Echocardiographic assessment of myocardial infarction: comparison of a rat model in two strains

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Summary

The purpose of this study was to induce myocardial infarction (MI) and compare the echocardiographic parameters and mortality ratio of Lewis inbred and Wistar outbred strain before and after the procedure to help choose the best one for MI studies. In this study MI was induced in 46 Lewis and 34 Wistar by occlusion of left anterior descending artery (LAD). Doppler, two-dimensional (2-D) and 2-D guided M-mode images were recorded from parasternal long-axis and parasternal short-axis and apical four-chamber views. The following parameters were acquired. Interventricular septum diastolic and systolic dimension (IVSd, s), diastolic and systolic left ventricular internal diameter (LVIDd, s), diastolic and systolic left ventricular posterior wall dimension (LVPWd, s), ejection fraction (EF), and fractional shortening (FS). The significant changes were observed in systolic IVS, LVID and EF and FS before and after MI and no significant difference was detected between Lewis and Wistar. The high mortality rate of 51% was seen in the procedure, including anesthesia in Lewis compared to 34% in Wistar. As a conclusion the echocardiographic parameters of these two strains were similar, but according to mortality rate and more cardiac anatomic variation in Lewis rats, Wistar is better for MI studies.

Key words: Echocardiography, Lewis, Mortality ratio, Myocardial infarction, Rat model

Introduction

Myocardial infarction (MI) is of the most common etiologies for death worldwide and biomedical researches attempt to discover new treatment strategies for this disease (White and Chew, 2008). One of the best models that mimics human MI is the rat model, specially inbred rats, by which scientists can determine the efficiency of new medical approaches and medications like gene and cell therapy. Echocardiography is a noninvasive and reproducible technique for assessment of cardiac anatomy, function and hemodynamic (Stein *et al*., 1997). These parameters are practical not only for MI investigation but also for the heart failure as the side effect of other treatments like radio, chemo and targeted therapy of cancer (Szmit *et al*., 2015; Zerna *et al*., 2015). So any attempt to discover new cardioprotective medications or to combat MI warrants research on animal models (Klocke *et al*., 2007; Liu *et al*., 2014).

Mortality of this model is high and different rat strains represent various responses to it. Previous researches reported echocardiographic parameters of different rat strains and some of them compare these parameters of different rat strains (Liu *et al*., 1997; Watson *et al*., 2004; Liao *et al*., 2012; Scheer *et al*., 2012). In other researches, changes in some of the cardiac parameters after intervention were reported (Matsumoto *et al*., 2005; Sun *et al*., 2007; Jin *et al*., 2009; Pieper *et al*., 2010; Holinski *et al*., 2011; Kusunose *et al*., 2012; Lee *et al*., 2012). The purpose of this study was to assess baseline echocardiographic parameter and compare echocardiographic parameters and mortality rate of MI in two common breeds of rats (Wistar and Lewis) and to determine which breed is the best for MI study. Induction of MI using left anterior descending artery (LAD) ligating and mortality rate were another purpose of our research.

Materials and Methods

Animal preparation

All procedures were based in accordance with the ethical standards of the animal research approved by the Iranian Ministry of Health and Medical Education. Lewis rats were supplied by the National BioResource Project-Rat, Kyoto University, Kyoto, Japan. They were mated in the laboratory and 46 males were used in the experiment. Thirty-four Wistar rats were provided from Laboratory Animal, Breeding and Husbandry Center, Iran University of Medical Sciences, Tehran, Iran. The

animals were kept at a controlled temperature (25°C), with daily exposure to a twelve-hour light-dark cycle and free access to standard laboratory food and water. Weight of rats was 250 to 350 g.

