Quantification of Myocardial Infarct Size
After Coronary Reperfusion by Serum Cardiac Myosin Light Chain II in Conscious Dogs

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The effects of early coronary artery reperfusion on the relation between the extent of myocardial infarction and serum levels of cardiac myosin light chain II or plasma creatine kinase levels were evaluated in the conscious dog. Hydraulic occluders were placed on the left anterior descending arteries of 38 dogs. Seven to 10 days later, myocardial infarction was produced. Coronary reperfusion was performed 3 hours (group A1, n=13) and 6 hours (group A2, n = 12) after the occlusion. In the other 13 dogs, coronary occlusion was sustained throughout the course of the experiment (group B). Seven days after the occlusion, the heart was cut from the apex to the base into 4-mm slices, and infarct size was determined macroscopically. Rapid appearance and early peaking of creatine kinase were observed in group A. Cumulative release of creatine kinase significantly correlated with infarct size in group A (infarct size ranged from 0.1 to 20.1 g, r=0.90) and group B (from 0.6 to 26.8 g, r=0.91). However, since creatine kinase release in group A was greater in comparison with that from infarcts of the same size in group B, the slope of the regression line for group A was significantly steeper (p<0.05). Cardiac myosin light chain II appeared as early as creatine kinase did and continued to be elevated for 7 days. A very close relation was observed between infarct size and total cardiac myosin light chain II release (r=0.87 for group A1, and r=0.88 for group B) or peak level of light chain II (r=0.85 for group A, and r=0.81 for group B). In addition, the slopes of the regression lines for infarct size and both peak and total release of light chain II did not differ between group A and group B. On histological examination, viable myocardium was frequently observed in the epicardium of the ischemic area in group A1; therefore, infarct size was greater in group B than in group A1 (p<0.05). Also, myocardial creatine kinase content in the epicardium of the center of the ischemic area in group A1 was greater than that in group B. Cardiac myosin light chain II release in group A1 was less than that in group B, whereas no difference was found in plasma creatine kinase release among groups A1, A2, and B. These results suggest that serum levels of cardiac myosin light chain II better quantitate the extent of infarction than plasma creatine kinase levels do. The relations stand regardless of the presence of coronary reperfusion, probably because of the gradual degradation of the myosin molecule. (Circulation Research 1989;65:684–694)
We have developed radioimmunoassays for sub-units of the cardiac myosin molecule, myosin light chain II,13,14 and light chain I.15,16 We have demonstrated that a linear relation exists between the extent of myocardial necrosis and serum levels of light chain II over a wide range of infarct sizes in experimental myocardial infarction without reflow.17,18 Our recent paper has shown the clinical usefulness of determining myosin light chain levels.19 We showed a close relation between peak level of myosin light chain I and left ventricular function after acute myocardial infarction. In addition, the relation was not affected by the presence of successful intracoronary thrombolysis. Also, this technique would be especially useful for the study of the effects of interventions that might protect ischemic myocardium.18

The liberation of cardiac myosin light chain from myofilaments in infarcted myocardium could be a much slower process than the leakage of cytoplasmic proteins such as creatine kinase, and this liberation continues for more than a week.13–19 Therefore, we hypothesized that the release of cardiac myosin light chain from infarcted myocardium might not be influenced by the changes in coronary flow. Khaw et al20 reported the time course of the liberation of total light chains from myocardium after coronary reperfusion. Their data suggest that this time course was similar to the time course observed in dogs without reperfusion. However, the relation between serum levels of light chains and infarct size has not been evaluated. The present study was designed to characterize the relation between the release into the serum of creatine kinase or cardiac myosin light chain II and the extent of myocardial necrosis after myocardial infarction in the presence or absence of early coronary reperfusion. We also evaluated the effects of early coronary reperfusion on the amount of cardiac myosin light chain II and creatine kinase released into the blood. To avoid the influence of anesthesia and the destruction of skeletal muscle, experiments were conducted in closed-chest, conscious dogs.

