Assessment of Cardiac Function and Gene Expression at an Early Phase after Myocardial Infarction

Akihisa Hanatani, MD, Minoru Yoshiyama, MD, Shokei Kim,1 MD, Takashi Omura, MD, Yoshiyasu Ikuno,2 MD, Kazuhide Takeuchi, MD, Hiroshi Iwao,1 MD, and Junichi Yoshikawa, MD

SUMMARY

The purpose of this study was to examine left ventricular function and cardiac gene expressions at an acute phase after myocardial infarction (MI). MI was induced in rats by ligation of the left coronary artery. Two days after MI, we performed Doppler-echocardiography and measured the systolic and diastolic function. We then analyzed the contractile protein and extracellular matrix mRNAs of cardiac tissues in the infarcted region, including the adjacent noninfarcted myocardium (the adjacent noninfarcted myocardium) and the remote noninfarcted myocardium, by Northern blot hybridization. Fractional shortening decreased significantly to 28%. Peak early diastolic filling wave (E wave) velocity increased in MI rats (MI; 90 ± 3 cm/s versus the control; 71 ± 2 cm/s, p < 0.05), and the deceleration rate of the E wave velocity was more rapid in MI rats (MI; 22.0 ± 2.6 m/s² versus the control; 16.5 ± 2.0 m/s², p < 0.01). Atrial filling wave (A wave) velocity decreased, resulting in a marked increase in the ratio of E wave to A wave velocity (MI; 3.1 ± 0.3 versus the control; 2.1 ± 0.2, p < 0.01). In the adjacent noninfarcted myocardium, mRNA levels for α-skeletal actin, atrial natriuretic polypeptide (ANP), transforming growth factor-β1 (TGF-β1), fibronectin, and collagen types I and III increased significantly. In the remote noninfarcted myocardium, mRNA levels for α-skeletal actin, ANP, and collagen types I and III increased, while mRNA levels for β-myosin heavy chain, TGF-β1 and fibronectin did not change. We observed left ventricular dysfunction and different gene expressions between adjacent noninfarcted myocardium and in the remote noninfarcted myocardium two days after MI. These findings suggest that cardiac gene expression after MI may be a compensation reaction for cardiac dysfunction induced by myocardial damage. (Jpn Heart J 1998; 39: 375-388)

Key words: Cardiac phenotype, Extracellular matrix, Myocardial infarction, Gene expression, Echocardiography

From the First Department of Internal Medicine and 1Department of Pharmacology, Osaka City University Medical School, Osaka, and 2Tane General Hospital, Osaka, Japan.

Address for correspondence: Minoru Yoshiyama, MD, First Department of Internal Medicine, Osaka City University Medical School, 1–5–7 Asahimachi, Abeno, Osaka 545, Japan.

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The loss of myocardium due to infarction demands that the surviving tissue sustain the pumping action of the heart. Ultimately, the recovery of cardiac function depends mainly on cellular responses, characterized by hypertrophy of myocytes (1) and hyperplasia of interstitial fibroblasts after myocardial infarction. Elucidating the changes in contractile protein and collagen is necessary for analyzing the mechanism of cardiac contraction and relaxation. The myocyte has two myosin isoforms, α-myosin heavy chain (MHC) and β-MHC, and two actin isoforms, α-cardiac actin and α-skeletal actin. These isogenes are known to be expressed in a different manner in cardiac hypertrophy and to be involved in the regulation of cardiac performance.4,21,22) Interstitium contains the collagen network and the majority of collagen proteins are fibrillar collagen types I and III27) and these changes may affect cardiac function. These gene expressions may be induced by cardiac dysfunction at an early phase after myocardial infarction. Our previous study reported that the expressions of fetal phenotypic gene and collagen gene were increased in the noninfarct left ventricular myocardium 1, 2 and 3 weeks after myocardial infarction.10 However, we did not analyze these gene expressions and did not measure cardiac function at an early stage after myocardial infarction.

