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REGULAR PAPER

Experimental Research

Sustained left ventricular diastolic dysfunction following ischemia reperfusion injury in an acute myocardial infarction rat model

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Abstract

The aim of this study was to investigate cardiac function in a rat myocardial ischemia/reperfusion injury model during the early phase using echocardiography. Adult male Sprague-Dawley rats underwent myocardial ischemia and reperfusion (I/R) injury by ligation of the left anterior descending coronary artery (LAD). Echocardiography was performed at 2 hours, 24 hours, 3 days, and 7 days after the induction of myocardial infarction (MI). The ejection fraction (EF) and fractional shortening (FS), indexes that reflect left ventricular systolic function, of the MI group were significantly decreased compared with those of the sham group at 2 hours and on day 1 ($P < 0.01$). To assess left ventricular (LV) diastolic dysfunction, we measured the peak velocity of the transmitral flow at early filling (E), the early diastolic mitral annular velocity (E') at the medial mitral annulus and the E/E' ratio. During the entire experimental period, the E' values were significantly decreased and the E/E' values were significantly increased in the MI group compared to the sham group. These results suggest that, unlike systolic function, diastolic dysfunction does not recover in the early phase of ischemic reperfusion injury in rats; this may be important for the development of effective therapies for acute MI. Moreover, this animal model and ultrasound-based assessment of cardiac function can be used in translational research and in the development of new heart failure drugs.

Introduction

Myocardial infarction (MI) is a major cause of cardiovascular morbidity and mortality worldwide and is characterized by the interruption of blood supply to a part of the heart, causing damage to the heart muscle[6,30]. Myocardial dysfunction can be evaluated by echocardiography, which is a well-established diagnostic tool for non-invasive and accurate evaluation of cardiac anatomy and hemodynamic function in clinical practice[34]. The best indicator of prognosis in MI patients is left

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ventricular (LV) function[15], and the patients with diastolic dysfunction has worse surgical outcomes than patients with only systolic dysfunction in the perioperative periods[27]. The rat model of myocardial ischemia and reperfusion (I/R) injury has been commonly used in mechanism studies and efficacy studies of new drugs or stem cells[8,16,29,31].

Recent technological advancements in echocardiography devices allow thorough echocardiographic examination even in laboratory rodents, which are small in size and have a rapid heart rate of over 300 beats per minute[4,12,33]. Therefore, there is increasing interest in using echocardiography as a basic research tool for the evaluation of cardiac function among standard laboratory animals[12,33].

Previous studies using echocardiography for the evaluation of MI models have focused on a relatively late phase of MI, as myocardial remodeling is a major topic in MI research[12,16,26,33]. However, it is necessary to evaluate myocardial function in the early phase after MI because I/R injury is a common cause of acute myocardial infarction, and therapeutic strategies for the prevention of myocardial I/R injury can improve clinical outcomes in patients[6,16,30,31].

In the present study, we evaluated myocardial function using echocardiography at 2 hours after reperfusion and on days 1, 3, and 7 after myocardial I/R injury in rats. This study aimed to show the significance of echocardiographic evaluation in the early phase after MI, which might be necessary for the development of effective therapies for acute myocardial infarction.

Materials and methods

Animals: Thirty-three adult male Sprague-Dawley rats (mean $\pm$ standard deviation (SD), 277.40 $\pm$ 9.48 g) were purchased from Koatech (Kyungki, South Korea) and kept on a 12-hr light/12-hr dark cycle for 7 days to acclimate to the animal facility. The rats were randomly divided into a sham group ($n = 15$) and myocardial I/R group ($n = 18$) as shown below (Fig. 1A).

All animal experiments were performed according to the Daegu-Gyeongbuk Medical Innovation Foundation guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF-16091201-00).

