Serum Cardiac Myosin Light Chain II in Acute Myocardial Infarction; Distinctive Pattern of its Appearance and Estimation of Infarct Size

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MANY kinds of enzymatic methods such as CPK, GOT and LDH or the radioimmunoassay of myoglobulin have now been widely used for the diagnosis of myocardial infarction and for the noninvasive estimation of infarct size. However, most of them are not necessarily sufficient in sensitivity and specificity. Furthermore, Cairns and his associates have recently reported that the larger a zone of infarction, the lower would be the fractional escape of CPK from its center, allowing underestimate large myocardial infarctions in the conventional analysis.¹ In this study, we reported a sensitive radioimmunoassay of a smaller subunit of cardiac myosin molecule, light chain II (LCII), which is a specific protein to the cardiac muscle with molecular weight of 20,000 and determined serially the LCII levels in the serum of dogs with coronary occlusion and patients with acute myocardial infarction by this assay, since detection of a structural protein of cardiac muscle in the serum which is expected to exit into the blood stream after myocardial infarction would provide a direct information concerning the degree of myocardial necrosis.

Radioimmunoassay of cardiac myosin light chain II

Cardiac myosin was extracted and purified from canine and human left ventricle by the

dilution technique² and the two species of light chains, with molecular weights of 27,000 (light chain I, LCI) and 20,000 (LCII) of cardiac myosin were fractionated by preparative disc gel electrophoresis.³ Antisera to LCII were prepared by immunizing rabbits with total light chains of dogs or human emulsified in complete Freund's adjuvant. LCII was labeled with ¹²⁵I by the chloramine T method. The detailed information on the radioimmunoassay procedures was given in our previous report.⁴ The radioimmunoassay method could detect as little as 0.1 ng of LCII. Cross-reactivity between LCII of canine cardiac and skeletal myosin was calculated as 16.0%.

Serum LCII levels in dogs after coronary occlusion

Serum LCII levels in 40 normal dogs were 2.70 ± 0.71 ng/ml. After the ligation of left anterior descending coronary artery, blood samples were obtained every 8 hours for 48 hours and then once daily until 7 days. Serum LCII levels began to rise within 6 hours in 21 of 26 dogs after coronary occlusion, and peaked between 2 and 3 days in all cases. Peak concentrations of LCII ranged from 0 to 180 ng/ml (59.7 ± 7.8 ng/ml). In 23 of 26 operated dogs, LCII levels stayed elevated at 7 days (30.6 ± 4.1 ng/ml, range 9 – 82 ng/ml) compared with the control levels before occlusion (0 – 9 ng/ml). In sham operated dogs (n = 4), maximum LCII levels were less than 23 ng/ml.

This distinctive pattern of serum LCII levels after coronary occlusion, rising rapidly and stay-

Key Words:

Myocardial infarction
Cardiac myosin light chain
Radioimmunoassay
CPK-MB
Infarct size

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Supported by Grant-in-Aid for Special Project Research (No.422004) and Grant-in-Aid for Scientific Research (No.357304) from the Ministry of Education, Science and Culture.

ing up for a long period, has not been observed in time-course studies of many kinds of serum enzymes or myoglobulin. Since exogenous LCII injected intravenously disappeared rapidly from the circulation (half life: 1.6 hours), this long time-course of LCII was suggested not due to accumulation, but mainly due to continuous liberation of LCII into the circulation. Our previous study of synthesis rates of light chains showed presence of a pool of uncombined free light chains in sarcoplasm, especially LCII, not assembled to myosin molecule.⁵ Thus, in the early stage of myocardial infarction, LCII may be released from this pool and then continuously liberated from degenerated myofibrils in the infarcted myocardium for longer than one week. Serial changes in histological appearance of ischemic myocardium showed that destruction of myofilaments was most prominent 2 and 3 days after occlusion, when serum LCII levels reached peak levels in most cases, and degenerating myofibrils were observed among apparently normal cells even 7 days after occlusion. Thus, the radioimmunoassay of LCII seems an unique method in that it can be applied to the diagnosis of acute myocardial infarction at early as well as late stage, and it provides a direct information concerning the degree of degeneration of myocardial structural protein.