Materials and Methods

Preparation

Fifty-four mongrel dogs weighing 8–17 kg underwent aseptic left lateral thoracotomy for the implantation of instruments. Anesthesia was induced with sodium pentobarbital (25 mg/kg i.v.). After intubation, thoracotomy was made in the fourth intercostal space, and then the pericardium was opened. The left anterior descending artery was dissected free from the surrounding tissue just proximal to the origin of the first diagonal branch, and then a hydraulic occluding cuff was implanted. A flow probe (model FH-020T, Nihon Kohden, Tokyo, Japan) was affixed proximal to the cuff. A heparin-filled catheter for blood sampling was inserted from the external jugular vein to the right atrium, then the thorax was closed, and the dog was allowed to recover.

Experiments were performed 7–10 days after the operation. Nine dogs that died before the experiments were excluded from the study. Morphine sulfate (0.2 mg/kg) was administered intravenously to produce mild sedation. Control blood samples were obtained. The occluding cuff was then inflated with saline. The dogs were randomly assigned to three groups. Coronary occlusion was maintained for 3 hours in 15 dogs (group A1), for 6 hours in another 15 dogs (group A2), and for 7 days in the remaining 15 dogs (group B). After these periods, coronary reperfusion was produced by deflating the occluders in the dogs in groups A1 and A2. Coronary flow was monitored frequently for 24 hours, and thereafter once a day for 6 days to ascertain coronary occlusion and reperfusion. Antiarrhythmic drugs were not administered. No specific attempts were made to prevent ventricular fibrillation. Electrocardiograms were monitored for at least 24 hours after occlusion.

Blood Samples

Serial samples of blood (6 ml) were withdrawn from the dogs over a 7-day period. The samples were taken every 1.5 hours for the first 6 hours, hourly for the next 9 hours, every 3 hours for the next 9 hours, every 6 hours for the next 24 hours, every 12 hours for the next 48 hours, and then once a day until the seventh day after occlusion.

Postmortem Examination of the Heart

Seven days after the production of myocardial infarction, the dogs were killed by an overdose of intravenous sodium pentobarbital. The heart was rapidly excised, washed in iced saline, wrapped in plastic wrap, and suspended in a freezer for approximately 30 minutes. Each heart was cut from apex to base into 4-mm thick slices. Identification of infarcted myocardium 7 days after infarction was not difficult by gross inspection. After the infarct margin had been determined, the infarcts were traced directly onto plastic sheets laid over the slices. The total cross-sectional area and the area of the infarction in each cross section were measured by a planimeter. The extent of infarction was expressed as the ratio of infarcted area to total cross-sectional area. The weight of infarction was calculated by the equation: infarction weight = sectional area of infarction/total sectional area × total ventricular weight. The procedure was verified by light-microscopic examination of histological sections. The left anterior descending artery was examined both macroscopically and microscopically for the presence or absence of thrombosis or other lesions resulting from the occluder or the flow probe. The transverse slices of the center of the infarct were examined for myocardial creatine kinase content. Several transmural specimens of the myocardium were obtained from the center of the infarc-
tion. Each specimen was divided into two equal parts: one endocardial, the other epicardial. A transmural specimen of normal myocardium from the same section was also examined.

Biochemical Measurements

Plasma creatine kinase activity was assayed at 37°C by the method of Rosalki.21 Myocardial creatine kinase was assayed by the method of Kjekshus and Sobel.22 Briefly, the myocardial samples were minced with scissors and homogenized in 20 vol/g iced 0.25 M sucrose, 0.001 M neutralized EDTA, and 0.1 mM mercaptoethanol in a Polytron PCU-2 apparatus (Kinematika, Switzerland). The homogenates were then centrifuged at 16,000g for 20 minutes. The supernatant fractions were diluted 1:200 in a buffer containing 0.2% bovine albumin and 0.01 M Tris base, pH 7.4, and assayed by the method described above. International units per milligram of wet tissue weight was calculated for each sample. The creatine kinase content was expressed as a percentage of creatine kinase activity as determined from the control samples of normal myocardium.