Myocardial ischemia/reperfusion injury: Animals were anesthetized with pentobarbital (65 mg/kg IP) and intubated. They were placed on a heated plate and monitored by ECG. The rat was monitored for 15 minutes to stabilize the heart status and then ventilated by a respirator (Harvard Apparatus Inspira, MA, USA) with a tidal volume of 3.0 ml/kg and at a rate of 60 bpm.

A 3-cm transverse incision was made at the left fourth intercostal space, and a chest retractor was positioned within the incision. The pericardium was opened to expose the left anterior descending coronary artery (LAD), which was ligated between the pulmonary cone and the left auricle using 6-0 silk (Fig. 1B). MI was confirmed by cyanosis of the apical region of the heart with S-T segment elevation on the ECG (Fig. 1C). After 30 minutes of ischemia via snaring, the suture on the polyethylene tube was loosened to allow reperfusion. The intercostal space was then closed with 3-0 white silk suture, and the skin incision was closed with 4-0 nylon suture. The sham group underwent the same surgical process without ligation.

Echocardiographic analysis: Echocardiography was performed 2 hours, 24 hours, 3 days, and 7 days after the induction of MI using Vevo2100 (VisualSonics Inc., Ontario, Canada). The rats were intraperitoneally injected with an additional dose of 25 mg/kg pentobarbital on the day of the surgery for 2 hours. For other time points, the animals were anesthetized with pentobarbital (65 mg/kg IP) and were placed in a supine position (Fig. 1D). The rats were then monitored by ECG
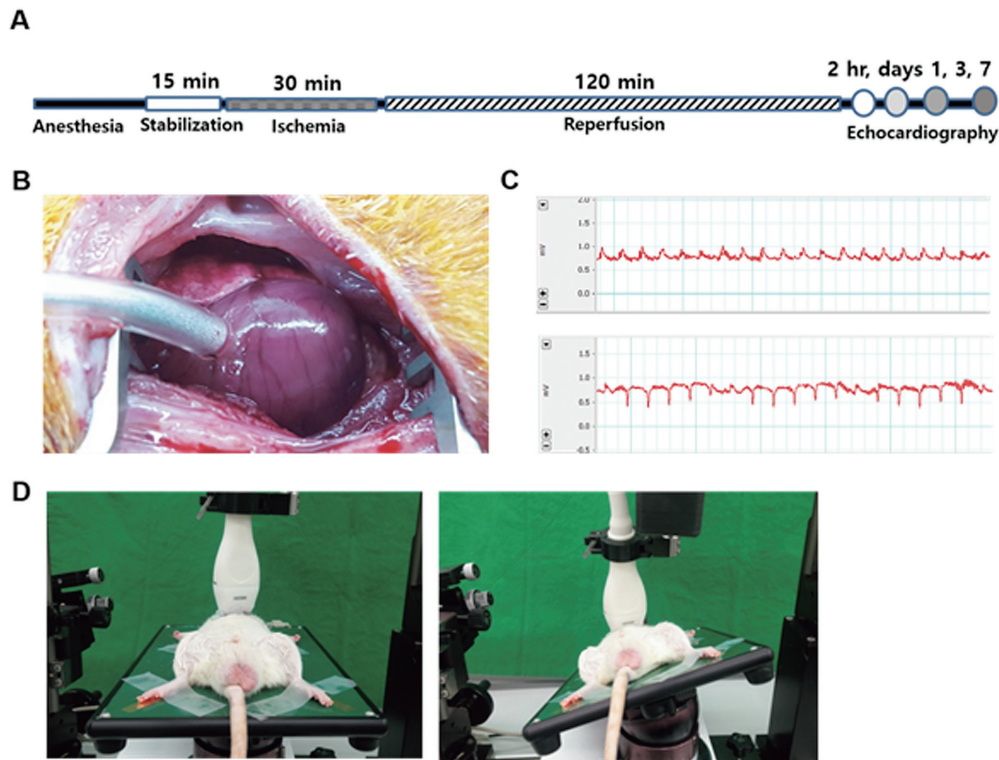
Fig. 1. Experimental design and surgical procedures for establishing the rat myocardial infarction/ reperfusion injury model. (A) Experimental design (B) Ligation of the left anterior descending (LAD) artery: a lateral thoracotomy was performed on the left side of the sternum through the 4th intercostal space. After ligation, a tube was placed, and a pale apical region was confirmed. (C) Electrocardiogram of the myocardial ischemia/ reperfusion animals: preoperation (upper) and postoperation (lower). (D) Positioning for echocardiography: parasternal short-axis view (left) and apical 4 chamber view (right).

and kept at 37°C.