Induction of myocardial infarction

Myocardial infarction was induced by ligating LAD using a 6-0 Prolene (Ethicon Inc., USA) suture as previously explained (Wu *et al*., 2011; Tavakoli *et al*., 2013; Darbandi Azar *et al*., 2014). Lewis rats were anesthetized before ligation by an intra-peritoneal injection of a mixture of 75 mg/kg Ketamine (K) and 5 mg/kg Xylazine (X). After anesthesia, the animals, orally intubated with a 16-gauge intravenous catheter, were placed in the supine position on a temperature control pad and ventilated with room air using a modified infant ventilator (Sechrist Industries, USA). Electrocardiographic monitoring was continuously done. The heart was exposed via a left thoracotomy by cutting the fourth and fifth ribs. The pericardial sac was opened and LAD was ligated permanently. To ensure the success of infarction, a pale discoloration of the left ventricular muscle was determined.

The muscle layer and skin incision were closed in 4-0 Prolene and 2-0 silk suture, respectively. At the end of surgery, Erythromycin (10 mg/kg, SC), Ketoprofen 10% (10 mg/kg IM.), and warm sterile saline (1-2 ml, SC) were injected and the rats were left on the heating pad until they had recovered from anesthesia. After that rats were extubated and removed from the ventilator. The dose of Flunixin was repeated every 6 to 12 h.

Echocardiography

Echocardiography was done in rats under Ketamine-Xylazine (K-X) anesthesia, before and after MI, using a Vivid 7 echocardiography machine (GE Healthcare Italia, Milano, Italy) equipped with 10 MHz phased array transducer. Doppler, two-dimensional (2-D) and 2-D guided M-mode images were recorded from parasternal long-axis and parasternal short-axis and apical four-chamber views. The measurements included:

Interventricular septum diastolic and systolic dimension (IVSd, s)

Left ventricular posterior wall thickness (systolic and diastolic) (LVPWs, d)

Left ventricle end diastolic and systolic volume (LVEDV, ESV)

Stroke volume (SV)

Ejection fraction and fractional shortening (EF, FS)

Animals with anatomic variation or reduced echocardiographic parameters were excluded from the experiment (7 rats).

Mortality ratio

Mortality ratio is defined as the ratio of dead animals during procedure to total animals in the study.

Statistical analysis

Statistical analysis was done using SPSS software V. 18 independent sample t-test was used for comparison of parameters between two strains and paired sample t-test was done for evaluation of parameters before and after MI.

Results

Echocardiographic 2-D views

Figure 1 represents a typical M-mode parasternal short-axis view. End-systolic and -diastolic dimension of the left ventricle are distinct in this view. Figure 2 displays 2-D parasternal short-axis view which represents right ventricle (RV), ventricular septum (VS), posterior wall (PW), and left ventricle (LV). This view was used for M-mode measurement as seen in Fig. 1. As can be illustrated in Fig. 3, parasternal long-axis view represents right ventricle (RV), ventricular septum (VS), left ventricular cavity (LV), aortic annulus (AO), mitral valve (MV), and left atrium (LA). In this study apical four-chamber view could be recorded in Fig. 4. It represents right ventricle (RV), left ventricle (LV), right atrium (RA) and left atrium (LA). Pulsed Doppler of left ventricular outflow was detected in four-chamber view, but it could not be detected in rats with heart rate above 230 bpm. Then, the acquired the Doppler image was stopped (Fig. 5).

Echocardiographic parameters

Table 1 represents the parameters for Lewis and Wistar strain before and after MI. Comparison of the

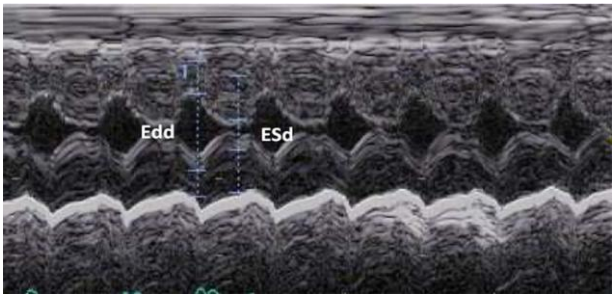

Fig. 1: M-mode parasternal short-axis. End-systolic dimension (ESd) and end diastolic dimension (Edd) of the left ventricle were shown in Lewis rat before MI

