our assay, 0.1–5.0 ng of LC II were effectively measurable. Serum LC II levels rose rapidly and stayed elevated long after coronary occlusion.

Infarct size was determined by gross inspection. In 24 dogs, infarct size ranged from 0.3 to 41.7 per cent of left ventricular weight. LC II release was calculated by the formula of Shell and associates. Regression analysis showed good correlation between infarct size and LC II release (r = 0.78). Infarct size also correlated with maximal LC II level (r = 0.77), and LC II level 24 hours after coronary occlusion (r = 0.69). Detection of circulating LC II is a useful method since it can be applied to the diagnosis of acute myocardial infarction at the early as well as late stage and infarct size can be assessed by analysis of serum LC II levels.

Since the work of Shell and coworkers, serial changes in activities of serum creatine phosphokinase (CPK), or CPK isozyme, CPK-MB, have been widely used for the noninvasive estimation of myocardial infarct size. Recently we have reported development of a sensitive radioimmunoassay of the smaller subunit of cardiac myosin molecule, light chain II (LC II), which is a protein specific to the cardiac muscle with molecular weight of 20 000. Detection of a structural protein of cardiac muscle in serum which is expected to exit into the blood stream may provide a direct information concerning the degree of myocardial necrosis. Thus, in this report we studied the correlation between LC II release and histologically determined myocardial infarct size.

METHODS

Purification of LC II

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

Key Words:
Acute myocardial infarction
Infarct size
Myosin light chain II
Radioimmunoassay

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Fig. 1. Immunodiffusion of partially purified cardiac myosin and of the purified LC II against rabbit antiserum.

**Preparation of antisera**

Antisera to LC II were prepared by immunizing a white rabbit weighing 2.4 kg with LC II. LC II (2.5 mg) were 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. Blood was collected from ear veins. After clotting, the blood samples were centrifuged at 2000 rpm for 20 min at 4°C, and the sera collected and stored at -60°C.

**Radioimmunoassay of LC II**

Five micrograms of LC II, dissolved in phosphate buffer saline (0.05 M potassium phosphate buffer, pH 7.4, 0.15 M NaCl) (PBS), was iodinated with $^{125}$I by the chloramine T method. LC II $^{[125]}$I was separated from inorganic $^{125}$I by gel filtration on a 50 × 1.0 cm column of Sephadex G-100. The specific activity of the iodinated LC II was of the order of 100 μCi/μg.

The radioimmunoassay procedure employed PBS containing 1% bovine serum albumin as buffer solution for all dilutions. Reagents were added into the 12 × 75 mm glass tubes in the following order; (a) 200 μl of diluting buffer, (b) a 100 μl sample to be assayed (suitably diluted serum or standard LC II solution), (c) 100 μl of antiserum, diluted 1:10,000 in PBS containing 1% normal rabbit serum to give a final dilution of 1:50,000, and (d) 100 μl of LC III$^{[125]}$I, about 10,000 cpm. After incubation in the dark at 4°C for 2 days, 100 μl of anti-rabbit gamma globulin antiserum was added to each tube. After an additional 18–24 hours of incubation, the tubes were centrifuged at 4°C. The supernates were immediately aspirated and precipitates were assayed for $^{125}$I. Using LC II $^{[125]}$I alone, with no added unlabeled LC II, and with the method described, 40% 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, using bovine serum albumin as a standard.

**Animal preparation and serum samples**

Mongrel dogs weighing 7–10 kg were anesthetized with pentobarbital sodium (25 mg/kg) and intubated. A left thoracotomy was performed, the pericardium opened, the left anterior descending coronary artery (LAD) was dissected free from adjacent tissue and occluded by a silk suture in 24 dogs. LAD was occluded at some point between its origin and the fourth diagonal branch, depending on the size of infarct desired. The chest was closed immediately after LAD was occluded, and the dog was allowed to recover. In 4 sham-operated dogs, the left anterior descending coronary artery was dissected, but not occluded. Blood samples were obtained before operation and 2, 4, 6, 12, 24, 48, 72, 120 and 168 hours after operation for serial LC II determinations.