Echocardiographic parameters were recorded in accordance with the American Society of Echocardiography Guidelines[2]. Images were obtained from the left parasternal short-axis views of the left ventricle at the level of the papillary muscles to define wall thicknesses and internal diameters during systole and diastole and to detect regional wall motion abnormalities in the LAD territory. The left ventricular posterior wall (LVPW) at diastole and systole and the interventricular septum (IVS) thicknesses at diastole and systole were measured. Additionally, the left ventricular internal diameter (LVID) at diastole and systole, ejection fraction (EF), fractional shortening (FS), stroke volume (SV), and cardiac output (CO) were measured to determine LV systolic function. In the apical four chamber views, the ratio of the early (E) to late

(A) ventricular filling velocities (E/A) and E/E' were calculated using color flow Doppler (CFD), pulsed wave Doppler, and tissue Doppler imaging to determine diastolic function.

Statistical analysis: Statistical analysis was performed with SPSS version 19 (SPSS, Inc., IL, USA). All data are expressed as the mean $\pm$ SD. The results for each group were compared by two-sample *t*-test and repeated measure two-factor analysis. And multiple comparison was performed with Bonferroni correction. The difference was considered statistically significant at $P < 0.05$.

Results

The experimental time schedule is shown in Fig. 1A; the rats were assigned to sham surgery
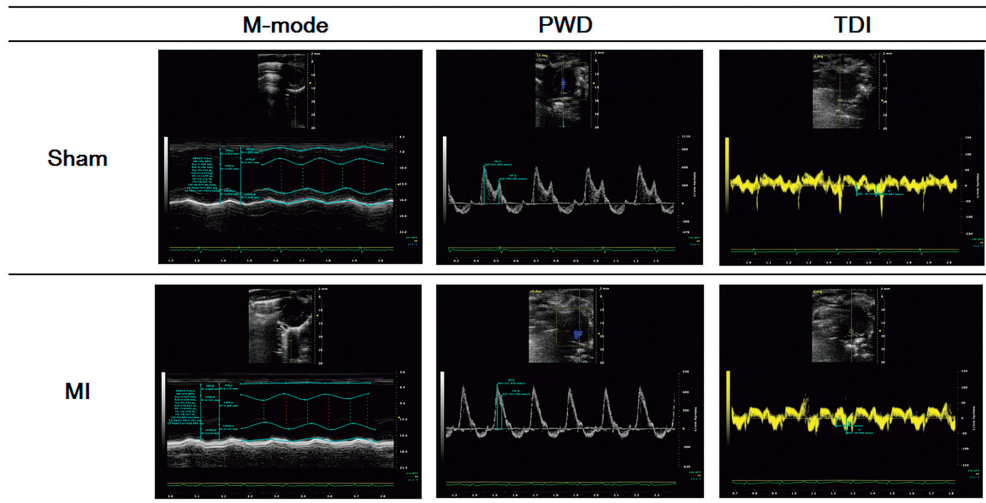
Fig. 2. Representative ultrasound images of rat hearts. Images of the mitral inflow velocity profile as determined by pulsed wave Doppler and tissue Doppler on 3 days are also shown. The E' value was significantly decreased and the E/E' value was significantly increased in the MI group compared to the sham group despite similar EF and FS values on 3 days. PWD: Pulse wave Doppler; and TDI: Tissue Doppler imaging.

or myocardial I/R injury (Fig. 1). The total mortality within 24 hours among rats subjected to coronary ligation was 16.6 %. None of the rats in the sham group died. Echocardiographic data were obtained from M-mode tracings, pulse wave Doppler, and tissue Doppler imaging (Fig. 2) and were summarized in Fig. 3 and Table 1.