The detailed procedure for radioimmunoassay of canine cardiac myosin light chain II has been reported.13,14 The release of cardiac myosin light chain II and creatine kinase was calculated by the formula of Shell, Roberts, Sobel, and coworkers5-6,23 as also reported previously by our laboratory.14 The equation was as follows: total release of creatine kinase (light chain II)=E₁+K₀(E₁-x+(E₁-x))/2 x t, where E₁ is plasma creatine kinase (serum cardiac myosin light chain II) at time t, K₀ is exponential disappearance rate, (E₁-x+(E₁-x))/2 is average creatine kinase (cardiac myosin light chain II) value during the preceding time interval, x. The K₀ of creatine kinase was determined by the exponential decay method from the downslope of individual creatine kinase curve.24 The K₀ of cardiac myosin light chain II was 0.0025 min⁻¹.14

Statistical Analysis

The Bonferroni method was used for multiple comparisons.25 A value of p>0.05 was considered nonsignificant. All data were expressed as mean±SEM. Linear regression was computed by the least-squares method. The slopes were compared by calculating residual variances and using the t distribution test for significant differences.

Results

Production of myocardial infarction was confirmed in 45 dogs by sustained S-T segment elevation in the precordial leads of the electrocardiogram immediately after occlusion and by serial elevations of plasma creatine kinase. Of the 45 dogs, three from group A1, two from group A2, and two from group B died of ventricular fibrillation. Thirty-eight dogs survived for 7 days. There was no statistical difference in mortality rate and heart rate among the three
Histological Examination

Infarct size in group A1 (9.8±2.7%/ventricular weight) was significantly less than that in group B (19.2±4.0%) (p<0.05) (Table 1). Damage or thrombosis of the left anterior descending artery at the site where the instruments were implanted was not noted in any dog. At 7 days, myocardial hemorrhage in the infarct area was not remarkable in the dogs of group B. Microscopic myocardial hemorrhage was frequently observed at the center of the infarct area in groups A1 and A2. But the area of hemorrhage was always confined to the area of infarction (Figures 1 and 2). Several dogs in groups A1 and A2 revealed a scattering of contraction band necrosis at the area of infarction.

By gross inspection, the epicardial wall of the ischemic area in the dogs with early reperfusion often showed scattered necrosis (Figure 3B). Light-microscopic observations showed degraded myocardial cells in the endocardium of the infarcted area in every dog. However, viable myocardium was frequently observed in a scattered distribution within the midwall in the center of the ischemic area in dogs treated with early coronary reperfusion (Figure 2). These observations were distinctly different from those of the permanently occluded dogs, in which necrosis was frequently present.
throughout the transmural sections (Figure 3A), although viable muscle was present in the subepicardial myocardium around small penetrating arteries and in thin layers of the subepicardial margin.

**Myocardial Creatine Kinase Content**

Myocardial creatine kinase content was measured in 31 dogs. Epicardial creatine kinase content in group A1 was significantly greater than that in group A2 \((p<0.01)\) and group B \((p<0.001)\) (Figure 4). Endocardial creatine kinase content in group A1 was also greater than that in group A2 and group B, but there was no statistical difference.

**Effects of Reperfusion on Serial Changes in Plasma Creatine Kinase and Serum Light Chain II Levels**

The time-activity curve of plasma creatine kinase in groups A1 and A2 was considerably different from that in group B (Figure 5). After reperfusion, creatine kinase was abruptly released into the plasma and rapidly reached its peak level. Therefore, the peak appearance time of creatine kinase increased in the following order: group A1, 7.5±0.6 hours; group A2, 10.1±0.5 hours \((p<0.01 \text{ versus group A1})\); group B, 16.9±1.3 hours \((p<0.001 \text{ versus group A1 and group B})\). There was no difference in the creatine kinase disappearance rate among the three groups.

