Infarct Sizing after Reperfusion by Two-dimensional Echocardiography and Serum Cardiac Myosin Light Chain II in Conscious Dogs: Dissociation between Early Left Ventricular Wall Motion and Ultimate Infarct Size

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The time course of recovery of left ventricular wall motion after coronary reperfusion and how that relates to anatomical infarct size, wall motion abnormality, and the amount of cardiac myosin light chain II release were evaluated in conscious dogs. One week after the implantation of hydraulic occluders on the left circumflex arteries, myocardial infarction was induced. Coronary reperfusion was performed 3 h after the occlusion in 9 dogs (R) and occlusion was sustained in 9 dogs (C). All dogs underwent serial 2-dimensional echocardiograms and determination of serum cardiac myosin light chain II. The infarct size was identified at 14 days. Systolic wall thickening at the center of the ischemic area (SWT) at 3 h was $-7.7 \pm 2.8\%$ (C), $-9.9 \pm 3.0\%$ (R). Systolic thinning was observed even at 14 days in C. Significant recovery of contraction was observed in R, but the improvement continued for as long as 2 days. SWT at 14 days was $-1.5 \pm 2.8\%$ (C) and $7.0 \pm 4.6\%$ (R) ($p < 0.05$). All of SWT or the extent of systolic thinning (EST) 3-hour and 14-day were correlated well with infarct size in C. In group R, 14-day SWT and 14-day EST correlated with infarct size but 3-hour SWT and 3-hour EST did not. Total release of serum cardiac myosin light chain II levels correlated well with infarct size ($r = 0.88$), 14-day SWT ($r = -0.90$) and 14-day EST ($r = 0.89$) in all dogs. These results indicate that both serum myosin light chain levels and the 2-dimensional echocardiography are noninvasive but quantitative techniques for evaluating infarct size after reperfusion. However, because of the presence of stunned myocardium, the magnitude of myocardial contraction abnormality early after reperfusion does not reflect ultimate infarct size.

Coronary thrombolysis is now widely accepted as an effective treatment for acute myocardial infarction. Although coronary reperfusion early after the onset of infarction reduces the ultimate infarct size and preserves left ventricular function, prolonged systolic dysfunction of salvaged myocardium is likely both in experimental and in clinical myocardial infarction. Since left ventricular function plays a crucial role in the management of patients with acute myocardial infarction, the effect of myocardial protection of coronary

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We have reported that serum levels of myosin light chain quantitatively reflect the extent of myocardial infarction irrespective of the presence or absence of coronary reperfusion in clinical\textsuperscript{10,11} and experimental myocardial infarction\textsuperscript{12,13} However, the relation between the impairment of systolic function of reperfused myocardium and light chain release has not been elucidated. In this investigation, we evaluated the time course of recovery of myocardial contractility after reperfusion using 2-D echocardiography in conscious dogs. Also, evaluated were relations among the levels of serum myosin light chain, wall motion abnormality of the left ventricle, and myocardial infarct size in the setting of coronary reperfusion.

METHODS

Animals: Thirty-seven mongrel dogs (8–15 kg) were aseptically implanted with a balloon occluder at the proximal portion of the left circumflex artery. The dogs were then allowed to recover. Seven days after the operation coronary occlusion was performed by inflating the occluding cuff with saline under mild sedation by intracutaneous administration of morphine sulfate (0.2 mg/kg). Six dogs died before the occlusion and were excluded from the study. Dogs were randomly assigned to two groups. In 15 dogs, coronary occlusion was maintained for 3 hours and then reperfusion was performed (R). In the other 16 dogs, coronary occlusion was maintained permanently (C).

Echocardiography and data analysis: Two-D echocardiography was performed in conscious dogs in the supine position by Toshiba SSH11A at 0, 3, 6, 24 hours, and 2, 7, 14 days. The ultrasonic beam was transmitted from the right fourth or fifth intercostal space. A short axis view of the left ventricle at the level of the mid-papillary muscle was obtained (Fig. 1) and videotaped for subsequent computer analysis. An electrocardiogram was recorded simultaneously.

Computer assisted digital analysis of left ventricular wall motion was performed using Cardio 80 and 200 (Kontron). End-diastolic and end-systolic epicardial and endocardial margins were determined using the electrocardiogram directly on the video display and entered into a computer by a digitizing pen. Percent systolic wall thickening at 15° intervals was calculated with a radial contraction model using the endo-
cardial center of mass as the center point and the position of posterior and anterior papillary muscles to align the end-diastolic and end-systolic contours. Systolic thickening in each section was calculated as:

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\text{End-systolic wall thickness} = \frac{-\text{end-diastolic wall thickness}}{\text{end-diastolic wall thickness}} \times 100
\]

The section in which systolic thickening was least at the 3-hour image, was regarded as the center of infarction and systolic thickening of this section was defined as systolic wall thickness (SWT). The radius of which systolic thickening was less than 5% was defined as the extent of systolic thinning (EST) and expressed in degrees.