The EF and FS, indexes that reflect LV systolic function, of the MI group were significantly decreased compared with those of the sham group at 2 hours and on day 1 ($P < 0.01$). To assess LV diastolic dysfunction, we measured the peak velocity of the transmitral flow at early filling (E), the early diastolic mitral annular velocity (E') at the medial mitral annulus, and the E/E' ratio, which exhibits relatively high reproducibility in humans[3]. The E' values were significantly decreased and the E/E' values were significantly increased in the MI group compared to the sham group.

Discussion

In this study, we investigated myocardial function in the early stage of myocardial I/R injury in rats using echocardiography. Significant decreases in EF and FS after myocardial infarction (MI) indicate MI induction as typical ischemic changes which have been reported[5,12,32]. In the sham group, the values of EF and FS at 2 hours were significantly higher than those at other time points (Fig. 3AB, Table 1); these hemodynamic effects may be attributed to sympathetic stimulation, a normal physiologic response to surgical pain[10,28]. In contrast, the values of EF and FS in the MI group were not increased at the same time point, which might reflect hemodynamic dysfunction in response to surgical pain in MI animals.

Transmitral inflow (E), which occurs during the rapid filling phase in early diastole, is sensitive to preload and increases with shorter deceleration time as diastolic function becomes worse with increasing filling pressure. However, E' is less sensitive to preload and decreases at all stages of diastolic dysfunction. In fact, reduced E' is usually the earliest manifestation of diastolic dysfunction in humans[2]. In clinical fields, LV filling patterns of transmitral flow and deceleration time, as revealed by echocardiography, are most commonly used to assess LV diastolic function and are dependent on preload variation, heart rate and heart rhythm disturbances[9,18]. In
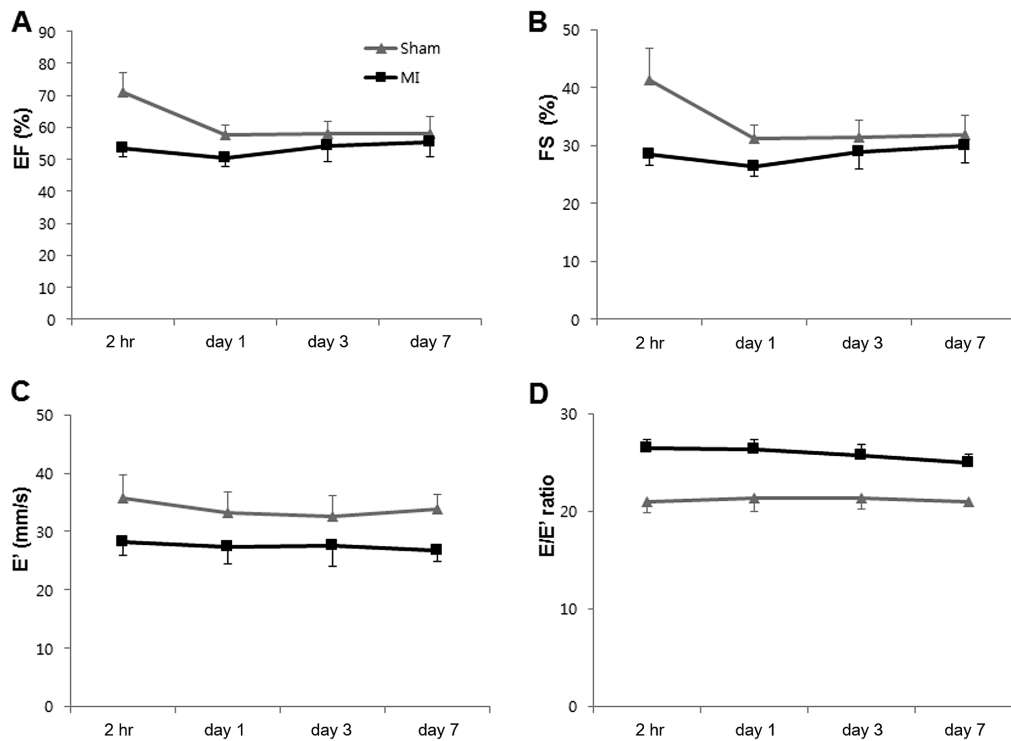
Fig. 3. Cardiac function as measured by echocardiography. (A) Fractional shortening, FS; (B) Ejection fraction, EF; (C) Early relaxation velocity on tissue Doppler, E'; (D) The E/E' ratio of diastolic function. The EF and FS determined by M-mode imaging reflect systolic dysfunction; the E' and E/E' ratios reflect determined by tissue Doppler imaging reflect diastolic dysfunction.