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- = undetected, * = peak level, S = sham-operated dog

Histological infarct size estimation
After 168 hours, dogs were sacrificed and the final infarct size was determined by gross inspection. At postmortem examination, the heart was removed and the left ventricle was sliced at 5 mm intervals from base to apex. Infarct area, determined by gross inspection, was excised, weighed and related to 100g of left ventricle.

Calculations
The formula of Shell and associates (1) was used for the calculation of LC II release into the circulation after infarction.

\[
\text{LC II release} = E_t + Kd \sum_{0}^{\infty} \frac{E_{t-x} + E_t}{2} \Delta t
\]

where \( E_t \) = serum LC II at time t, \( Kd \) = exponential disappearance rate, \( (E_{t-x} + E_t)/2 \) = average LC II value during preceding time interval x, and \( \Delta t \) = time interval.

The disappearance rate of LC II was studied in 4 normal dogs and in other 4 dogs at 48 hours after coronary artery occlusion by injecting LC II\[^{125\text{I}}\] intravenously (2.0 \times 10^6 \text{cpm/kg}).

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RESULTS

Anti-LC II antiserum
The antiserum obtained after immunization of a rabbit with LC II gave a single precipitin line against either purified LC II or against partially purified cardiac myosin, and the lines showed a reaction-of-identity with each other (Fig. 1).

Characteristics of radioimmunoassay.
Fig. 2 shows the displacement of LC II \(^{125}I\) from anti-LC II antibody by increasing amounts of unlabeled pure LC I, LC II and total light chains of dog skeletal muscle. Amounts of 0.1 ng of LC II were effectively measurable. Cross-reactivity between LC II and total light chains of dog skeletal muscle was calculated as 16.0%. Cross-reactivity between LC I and LC II was 4%. Serial dilutions of serum sample of a dog with coronary artery occlusion gave results indistinguishable from the standard curve. Serum samples were routinely assayed in duplicate, and the intraassay agreement was 4.8%. When 10 determinations from the same sample were made on different days during five months, the coefficient of variation was 10.9%.

Serum LC II levels in dogs after operation
Serum LC II levels in 40 normal dogs were 2.70 ± 0.71 ng/ml (mean ± SEM). Serum LC II levels after operation are summarized in Table I. LC II levels began to rise within 6 hours in most cases and peaked at 24 hours in 1 case, at 48 hours in 3 cases, at 72 hours in 14 cases, at 120 hours in 3 cases and at 168 hours in 1 case. Peak concentrations of LC II ranged from 0 to 98 ng/ml (49.3 ± 4.9 ng/ml). In 21 dogs, LC II levels stayed elevated at 7 days (27.8 ± 3.7 ng/ml, range 9–70 ng/ml).

Disappearance of exogenous LC II from the circulation
In 4 normal dogs, radioactivities in serum and those precipitated with trichloroacetic acid declined in parallel (Kd = 0.0025 ming⁻¹ ± 0.0003, mean ± SEM). Similar results were obtained in 4 dogs with coronary occlusion (Kd = 0.0023 ming⁻¹ ± 0.0003). Since acid precipitable radioactivities appeared in urine at 7 hours after injection, renal excretion may contribute to clearance of LC II from the circulation. However, because serum creatinine levels did not elevate in all of 24 cases after infarction (maximal creatinine concentration = 1.12 ± 0.04 mg/dl, mean ± SEM),
renal perfusion could be so well preserved as not to influence on serum LC II levels. In this study the mean value of Kd, which was obtained from the decrease rate of acid precipitable radioactivity in dogs with coronary artery occlusion, was adopted for the calculation.