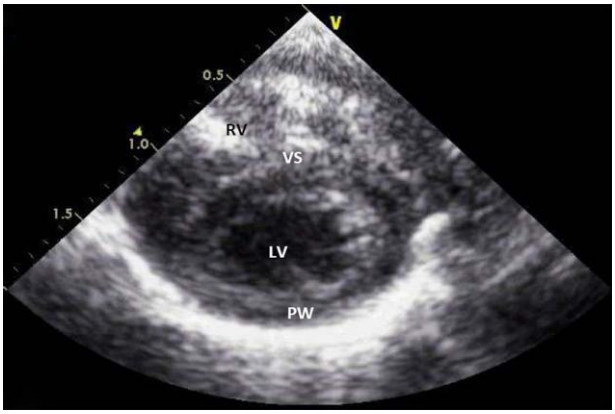

Fig. 2: Parasternal short-axis view. Represents (V), right ventricle (RV), ventricular septum (VS), posterior wall (PW), and left ventricular (LV) in Lewis rat before MI

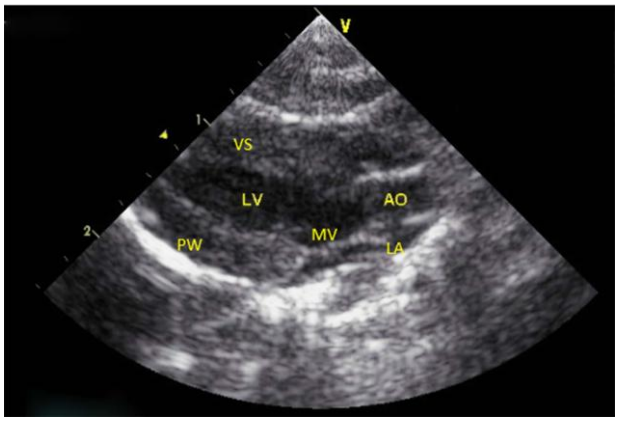

Fig. 3: Parasternal long-axis view. Represents (V), (PW), ventricular septum (VS), left ventricular cavity (LV), aortic annulus (AO), mitral valve (MV), and left atrium (LA) in Lewis rat before MI

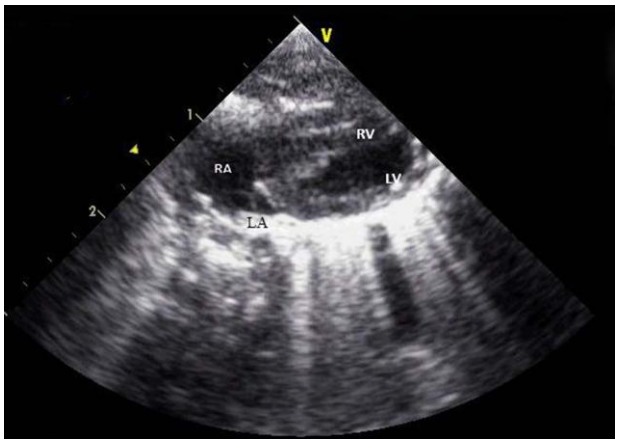

Fig. 4: Apical four-chamber view represents (V), right ventricle (RV), left ventricular (LV), right atrium (RA), and left atrium (LA) in Lewis rat before MI

corresponding parameters between two strains is as follows.

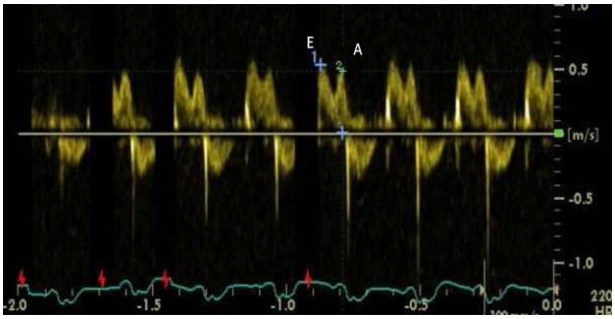

Fig. 5: Pulsed Doppler of left ventricular outflow in Lewis rat before MI. Represents (E), and (A)

Interventricular septum diastolic dimension (IVSd)

Significant changes were observed between Lewis and Wistar breeds (P=0.0001). IVSd in Lewis is thicker than Wistar. This parameter was significantly different before and after MI in Lewis (P=0.0001).