comparison, E' measurement with tissue Doppler imaging of the mitral annulus is considered a more sensitive tool in the assessment of diastolic dysfunction and becomes preload-independent, since E' reflects the myocardial velocity and not the velocity of blood flow[7,25]. In this experiment, we measured E' in the medial annulus because it is easy to measure with transthoracic echocardiography in humans. Usually, the values of E' at the medial annulus are smaller than those at the lateral annulus, which exhibits increased freedom from its surrounding tissues and, thus, more mobility[22]. As E' indicates early active diastolic relaxation of the left ventricle but not its compliance, the E/E' ratio reflects mean left atrial pressure, which indicates LV filling pressure. In humans, elevation of LV filling pressure is the most important factor for poor outcomes, including mortality, morbidity and length of stay in the ICU/hospital[17,19]. Owing to E', E/E' is also very accurate and relatively

independent of LV systolic function, rhythm abnormalities and LV hypertrophy[21,23]. Of course, there are some limitations to E' and E/E', which show only the global function of the left ventricle. However, we can use strain, strain rate, speckle tracking and velocity vector imaging with color tissue Doppler to assess regional function and the filling dynamics of the left ventricle[11,20].

During the entire experimental period, the E' values were significantly decreased and the E/E' values were significantly increased in the MI group compared to the sham group (Fig. 3CD, Table 1). These findings indicate that ventricular diastolic function does not recover throughout the experimental period, but left ventricular systolic function is able to recover beginning 3 days after MI, which has been previously reported in MI patients[22,24]. In clinical cases, patients with diastolic dysfunction or diastolic heart failure with normal EF account for nearly one-third of elderly patients undergoing surgery. Patients