Serial changes in the mean values of serum cardiac myosin light chain II after myocardial infarction are shown in Figure 6. In group B, cardiac myosin light chain II began to increase by 3–12 hours, reached its peak level 61.9±11.2 hours after the occlusion, and thereafter decreased gradually. The appearance and elevation of serum cardiac myosin light chain II in groups A1 and A2 were similar to those in group B. But the peak appearance times of group A1 \((17.5±2.2 \text{ hours})\) and group A2 \((35.2±8.6 \text{ hours})\) were significantly earlier than in group B. Serum levels of light chain II in group A1 decreased earlier than did those in group B. Total release and second, third, fourth, and sixth day levels of serum cardiac myosin light chain II in group A1 were significantly less than those in group B \((p<0.05)\).

**Relation Between Infarct Size and Plasma Creatine Kinase Level**

A linear relation was observed between infarct size as determined by gross inspection and creatine kinase release in both group A and group B: $IS(g)=0.0014 \times \text{creatine kinase release}+1.22 \ (r=0.90) \ (\text{group A})$, and $IS(g)=0.0032 \times \text{creatine kinase release}−1.71 \ (r=0.75) \ (\text{group B}) \ (\text{Figure 7})$, where $IS$ is infarct size, and creatine kinase release is cumulative release of creatine kinase. However, the slopes of their regression lines were significantly different \((p<0.05)\). The amount of creatine kinase release after coronary reperfusion was greater than that observed in myocardial infarcts of the same size without reperfusion.

A linear relation was also observed between infarct size and peak creatine kinase in the two groups: $IS(g)=0.0029 \times \text{peak creatine kinase}+1.22 \ (r=0.91) \ (\text{group A})$, and $IS(g)=0.0040 \times \text{peak creatine kinase}+0.060 \ (r=0.73) \ (\text{group B})$. The slope of
FIGURE 3. Photomicrographs of left ventricular slices (×2). Panel A: A transverse left ventricular slice from a dog that underwent 7 days of left anterior descending artery occlusion. Transmural infarction is observed. Panel B: A slice from a dog that underwent 3 hours of occlusion followed by 7 days of reperfusion of the left anterior descending artery. Viable muscle salvaged by reperfusion is present in a patchy distribution within the midwall and outer wall of the ischemic area.

The regression line for group A was also significantly steeper than that for group B.

Relation Between Infarct Size and Serum Cardiac Myosin Light Chain II Level

A close relation was observed between infarct size and light chain II release in the group A and group B (Figure 8). IS(g)=0.017×light chain II release+0.91 (r=0.87) (group A), and IS(g)=0.017×light chain II release−1.57 (r=0.88) (group B), where light chain II release is cumulative release of cardiac myosin light chain II. In addition, the slopes of the regression lines were not different. The regression equation in all dogs was IS(g)=0.016×light chain II release+0.53 (r=0.86).

The relation between infarct size and peak light chain II was also good (Figure 9). IS(g)=0.19×peak light chain II+0.34 (r=0.85) (group A), and IS(g)=0.20×peak light chain II−1.25 (r=0.81) (group B). The slopes of the regression lines were quite similar.

Discussion

The results of the present study show that the quantitative relation between serum level of cardiac myosin light chain II and the extent of myocardial infarction is not influenced by early coronary reperfusion. Release of cardiac myosin light chain II is reduced reflecting epicardial salvage by early reperfusion. These findings are distinctly different from
Since the report of Shell, Roberts, Sobel, and coworkers, the analysis of serial changes in plasma creatine kinase has been widely used as a noninvasive means of quantifying infarct size. However, the experimental and mathematical basis for the enzymatic model as a measure of infarct size has been a matter of controversy. The major problem of this technique at present is the influence of coronary reflow on the release of creatine kinase. Jarmakani et al reported that infarct size estimated from total plasma creatine kinase values is greater than necropsy-defined infarct size after coronary reperfusion. They suggested that reperfusion causes the delivery of a greater proportion of the enzyme from necrotic tissue to the serum. Vatner et al showed that the rapid appearance and the greater recovery of creatine kinase in blood per unit of infarcted myocardium is due to a washout phenomenon after complete reperfusion 1–3 hours after coronary occlusion. Swain et al also mentioned that regional blood flow to the ischemic region influences the appearance of creatine kinase.