Interventricular septum systolic dimension (IVSs)

IVSs in Lewis is thicker than Wistar, significantly (P=0.0001). There is a significant change between IVSs parameters before and after MI in both strains (P=0.0001).

Diastolic left ventricular internal diameter (LVIDd)

The corresponding parameters in Lewis and Wistar were not statistically different. But, significant changes were observed before and after MI in Wistar.

Systolic left ventricular internal diameter (LVIDs)

The difference between LVIDs before and after MI was significant in Lewis and Wistar (P=0.0001 and 0.0001). No significant differences were observed before and after MI.

Diastolic left ventricular posterior wall dimension (LVPWd)

This parameter in Lewis and Wistar was statistically

Table 1: Summary of echocardiographic parameters before and after myocardial infarction in Wistar and Lewis rats

Parameter	Wistar (n=34)				Lewis (n=46)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Before MI								
IVSd	0.176	0.0239	0.145	0.200	0.197	0.025	0.150	0.250
IVSs	0.285	0.0345	0.230	0.333	0.304	0.035	0.25	0.40
LVIDd	0.541	0.099	0.385	0.700	0.55	0.059	0.45	0.65
LVIDs	0.245	0.052	0.200	0.350	0.262	0.039	0.20	0.333
LVPWd	0.177	0.027	0.135	0.200	0.191	0.039	0.10	0.30
LVPWs	0.297	0.043	0.235	0.375	0.268	0.043	0.20	0.35
EF	88.7	3.454	84.2	93.75	88.1	2.6	84.66	93
FS	54.04	5.15	47.145	62	52.58	4.238	39.5	59.75
After MI								
IVSd	0.160	0.021	0.14	0.20	0.177	0.028	0.125	0.20
IVSs	0.247	0.034	0.175	0.30	0.214	0.037	0.180	0.30
LVIDd	0.592	0.117	0.44	0.793	0.57	0.111	0.36	0.742
LVIDs	0.377	0.104	0.273	0.55	0.386	0.822	0.20	0.485
LVPWd	0.157	0.020	0.127	0.2	0.168	0.028	0.125	0.20
LVPWs	0.216	0.030	0.170	0.275	0.222	0.056	0.14	0.32
EF	71.31	10.33	52.5	84.53	66.45	11.48	51	82.4
FS	36.7	8.06	23.5	48.3	33.3	8.19	23.75	46.0

different (P=0.002). LVPWd was the same before and after MI.

Systolic left ventricular posterior wall dimension (LVPWs)

This was significantly different between two strains. It was changed meaningfully before and after MI in Wistar.

Ejection fraction (EF) and fractional shortening (FS)

EF and FS were similar between both strains and their difference before and after MI was significant in both.

Other parameters

In Doppler echocardiography, early and late diastolic velocity (E, A) pick could be achieved just in cases with lower heart rate. Most of the animals had higher heart rate and the E and A pick were fused and not detectable. We stopped measuring Doppler pick.

Mortality ratio

In the present study, 23% of Lewis rats died immediately after injection of X-K or after intubation. After echocardiography, it was demonstrated that 8.6% of rats had an anatomic variation or reduced echocardiographic parameters which were excluded from occlusion procedure. MI induction was done on remaining animals and mortality after MI within 48 h was 51%. Survival of the procedure with Lewis rat was 34.8%. X-K mortality and reduced echocardiographic parameters were not seen in Wistar strain and the survival rate of the procedure was 47%.