The investigation of cardiac gene expression and function in myocardial infarcted (MI) rats may help elucidate myocardial infarction. The present study was undertaken to examine left ventricular function and cardiac gene expression at an acute phase after myocardial infarction. We performed Doppler-echocardiography and investigated the mRNA expressions of contractile proteins, atrial natriuretic polypeptide (ANP), transforming growth factor-β1 (TGF-β1) and extracellular matrix (fibronectin, collagen types I and III) in the adjacent noninfarcted myocardium and the remote noninfarcted myocardium two days after myocardial infarction.

Materials and Methods

Experimental protocol: Myocardial infarction was produced in male Wistar rats, weighing 290–310 g (Clea Japan Inc., Osaka, Japan), by a previously described method of coronary artery ligation.6,7 In brief, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (35 mg/kg body weight), and after intratracheal intubation a left thoracotomy was performed under volume-controlled mechanical ventilation. The heart was raised from the thorax and a ligature with a 6-0 prolene suture was placed around the proximal left anterior descending coronary artery. The chest was then closed. The same surgical procedures were also performed in the control rats, except that the suture around the coronary artery was not tied.
Doppler-echocardiographic studies: Transthoracic echocardiography was performed on each rat by modifying the previously described study. In brief, the rats were anesthetized with ketamine HCl (25 to 50 mg/kg body weight) and xylazine (5 to 10 mg/kg body weight). Echocardiograms were performed with a commercially available echocardiographic system equipped with a 7.5-MHz phased-array transducer (Hewlett Packard SONOS 2500, Andover, MA, USA).

A two-dimensional short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles. M-mode tracings were recorded through the anterior and posterior LV walls at a paper speed of 100 mm/s. The anterior and posterior end-diastolic and end-systolic LV internal dimensions were measured by the American Society of Echocardiology (ASE) leading-edge method. At least three dimensions were analyzed in consecutive cardiac cycles on the M-mode tracings and the percent fractional shortening (%FS) calculated. All measurements were performed by two observers and analyzed using the analysis software present on the echocardiography machine.

Pulse-wave Doppler spectra of mitral inflow velocities were recorded from the apical four-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximal and the flow pattern laminar. Sample volume was set at the smallest size available. The left atrium was then examined with pulsed-wave Doppler for the presence of mitral regurgitation. All Doppler spectra were recorded at a paper speed of 100 mm/s and analyzed off-line as previously described. The results represent the mean of at least three consecutive cardiac cycles.

Hemodynamic studies: Two days after the operation, the systolic, diastolic and mean blood pressures of the conscious rats were measured by the tail-cuff method using a sphygmomanometer (Riken Development Co., Ltd., Tokyo, Japan). Each value is the average of three consistent readings. After that, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (35 mg/kg body weight) (Each group; n = 6). The hemodynamic measurements were performed 5 minutes after an injection of pentobarbital sodium. Reasonable hemodynamic data were obtained in our previous studies, with the same dose as that in this study. When the level of the anesthesia was shallow or deep, we added pentobarbital sodium at 20 mg/kg body weight or waited for a while, respectively. A stable hemodynamic state in our model could be achieved in this way. Body temperature was maintained at about 37°C with a heating pad. The method of hemodynamic measurement has been described in detail. In brief, LV end-diastolic pressure was recorded by inserting a polyethylene tubing catheter (0.58 mm internal diameter, PE-50) into the right carotid artery and advanced into the left ventricle. A water-filled catheter was connected to polyethylene tubing connected to a water-filled pressure transducer (Model P23 ID, Gould
Inc., CA, USA). Pressures were recorded on a physiological recorder (Polygraph MIC-9800 and Thermal Recorder RF-85, Fukuda Denshi, Tokyo) with rats breathing spontaneously. LV end-diastolic pressure was obtained by averaging the values for 10 beats.

After a hemodynamic measurement, the rats were decapitated and the hearts rapidly removed. Myocardial infarct size was measured, as previously described. Rats with less than 20% of the infarct size were excluded from analysis. After determination of the infarct size, the left ventricle was divided into two parts, the myocardial infarcted region including two mm around the infarcted zone (adjacent noninfarcted myocardium) and the remote noninfarcted myocardium. After weighing, the tissues were rapidly frozen in liquid nitrogen and stored at −80°C until use.