Our results are consistent with these observations. The time activity curve of plasma creatine kinase was significantly different among the three groups. In groups A1 and A2, creatine kinase was released immediately after the reperfusion and reached its peak level earlier than in group B. Although a close relation between anatomically determined infarct size and total creatine kinase release was observed in the two groups, the cumulative release of creatine kinase in the early reperfusion group was greater in comparison with that from myocardial infarction of the same size without reperfusion. As mentioned by Vatner et al., these differences are probably due to the enhanced recovery rate of creatine kinase by the washout phenomenon. It is clear from these results that the mathematical basis for estimates of infarct size by creatine kinase level, which rest on a permanent occlusion model, cannot be applied to infarcts with coronary reflow.
Predictions of infarct size from the time-activity curve of creatine kinase after early reperfusion should be made with an appropriate rectification.

Although cardiac myosin light chains are the structural proteins of cardiac muscle, after myocardial infarction they are liberated from infarcted myofilaments as early as creatine kinase is released, and their serum level continues to be elevated for more than a week. The early release of light chain after reperfusion has been shown to be due to a soluble pool of unassembled light chains in the cytoplasm of cardiac myocytes. The pattern of the release of light chain I or II after infarction is similar to the results from other laboratories although the other investigators measured light chains together. Since myosin light chains are low molecular weight proteins, they disappear rapidly from the glomerulus into the urine. Therefore, the persistent elevation of myosin light chain levels in the serum after myocardial infarction indicates that infarcted myofilament continues to be degraded and to release its fragments into the serum.

We have repeatedly demonstrated that serum levels of cardiac myosin light chain quantitatively reflect the extent of myocardial necrosis in experimental animals without reflow. In the present study we show that the slopes of the regression lines for myosin light chain release and anatomically determined infarct size are similar in reperfused and nonreperfused infaracts. Reports of Khaw et al also show that the patterns of total light chain release do not differ in reperfused and nonreper-

![Figure 6. Graph showing serial changes in serum cardiac myosin light chain II after coronary occlusion and subsequent reperfusion. The appearance and elevation of myosin light chain II are similar among the three groups, but peak levels are reached earliest in the 3-hour occlusion group. The second, third, fourth, and sixth day levels of myosin light chain II are greater in the 7-day occlusion group than in the 3-hour occlusion group. Vertical bars indicate mean±SEM. *p<0.05 versus 3-hour occlusion group; tp<0.001 versus 3-hour occlusion group.](Image)

![Figure 7. Graphs showing creatine kinase release plotted against infarct size in dogs with 7-day occlusion (left panel) and dogs with early reperfusion (right panel). Although significant correlations are observed in the two groups, the slope of the regression line in the early reperfusion group is significantly steeper (p<0.05).](Image)
fused dogs. The fact that the release of cardiac myosin light chain II is insensitive to washout by reperfusion indicates that the release rate of light chain is predominantly limited by the rate of degradation of the myosin molecule and not by the rate of myocardial perfusion. The release of cardiac myosin light chain II is such a gradual process that it might not be disturbed by the drastic changes in coronary flow early after myocardial infarction.