**Determination of cardiac myosin light chain II**: Blood samples were withdrawn through a catheter inserted into the jugular vein 0 h, 24 h, and daily until the 14th day. Detailed radioimmunoassay of cardiac myosin light chain II has been reported previously. Total release of cardiac myosin light chain II was calculated following the formula of Shell et al. The equation was as follows: Total release of light chain II = E_t + Kd (E_{t-x} + E_t)/2 \times t, where E_t = serum cardiac myosin light chain II at time t, Kd = exponential disappearance rate, (E_{t-x} + E_t)/2 = average cardiac myosin light chain II value during the preceding

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time interval, x. The Kd of cardiac myosin light chain II was 0.0025 min⁻¹.¹²

Post mortem examination of the heart: Infarct size was determined as mentioned elsewhere.¹³ Briefly, the dogs were killed 14 days after the production of myocardial infarction. The hearts were rapidly excised, washed in iced saline, wrapped in plastic, and suspended in a freezer for 30 min. Each heart was cut from apex to base into 4-mm slices. After the infarct margin was 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.

Statistical analysis: All values are expressed as mean ± standard deviation. The unpaired t test was used to compare differences between the two groups. A p value of less than 0.05 was considered statistically significant. Linear regression was computed by the least squares method.

RESULTS

Thirteen dogs were excluded from the study because they did not show electrocardiographic evidence of ST segment elevation at the occlusion,¹ died from ventricular fibrillation,⁵ did not yield 2-D echocardiogram of adequate quality for precise analysis.⁶ The results are based on the 18 remaining dogs, 9 dogs in group R and 9 in group C.

1. Serial changes in left ventricular wall motion at the infarcted area.

Left ventricular wall motion at the center of the ischemic area showed systolic thinning and
paradoxical motion beginning immediately after the coronary occlusion. SWT at 3 hours in groups C and R was $-7.7 \pm 2.8\%$ and $-9.9 \pm 3.0\%$, respectively. Thinning was developed at 6 hours in group C ($-10.6 \pm 3.2\%$). In this group, systolic thinning gradually improved, but thinning was observed even at 14 days (Fig. 2). In group R, slight return of myocardial contractility was seen at the first determination performed at 3 hours of reperfusion ($-8.0 \pm 3.0\%$). After all, considerable recovery of contraction was observed, but the improvement in contractility continued for as long as 2 days. Active thickening did not occur until 2 days after reperfusion. Also, SWT in group R became greater than that in group C for the first time at day 2. SWT on the day of sacrifice was $7.0 \pm 4.6\%$ (group R) and $-1.5 \pm 2.8\%$ (group C) ($p < 0.05$).

As shown in Fig. 2, the recovery of EST followed a similar pattern. After 2 days, EST eventually returned to normal in group R.

2. Relation between infarct size and left ventricular wall motion.

Relations between SWT or EST and histological infarct size at the time of sacrifice (14-day) were evaluated (Fig. 3). In group C, both 3-hour SWT and 14-day SWT correlated well with infarct size. Correlation coefficients were $-0.90$ and $-0.85$, respectively. In group R, 14-day SWT correlated with infarct size ($r = -0.73$), but 3-hour SWT did not. EST showed similar relations between infarct size. In group C, 3-hour EST and 14-day EST correlated well with

infarct size ($r = 0.74$, $r = 0.93$, respectively). In group R, 14-day EST correlated with infarct size ($r = 0.91$) but 3-hour EST did not.

3. Relation between infarct size and cardiac myosin light chain II.

As shown in Fig. 4, both peak cardiac myosin light chain II levels and total release of light chain II correlated very well with infarct size as determined on the day of sacrifice ($r = 0.86 = 0.88$, respectively) in all dogs. Fig. 5 shows relations between total release of myosin light chain and left ventricular wall motion at 14 days. There were quite good correlations among them ($r = -0.90$ vs 14-day SWT, $r = 0.89$ vs 14-day EST) irrespective of the presence of reperfused infarcts.