Oligonucleotide and cDNA probes: Synthetic oligonucleotide probes complementary to the unique 3’ untranslated regions of the two MHC and two α-actin mRNAs were used, as previously described. In addition, to monitor the RNA content of the different lanes, we hybridized the blots with a 24-base oligonucleotide probe complementary to rat 18S ribosomal RNA. The sequences of the oligonucleotide probes were as follows:

- α-MHC, 5’-TTGTGGGATAGCAACAGCGA-3’;
- β-MHC, 5’-GTCTCAGGGCTTCACAGG-3’;
- α-skeletal actin, 5’-GCAACCATAGCACGATGGTC-3’;
- α-cardiac actin, 5’-TGCACGTGTGAACAAACT-3’;
- 18S, 5’-ACGGTATCTGATCGTCTTCGAACC-3’;

The oligonucleotide probes were labeled with (γ-32P)-ATP (6,000 Ci/mM) at the 5’ end, using T4 polynucleotide kinase, and the labeled probes were purified by chromatography on a Bio-Spin 6 column (Bio-Rad, Richmond, CA, USA).

The cDNA probes used were rat transforming growth factor-β1 (TGF-β1) (a 1.0 kb HindIII/XbaI fragment), rat fibronectin cDNA (a 0.27 kb HindIII/EcoRI fragment), rat α1(I) collagen cDNA (a 1.3 kb PstI/BamHI fragment), mouse α1(III) collagen cDNA (a 1.8 kb EcoRI/EcoRI fragment), and rat ANP cDNA (a 0.825 kb fragment).

The cDNA probes were labeled with (32P)-dCTP (specific activity 3000 Ci/mM, New England Nuclear, Boston, MA) by a random primer extension method, using a Random Primer DNA Labeling Kit (Takara, Kyoto).

Extraction of total RNA: The total RNA extraction method used has been previously described in detail. The RNA concentration was spectrophotometrically determined by absorbance at 260 nm.

Northern blot hybridization: Twenty micrograms of total RNA were denatured by incubating with 1 M deionized glyoxal and 50% dimethyl sulfoxide at
50°C for 1 hour, electrophoresed on a 1% agarose gel and transferred to a nylon membrane (Gene Screen Plus, E.I. du Pont de Nemours and Co., NEN Products, Boston, MA), as previously described. The 28S and 18S ribosomal RNAs in gels were stained with ethidium bromide to demonstrate the integrity of applied RNA and verify that the same amount of RNA was applied to each lane. The method of Northern blot analysis with an oligonucleotide probe and a specific cDNA probe has been previously described.

All RNA samples were denatured at the same time, electrophoresed on the same gel and transferred to the same nylon membrane. Furthermore, hybridization, washing and autoradiography of the membrane were carried out at the same time. Our Northern blot analysis procedure therefore allowed for an accurate comparison between all samples.

Quantification of mRNA: To evaluate mRNA levels, an optical scanner (EPSON GT-8000, Seiko, Tokyo) was utilized for digitizing autoradiograms. The densities of the autoradiogram bands in the digitized image were measured using a public domain NIH image program and a computer (Macintosh LC-III, Apple Computer, Inc., CA), as previous described. For all the RNA samples, the density of an individual mRNA band was divided by that of an 18S mRNA band to correct for the difference in RNA loading and/or transfer.

Statistics: Results are expressed as mean ± SE. Statistical significance was determined using ANOVA and Duncan’s multiple range test. A difference was considered statistically significant at a value of p < 0.05.

RESULTS

Changes in weight and hemodynamics: As shown in Table I, there were no significant differences in body weight, LV weight/body weight, mean blood pressure, or heart rate between the control and the MI rats. In MI rats, however, LV end-diastolic pressure significantly increased to 20 ± 3 mmHg compared with the control rats (p < 0.01).