Although good relations were found between infarct size and light chain levels, estimation of infarct size from light chain II levels was not feasible in this study because of scatter in data and positive y-intercepts. The release of biochemical substances from necrotic myocytes into the blood stream should be influenced by many factors in addition to infarct size. Coronary circulation, extent of no reflow, degree of cell infiltration, and enzymatic proteolysis could all affect the release of biochemical substances from necrotic tissue. Postinfarct coronary circulation is affected by factors such as collateral blood flow, coronary spasm, ventricular function, systemic blood pressure, and systemic blood volume; variability in these factors from dog to dog may account for the scatter in the data. Also, since the disappearance of light chain from the circulation is rapid, the number of blood samplings may not have been sufficient to allow an estimation of the true total light chain release. Swain et al. indicated that over a broad range of infarction the relation between creatine kinase estimates of infarction and histologic extent of infarc-

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**FIGURE 8.** Graphs showing cardiac myosin light chain II release plotted against infarct size in dogs with 7-day occlusion (left panel) and dogs with early reperfusion (right panel). Close correlations are observed in both panels. In addition, the slopes of the regression lines are not statistically different.

**FIGURE 9.** Graphs showing peak levels of cardiac myosin light chain II plotted against infarct size in dogs with 7-day occlusion (left panel) and dogs with early reperfusion (right panel). Significant correlations are observed in both panels. The slopes of the regression lines are not statistically different.
The time course of cardiac myosin light chain II release after coronary reperfusion requires discussion. The peak level of serum light chain II appeared earliest in the 3-hour occlusion group (A1). Total release and the second, third, fourth, and sixth day levels of cardiac myosin light chain II in this group were also lower than those in the permanently occluded group (B). Several factors could contribute to this difference. The extent of infarct and the degree of coronary flow in the infarcted area are the most probable causes of the difference in the timing of peak release. Smaller, scattered infarcts in the early reperfusion group probably attained more complete flow, which could allow the myosin light chains to reach the blood stream earlier. On the other hand, in larger infarcts the myosin light chains must diffuse out of the areas of no reflow. Also, the early peaking may relate to an alteration in the time course of the degradation of the myosin molecule early reperfusion may relate to an alteration in the time course of cardiac myosin light chain release after early reperfusion. The present data do not allow evaluation of this problem. Further investigations are needed to explore the factors that affect the time course of cardiac myosin light chain release after myocardial infarction.

Many studies have documented the limitation of experimental infarct size by early coronary reperfusion. Our experimental design was not appropriate for an evaluation of the changes in infarct size because we failed to measure the areas at risk and collateral blood flow to these areas. However, infarct size in group A1 was smaller than that in group B. Histological examinations showed frequent preservation of viable myocardium in the epicardial region of the ischemic area in the dogs with 3 hours of occlusion followed by reperfusion. These observations were considerably different from those made on the dogs not reperfused, in which necrosis was frequently present throughout the transmural section. Also, myocardial creatine kinase content in the epicardial region of the ischemic area was greater in the early reperfusion groups than in the permanently occluded group. Though smaller vascular areas at risk or greater collateral blood flow possibly related to the reduction of infarct size in group A1, our results are in general agreement with previous studies that show the epicardial salvage of myocardial infarct by early coronary reperfusion. Therefore, the significant reduction in total release of cardiac myosin light chain II in group A1 seems to reflect epicardial salvage in the ischemic area. On the other hand, no statistical difference was obtained for total creatine kinase release among the three groups. Release of creatine kinase from the scattered infarcts in group A1 may have been enhanced by early reflow, whereas that from group B may have been reduced by severely diminished blood flow. As a matter of fact, Cairns et al. reported that the recovery rate of creatine kinase is much lower in homogeneous as opposed to scattered infarcts. Therefore, we think the washout of creatine kinase also accounts for the absence of a difference in creatine kinase release among the three groups.

In the present study, we demonstrate that serum levels of myosin light chain II quantitatively reflect the extent of experimental infarct size, regardless of the presence of coronary reperfusion. On the other hand, reperfusion significantly affects plasma creatine kinase estimates of infarct size because of the washout phenomenon. Thus, serial determinations of serum light chain II levels appear to facilitate the assessment of the extent of early coronary reperfusion on infarct size. Further evaluation of the usefulness of this method will require clinical studies of patients who have received thrombolytic therapy for acute myocardial infarction.

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