DISCUSSION

The major findings of the present study were that recovery of reperfusion-salvaged myocardial contractility takes as long as 2 days and the magnitude of myocardial contraction abnormality early after reperfusion does not reflect ultimate infarct size. We also showed that serum levels of myosin light chain quantitatively reflect the impairment of systolic function of the ischemic area regardless of the presence of reperfusion.

Coronary reperfusion early after the onset of myocardial infarction results in a reduction in infarct size$^{16-18}$ However, it has recently been reported that the recovery of contractility of transiently ischemic myocardium can take several days and the salvaged myocardium acts as if it has been stunned during that period$^{9-7}$ Although coronary thrombolysis is widely accepted as a way of reducing the mortality of acute myocardial infarction$^{2,3}$ many patients still die of failure in pump function of the heart. Since restoration of cardiac pump function is one of the goals of coronary thrombolytic therapy, time course of recovery of contractility of myocardium that is salvaged by reperfusion is important for patients’ care.

Our results indicate that reperfusion within 3 hours after infarction results in significant improvement of myocardial contractility. Although improvement was observed even in the control group, ultimate myocardial contractility was significantly greater in the reperfused group. Myocardial function, as determined by SWT, recovered to less than half of the preocclusion value in the reperfused dogs. EST recovered to within normal range in this group. Thus, reperfusion within 3 hours after infarction effectively improves cardiac function. However, this improvement takes as much as 2 days after reperfusion. These results are in general agreement with the results of previous studies$^{5,7,9,19-21}$

Detection and quantification of the extent of acute myocardial infarction using 2-D echocardiography have received considerable attention in recent years. Wyatt et al. reported that 2-D echocardiographic dysynergic areas correlated reasonably with infarct areas in dogs$^{20}$ Nieminen et al$^{21}$ also demonstrated that 2-D echocardiographic wall motion abnormalities improved and correlated better with infarct size at 48 hours than at 2 hours in a canine model of coronary occlusion. However, few studies have assessed the relation between the extent of reperfused myocardial infarction and wall motion abnormality as imaged by 2-D echocardiography$^{8,9}$ The results of the present study confirmed the good correlation between anatomical infarct size and 2-D echocardiographic estimates of infarct size in the setting of permanent coronary occlusion. Good correlations were observed both in the 14-day value of SWT and EST, as well as in the 3-hour value of SWT or EST in this group. However, the relation was different in the reperfused dogs. Although a good correlation was shown between wall motion abnormality at 14 days and infarct size, wall motion at 3 hours did not correlate with infarct size in this group. This discrepancy seems to stem from the presence of stunned myocardium. Therefore, the estimation of infarct size by 2-D echocardiography early after reperfusion should be made with caution.

We have shown that serum levels of myosin light chain quantitatively reflect both the extent of experimental myocardial infarction$^{12,13,22}$ and the impairment of left ventricular function in acute myocardial infarction$^{10,11}$ In the present study, we attempted to define the relation between light chain release and the degree of wall motion impairment in dogs with and without reperfusion. We found good correlations among serum levels of myosin light chain, SWT or EST at 14 days, and anatomical infarct size. In addition, these relations stood for the presence of coronary reperfusion. Although myosin light chain is a component of structural protein, its serum level rises rapidly after the onset of acute
myocardial infarction and remains elevated for more than a week. Since the release of light chain is a very gradual process, drastic changes in coronary flow might not influence the release from the necrotic myocardium. Accordingly, serum light chain levels could reflect the changes in left ventricular function even after coronary reperfusion.

Our findings may have important clinical implications. This study demonstrates the restoration of function in the salvaged myocardium. However, the recovery of systolic function is delayed for days after reperfusion. Protective effects of reperfusion on ischemic myocardium, therefore, should be evaluated not only from the reduction of infarct size, but also from the rate of recovery of contractility. There have been several studies concerning the protective effects of pharmacological interventions on the contractile function of reperfusion-salvaged myocardium. It has been shown that stunned myocardium possesses functional reserve to respond to stimulation of inotropic agents. The combined administration of propranolol and diltiazem is reported to increase segmental contractile function of the ischemic area during reperfusion. We have also observed that administration of 1-carnitine chloride improves the contractile function of the stunned myocardium and suppresses the release of myosin light chain at an early stage after infarction.

These observations indicate that the administration of drugs in addition to coronary reperfusion may be effective in improving the myocardial contractility of the previously ischemic myocardium. As shown in this study, serum cardiac myosin light chain is useful for the study of the effects of coronary reperfusion on infarct size because of its insensitivity to reperfusion, and serial 2-D echocardiography is suited for evaluating functional recovery of stunned myocardium.

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