Doppler-echocardiographic assessments: M-mode echocardiograms of the

| Table I. Changes in Body Weight and Hemodynamics of the Control and Myocardial Infarcted Rats |
|-----------------------------------------------|-----------------|
| Control                                      | MI              |
| Body weight (g)                              | 306 ± 5         | 302 ± 6         |
| LV weight.Body weight (g/kg)                 | 2.08 ± 0.07     | 2.02 ± 0.06     |
| Mean blood pressure (mmHg)                  | 110 ± 4         | 104 ± 4         |
| Heart rate (beats/min)                       | 363 ± 7         | 365 ± 6         |
| LVEDP (mmHg)                                 | 4 ± 1           | 20 ± 3**        |
| Myocardial Infarcted size (%)               |                 | 35 ± 4          |

**p < 0.01 v.s. control
Control

Figure 1. Examples of the M-mode echocardiograms from an anesthetized control and myocardial infarcted rat left ventricle two days after myocardial infarction. AW = anterior wall; PW = posterior wall.

Table II. Doppler Echocardiographic Measurements of the Control and Myocardial Infarcted Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDd (mm)</td>
<td>5.4 ± 0.2</td>
<td>7.0 ± 2.1**</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>3.3 ± 0.3</td>
<td>5.0 ± 0.3**</td>
</tr>
<tr>
<td>FS (%)</td>
<td>39 ± 2</td>
<td>28 ± 2**</td>
</tr>
<tr>
<td>E Velocity (cm/s)</td>
<td>71 ± 2</td>
<td>90 ± 3**</td>
</tr>
<tr>
<td>A Velocity (cm/s)</td>
<td>35 ± 3</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>E/A</td>
<td>2.1 ± 0.2</td>
<td>3.1 ± 0.3*</td>
</tr>
<tr>
<td>E Deceleration (m/s²)</td>
<td>16.5 ± 2.0</td>
<td>22.0 ± 2.6**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 v.s. control

Figure 2. Examples of the pulsed Doppler spectra of the mitral inflow pattern from the control and myocardial infarcted rats. E = early diastolic filling wave velocity; A = atrial filling wave velocity.
left ventricle of a control rat and an MI rat are shown in Figure 1. In these images, there is a symmetric thickening of the anterior and posterior walls in the control rat, while in the MI rat, the anterior wall is thinned and akinetic, and the left ventricular cavity is remarkably dilated compared with the control rat. The results of echocardiographic assessments of LV geometry and systolic function for both groups of rats are shown in Table II. LV diastolic dimension (LVDd) was significantly increased from 5.4 to 7.0 mm compared with that of the control rat (p < 0.01). LV systolic function was abnormal in the infarcted hearts. LV systolic dimension (LVDs) was significantly increased from 3.3 to 5.0 mm and fractional shortening (FS) remarkably decreased from 39% to 28% compared with those of the control rat (p < 0.01).

Examples of pulsed-wave Doppler recordings of mitral inflow from a control rat and an MI rat are shown in Figure 2. MI rats also had diastolic dysfunction, as defined by an increased peak early diastolic filling wave (E wave) velocity, deceleration rate of the E wave velocity, a decreased atrial filling wave (A wave) velocity, and an increase in the ratio of E wave to A wave velocity. Peak E wave velocity increased in MI rats (MI; 90 ± 3 cm/s versus the control; 71 ± 2 cm/s, p < 0.05), and the deceleration rate of the E wave velocity was more rapid in MI rats (MI; 22.0 ± 2.6 m/s² versus the control; 16.5 ± 2.0 m/s², p < 0.01). A wave velocity was decreased (MI; 29 ± 2 cm/s versus the control; 35 ± 3 cm/s, N.S.), resulting in an increase in the ratio of E wave to A wave velocity (MI; 3.1 ± 0.3
versus the control; 2.1 ± 0.2, \( p < 0.01 \).