**Correlation of LC II release with histologically determined infarct size**

Infarct size estimated by gross inspection ranged from 0.3 to 41.7% of left ventricular weight. Table I shows LC II level after operation, peak LC II level, LC II release and infarct size in 24 dogs with coronary occlusion and in 4 sham-operated dogs. The correlation between LC II release and infarct size is shown in Fig. 3. Linear regression analysis between them resulted in correlation coefficient of 0.78 (p < 0.001). Infarct size also correlated with the peak serum LC II level (r = 0.77, p < 0.001) and LC II level at 24 hours (r = 0.69, p < 0.001) (Figs. 4 and 5).

**DISCUSSION**

This report describes assessment of myocardial infarct size by serial changes in circulating LC II. Recently the radioimmunoassay of cardiac myosin light chains has been developed in our laboratory and others. Traher et al. reported the application of a radiolabeled antibody for human LC I in the diagnosis of acute myocardial infarction. However, determination of LC I may be inaccurate because LC I and LC II were not separated. Khaw et al. reported a radioimmunoassay for total light chains of dog cardiac myosin and studied time course of serum light chains levels after experimental myocardial infarction. Our method effectively measures the smaller subunit of light chains, LC II, in the amount of as little as 0.1 ng. By using this method, we studied appearance of LC II in the serum after coronary occlusion in greater detail and the relationship between infarct size and LC II release.

Serum LC II rose rapidly and stayed up during a long period after coronary artery occlusion. This distinctive pattern of appearance of LC II in the serum has not been observed in time course studies of many kinds of serum enzymes or myoglobin. Since disappearance rate of LC II was quite rapid and renal perfusion was well preserved in every case, this long time course of LC II was suggested to be caused, not by accumulation of LC II, but mainly by continuous liberation of LC II into the circulation. Our previous study of synthesis rate of light chains showed presence of a pool of uncombined free light chains in sarcoplasm, especially LC II not assembled to myosin molecule. Thus, in the early stage of myocardial infarction, LC II may be released from this pool and then continuously liberated from degenerated myofibrils. From the study of serial changes in histological appearance of ischemic myocardium (unpublished data), destruction of myofilaments was revealed to be most prominent at 48 to 72 hours after infarction, when serum LC II levels reached maximum in most cases. Degenerating myofibrils were observed even at 168 hours among apparently normal cells.

LC II release correlated well with histologically determined infarct size. In this study, serum LC II levels until 168 hours were used for the calculation of LC II release. However, infarct size could be estimated at the earlier stage because of general correlation between infarct size and LC II level at peak and at 24 hours after infarction. In the report of Khaw et al. they failed to find the correlation between infarct size and time of first appearance of light chains, time of peak concentration or maximal light chains.
levels. Several problems may be responsible for it; 1) their method was not sensitive enough to detect small amounts of light chains, 2) light chains levels could not be defined sufficiently since two species of light chains were not fractionated, and 3) infarct size was determined by triphenyl tetrazolium chloride method, which underestimates the infarct area at 7 days after infarction.

The relationship between myocardial infarct size and release of CPK into the circulation has been well investigated by Shell and coworkers. However, Cairns et al. have recently proposed that the larger a zone of infarction, the lower would be the fractional escape of CPK from its center, allowing difficulties to distinguish large from small infarction. Bishop et al. showed the blood flow in the ischemic myocardium recovered during four days after infarction Therefore, serum CPK levels may be influenced by the washout rate of the enzyme from the infarcted area, because CPK is released mainly during this period. On the other hand, release of LC II is more gradual than that of CPK, reaching maximum at 3 to 5 days after infarction. Thus serum LC II levels seem to be less influenced by changes in the blood flow in the ischemic area than those of CPK, reflecting well the degree of myocardial necrosis.

The radioimmunoassay of LC II seems a unique method in that it can be applied to the diagnosis of acute myocardial infarction at the early as well as late stage, and that it provides a direct information concerning the degree of degeneration of myocardial structural protein.

Acknowledgements

We wish to acknowledge the technical assistance of Yukiko Hadano.

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