Correlation between LCII release and infarct size

The determination of total LCII release (Σ LCII) into the circulation from the infarcted heart was calculated according to the formula of Shell and his associates.⁶ Infarct size was determined histopathologically by the nitroblue tetrazolium staining method. In 26 dogs, infarct size ranged from 0.3 to 51% of left ventricular weight. Linear regression analysis showed a good correlation between infarct size and calculated cumulative LCII in serum with a correlation coefficient of 0.84, although, in this study, serum LCII levels until 7 days were used for the calculation of LCII release.⁷ Furthermore, in contrast to CPK release, cumulative LCII serum entry was not variable in the case of larger size of infarction. Also, infarct size could be estimated at the earlier stage because of general correlation between infarct size and LCII level at peak ($r = 0.77$) and at 24 hours ($r = 0.75$) after coronary occlusion. On the other hand, cumulative CPK-MB release in the serum less correlated with infarct size determined histologically ($r = 0.68$).

Clinical assessment of serum LCII

A radioimmunoassay for serum human LCII has been developed in order to evaluate the time-course of serum LCII level and correlation between LCII release and clinical and biochemical estimates of the severity in patients with acute myocardial infarction. Amounts of 0.2 – 10 ng of human LCII were effectively measurable. Antibody cross-reactivity with skeletal muscle light chains was 8%. Values for LCII in normal human sera ($n = 20$) were 4.20 ± 0.75 ng/ml. Serum samples were obtained every 8 hours for 48 hours after admission and then once daily for 16 days. LCII from the infarcted heart first appeared in the serum within 6 hours after infarction and reached peak level between 2 and 3 days (15 – 110 ng/ml) and stayed up until 10 days. This pattern of appearance of LCII in the serum after acute myocardial infarction is closely parallel to that of dog LCII after coronary occlusion and distinctive from time-courses of many kinds of serum enzymes and myoglobulin. Cumulative LCII release in the serum correlated well with clinical observation and peak levels of CPK-MB. Since the pattern of LCII appearance in the serum was much smoother than that of CPK, blood sampling once daily could be sufficient for the estimation of total cumulative LCII in the serum. Thus, LCII determination appears to be clinically useful in the quantification of infarct size as well as the diagnosis of acute myocardial infarction. In addition, it could provide retrospective diagnosis of myocardial infarction in patients several days after the clinical moment of infarction. In the case of angina pectoris, we could not detect significant elevation of serum LCII.

REFERENCES

1. CAIRNS, J. A., MISSIRLIS, E., & FALLEN, E. L.: Myocardial infarction size from serial CPK: Variability of serum entry ratio with size and model of infarction. *Circulation* 58: 1143, 1978.
2. YAZAKI, Y., & RABEN, M. S.: Effect of the thyroid state on the enzymatic characteristics of cardiac myosin: A difference in behavior of rat and rabbit cardiac myosin. *Circ. Res.* 36: 208, 1975.
3. YAZAKI, Y., MOCHINAGA, S., & RABEN, M. S.: Fractionation of the light chains from rat and rabbit cardiac myosin. *Biochim. Biophys. Acta* 328: 464, 1973.
4. NAGAI, R., UEDA, S., & YAZAKI, Y.: Radioimmunoassay of cardiac myosin light chain II in the serum following experimental myocardial infarction. *Biochem. Biophys. Res. Comm.* 86: 683, 1979.

5. YAZAKI, Y., & RABEN, M. S.: Comparison of the synthesis of light and heavy chains of cardiac myosin. In *Recent Advances in Studies on Cardiac Structure and Metabolism*, edited by Kobayashi T., Ito Y. and Rona G. vol. 12, University Park Press, Baltimore, 1978, p.39.
6. SHELL, E. E., KJEKSHUS, & SOBEL, B. E.: Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity. *J. Clin. Invest.* 50: 2614, 2971.
7. YAZAKI, Y., & NAGAI, R.: Serial determination of serum cardiac myosin light chain II: a new method for estimation of myocardial infarction size. *Circulation* 60: II-139, 1979.