**Gene expressions of contractile proteins and ANP RNAs after myocardial infarction:** mRNA expression in the infarcted region, including the adjacent noninfarcted myocardium, represent the adjacent region because the infarcted region is thin tissue and contains little mRNA. As shown in Figures 3 and 4, in the adjacent noninfarcted myocardium, \( \alpha \)-MHC, \( \beta \)-MHC, and \( \alpha \)-cardiac actin mRNA levels decreased by about 0.2-, 0.4-, and 0.7-fold, respectively, at 2 days (\( p < 0.01 \)), while \( \alpha \)-skeletal actin and ANP mRNA levels increased by 2.1- and 4.5-fold (\( p < 0.01 \)), respectively. In the remote noninfarcted myocardium, \( \alpha \)-MHC, \( \beta \)-MHC, and \( \alpha \)-cardiac actin mRNA levels did not show significant
Figure 5. Typical autoradiograms of Northern blot analysis of the adjacent noninfarcted myocardium and the remote noninfarcted myocardium mRNAs for transforming growth factor-β1 (TGF-β1), fibronectin, collagen types I and III and 18S ribosomal RNA two days after myocardial infarction. C = sham-operated rats; Aj = adjacent noninfarcted myocardium; Re = remote noninfarcted myocardium.

Figure 6. Bar graphs show the left ventricular mRNA levels two days after myocardial infarction. Control = sham-operated rats; Adjacent = adjacent noninfarcted myocardium; Remote = remote noninfarcted myocardium. Each mRNA value was normalized for the 18S mRNA value. Each bar represents mean ± SE (n = 6 in each group). *p < 0.05, **p < 0.01 vs. control.
change, while α-skeletal actin and ANP mRNA levels increased by 1.7- and 3.8-fold (p < 0.01), respectively, as in the adjacent noninfarcted myocardium.

**Gene expressions of TGF-β1, fibronectin and types I and III collagen mRNAs:** Figures 5 and 6 show the Northern blot analysis of extracellular matrix components and TGF-β1. In the adjacent noninfarcted myocardium, mRNA levels for TGF-β1, fibronectin, and types I and III collagen significantly increased by 2.9-, 6.8-, 2.7-, and 2.6-fold (p < 0.01), respectively. In the remote noninfarcted myocardium, TGF-β1 and fibronectin mRNA levels did not show significant changes, while types I and III collagen mRNA levels increased by 1.7- and 1.7-fold (p < 0.01), respectively, in the adjacent noninfarcted myocardium.

**DISCUSSION**

We found evidence of an increase in left ventricle internal diastolic dimension, a decrease in fractional shortening, an increase in peak E wave velocity, and a more rapid deceleration rate of E wave velocity. These findings were associated with higher levels of mRNA for α-skeletal actin, ANP, and collagen types I and III in the adjacent noninfarcted myocardium and the remote noninfarcted myocardium.

Previous investigators have demonstrated that ligation of the left coronary artery in the rat results in transmural anterior infarction that is followed by marked hemodynamic alteration and progressive increases in LV volume similar to those seen in patients with anterior myocardial infarction.1,18,19) Litwin et al. demonstrated the technical feasibility of using serial transthoracic echocardiography following postinfarction in the rat.17) We also demonstrated the appearance of chamber dilatation, systolic dysfunction, and an abnormal LV filling pattern after myocardial infarction, which is consistent with Litwin’s study. Fractional shortening can be used as systolic function. On the other hand, many aspects of diastolic function have been inferred from the pattern of transmitral flow velocity seen on pulsed-wave Doppler.9,28) In this rat model, increased peak E wave velocity, decreased peak A wave velocity (or absent A wave velocity), and rapid E wave velocity deceleration were observed, and they are similar to the transmitral flow profiles observed in patients with heart failure with a restrictive filling pattern. Increased peak E wave velocity probably results from increased left atrial pressure, and rapid E wave velocity deceleration appears to be most strongly associated with an increase in operating chamber stiffness.25) Although we were not able to directly measure left atrial pressure, we found a marked increase in LV end-diastolic pressure in our infarcted rats. Litwin et al. demonstrated an increased stiffness of the surviving, noninfarcted myocardium which may be due to accumulation of interstitial collagen.17) Therefore, we believe this
cardiac dysfunction may be partially related to the induction of cardiac gene expression after myocardial infarction.