Table 1. Cardiac function measured by echocardiography throughout the study

Cardiac function	2 hours		Day 1		Day 3		Day 7	
	Sham	MI	Sham	MI	Sham	MI	Sham	MI
EF (%)	68.82 ± 4.37^{ff}	53.71 ± 4.22**	56.76 ± 3.18	52.26 ± 3.72**	57.39 ± 3.43	54.78 ± 4.27	58.51 ± 3.88	55.91 ± 4.21
FS (%)	39.33 ± 3.50^{ff}	28.47 ± 2.67**	30.60 ± 2.18	27.59 ± 2.80**	31.13 ± 2.36	29.36 ± 2.80	31.99 ± 2.62	30.23 ± 2.81
SV (µl)	110.34 ± 15.32	107.95 ± 13.48	127.87 ± 18.82	124.15 ± 29.90	146.71 ± 19.08	138.14 ± 19.07	159.07 ± 27.52	162.98 ± 23.79
CO (mL/min)	46.52 ± 6.07	46.60 ± 9.45	55.65 ± 7.88	60.21 ± 18.96	57.97 ± 7.87	70.27 ± 32.71	62.61 ± 8.48	62.07 ± 11.55
LVIDd (mm)	5.59 ± 0.43	6.22 ± 0.44**	6.44 ± 0.56	6.75 ± 0.67	6.91 ± 0.45	6.89 ± 0.49	7.05 ± 0.59	7.33 ± 0.49
LVIDs (mm)	3.53 ± 0.42	4.46 ± 0.46**	4.56 ± 0.50	4.86 ± 0.51	4.78 ± 0.41	4.92 ± 0.51	4.86 ± 0.53	5.14 ± 0.55
IVSd (mm)	1.89 ± 0.30	1.83 ± 0.25	1.79 ± 0.24	1.83 ± 0.19	1.83 ± 0.32	1.79 ± 0.23	1.78 ± 0.24	1.78 ± 0.16
IVSs (mm)	2.75 ± 0.52	2.52 ± 0.32	2.45 ± 0.43	2.48 ± 0.24	2.52 ± 0.35	2.47 ± 0.42	2.54 ± 0.27	2.54 ± 0.32
LVPWd (mm)	2.30 ± 0.31	2.30 ± 0.28	2.07 ± 0.30	1.99 ± 0.22	1.94 ± 0.17	1.94 ± 0.17	1.88 ± 0.37	1.87 ± 0.13
LVPWs (mm)	3.08 ± 0.34	2.96 ± 0.41	2.65 ± 0.41	2.67 ± 0.17	2.63 ± 0.30	2.70 ± 0.20	2.51 ± 0.52	2.64 ± 0.17
E'	35.03 ± 3.88	28.26 ± 2.37**	33.47 ± 3.56	27.36 ± 2.83**	32.75 ± 3.89	27.64 ± 3.56**	34.08 ± 2.52	26.75 ± 1.78**
E/A	1.50 ± 0.25	1.55 ± 0.16	1.51 ± 0.24	1.39 ± 0.27	1.40 ± 0.19	1.50 ± 0.18	1.34 ± 0.20	1.42 ± 0.28
E/E'	21.16 ± 0.85	26.71 ± 0.92**^{ff}	20.99 ± 1.10	26.49 ± 0.93**^{ff}	21.20 ± 1.18	25.88 ± 0.94**	20.86 ± 0.66	25.35 ± 0.85**

EF: ejection fraction; FS: fractional shortening; SV: stroke volume; CO: cardiac output; LVIDd: left ventricular internal diameter at diastole; LVIDs: left ventricular internal diameter at systole; IVSd: interventricular septal thickness at diastole; IVSs: interventricular septal thickness at systole; LVPWd: left ventricular posterior wall thickness at diastole; LVPWs: left ventricular posterior wall thickness at systole; E': early diastolic tissue Doppler velocity; E/A: the ratio of the early (E) to late (A) ventricular filling velocities; and E/E': the ratio of the early (E) to early diastolic tissue Doppler velocities. ** indicates a significant difference compared to rats of the sham group by two sample t-test ($P < 0.01$). ^{ff} indicates a significant difference by multiple comparison with Bonferroni correction ($P < 0.01$).

with poor ventricular diastolic function tend to have worse postoperative outcomes than patients with only poor ventricular systolic function. Moreover, the incidence of ischemic heart disease may increase in the near future, especially in South Korea, due to increases in the major risk factors for vascular disease, such as diabetes and hypercholesterolemia[1,13,14,25].

The goal of this study was to investigate cardiac function in the early phase following myocardial I/R injury in rats using echocardiography. To the best of our knowledge, this is the first study to show measurements of cardiac function in the early phase following acute MI in rats. Evaluation of short-term changes in cardiac function in the ischemia/reperfusion rat model by echocardiography may provide insight on the status of human cardiac function following MI. Additionally, our current study provides a strong rationale for the use of echocardiography on the rat MI animal model in the early phase to develop new drugs for high-risk MI patients.

Acknowledgments

Conflicts of interest

The authors declare that they have no conflicts of interest.

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