Our previous studies showed that $\beta$-MHC, $\alpha$-skeletal actin and ANP mRNAs increased after myocardial infarction in the noninfarcted myocardium. These data suggest that systolic dysfunction is certainly related in part to the induction of qualitative change in myocardium, such as modulation of contractile protein or increased interstitial collagen. Two days after myocardial infarction, ANP and $\alpha$-skeletal actin mRNAs had already increased in the adjacent noninfarcted myocardium and the remote noninfarcted myocardium. On the other hand, the mRNA expressions of $\alpha$-MHC, $\beta$-MHC, and $\alpha$-cardiac actin decreased in the adjacent noninfarcted myocardium. The gene expressions of the contractile protein and ANP in the adjacent noninfarcted myocardium are supposed to be located in the adjacent noninfarcted myocardium. This study suggests that ANP and $\alpha$-skeletal actin may be sensitive to a shift of myocytes to fetal phenotype in the noninfarcted myocardium after myocardial infarction. Interestingly, $\beta$-MHC mRNA was decreased in the adjacent noninfarcted myocardium and was not changed in the remote noninfarcted myocardium. The gene expression mechanism of $\beta$-MHC may be different from those of ANP and $\alpha$-skeletal actin genes. ANP and $\alpha$-skeletal actin are thought to be useful markers for changes in myocardial properties.

The change in the left ventricle after myocardial infarction is associated with not only the phenotypic modulation of noninfarcted myocardium but also with the infarcted region, which is characterized by increased deposition of extracellular matrix such as collagen (mainly composed of types I and III collagen) and fibronectin. Fibronectin is one of the main extracellular matrix proteins and is thought to have a functional role in diverse cellular processes, including wound healing. It is localized along the surface of cardiac myocytes and connects cardiac myocytes to perimyotic collagen and stimulates fibroblast proliferation. These properties may contribute to the acute phase of the repair process postinfarction where cell slippage is thought to contribute to ventricular dilatation and infarct expansion before collagen begins to accumulate to form the final scar. In our data, although mRNA of fibronectin increased in the adjacent noninfarcted myocardium, there was no significant increase in the remote noninfarcted myocardium. So fibronectin may have an important role in scar formation after myocardial infarction.

Our data showed that gene expression of collagens in the adjacent noninfarcted myocardium increased much more than gene expression in the remote noninfarcted myocardium. Collagen types I and III are the most abundant forms of collagen within cardiac tissue. In the latter stage of cardiac wound healing after myocardial infarction, these collagen types comprise the scar tissue
that replaces necrotic myocytes. Therefore, gene expression of collagens in the adjacent noninfarcted myocardium increased much more than it did in the remote noninfarcted myocardium. In our study, the increases in collagen types I and III mRNA in both regions had already occurred two days after myocardial infarction. Thus it may be surmised that interstitial fibrosis in the noninfarcted myocardium starts two days after myocardial infarction.

TGF-β1 is a multifunctional growth factor which regulates cell growth and stimulates the production of collagen and fibronectin, thereby contributing to the healing process after myocardial infarction. In this study, TGF-β1, fibronectin, and type I and III collagen mRNAs increased in the adjacent noninfarcted myocardium at an early stage after myocardial infarction. However, in the remote noninfarcted myocardium TGF-β1 mRNA did not increase significantly. Thus, we believe that TGF-β1 may have an important role in scar formation in an infarcted region.

**Study limitations:** In this study, we did not show the relationship between mRNA and protein levels. mRNA levels may not necessarily be representative of protein levels. Therefore, they cannot provide sufficient data for us to analyze progressive cardiac dysfunction after myocardial infarction. In future studies, it will be necessary to measure protein levels in the noninfarcted myocardium after myocardial infarction.

In conclusion, we observed left ventricular dilatation and different expressions of fetal phenotypic and extracellular matrix genes in adjacent noninfarcted myocardium and remote noninfarcted myocardium two days after myocardial infarction. These findings suggest that cardiac gene expression after myocardial infarction may be a compensatory reaction for the cardiac dysfunction induced by myocardial damage.

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