Discussion

In the present study, the baseline echocardiographic values of Lewis and Wistar as one of the inbred and outbred rats were compared before and after MI using LAD ligation. Measurements of LV systolic and diastolic thickness by M-mode and 2-D echocardiography and comparison before and after MI represented that systolic IVS, LVID and EF and FS were changed significantly after MI in Lewis and IVSs, LVID, LVPWs, EF and FS were changed significantly in Wistar. The parameters which were dissimilar in Lewis and Wistar were IVS and LVPW. It seems that the posterior wall and septum are thicker in Lewis. In our study the EF and FS before MI for Lewis were 88% and 52%, respectively; the corresponding value for Wistar was the same. Comparison of these two parameters with other reports in this field represents similarity between some of them, while other studies represent lower EF and FS. For example, in three studies EF was reported 60%, 75%, 91% and the corresponding value for FS was 48%, 41% and 56% (Pawlush *et al*., 1993; Watson *et al*., 2004; Scheer *et al*., 2012). Different rat strain, sex, age, echocardiographic transducer or technique can account for a different reported ejection fraction in the above mentioned reports in comparison with the present study. In this study different rat strains have the same parameters, but 8% of Lewis represents EF lower than 80%.

The mortality of MI in the present study was 66% for Lewis and 42% for Wistar, which agreed with 40-60% mortality reported in other studies (Wollert *et al*., 1994; Leenen and Yuan, 2001; Zornoff *et al*., 2009). But with counting the excluded animals due to X-K mortality and cardiac cardiomyopathy, Wistar is better for MI studies. As mentioned in previous studies heart response to MI is influenced not only by the infarction size, but also by genetic variation within or between species (Liu *et al*., 1997; van den Borne *et al*., 2009; Zornoff *et al*., 2009). The present study showed 21% mortality in anesthesia with X-K, while such a high mortality rate was not reported previously in this strain. In a study of other rat strains, it was reported that ACI and BN strains died soon after X-K injection (Avsaroglu *et al*., 2007; Avsaroglu *et al*., 2008). Anesthetics cause mortality in laboratory rodents by inhibiting thermoregulatory mechanism, bradycardia, cardiac arrhythmia and cardiac depression (Wixson *et al*., 1987; Henke *et al*., 2005; Avsaroglu *et al*., 2007). The authors suggest that the high degree of inbreeding in Lewis may unmask deleterious traits, of which hypersensitivity to anesthetic agents is one of them.

The inbreeding in animals guarantees a consistent and uniform genetic background in animal models for experimental purposes. This enable researchers to reduce variations subsequent from individual differences in the results. Moreover, inbred models are an inseparable part in cell transplantation studies, among which rejection is a great obstacle. But inbreeding is very important in sensitivity of animals to the procedure. It can lead to greater recessive mutational load which has the important effect of the survival problem in biology (Meagher *et al*., 2000). Recessive alleles in homozygous animals may result in high mortality rates during the experiment.

Mitral inflow velocity patterns could be achieved in few rats since tachycardia in the rat results in fused early and late diastolic velocity (E, A) pick. Using inhalant anesthetics like isoflurane, which is more controllable and can slow the mitral valve instead of X and K would help to get the Doppler pattern. Unfortunately, in this experiment, it was one of the limitations.

To decide about the effectiveness of a new procedure in treatment, comparison of parameters after treatment with baseline cardiac parameters is necessary. Echocardiography is a procedure which needs special equipment and skilled sonographer, particularly in small animals like rats to obtain accurate and reproducible results. So, performing the procedure for all the animals in experimental groups without any background about the baseline parameters may be time consuming and may not be possible for all experiments. Knowledge of base line parameters in these animals can help other researchers decide about the accuracy of their measurements.

Our study is limited by using single sex rats. According to Cittadini *et al*. (1996), sex difference

represents one of the reasons. Echocardiographic parameters may be different in females and also other strains.

The study was attempted in order to prepare a myocardial infarction model based on routine protocols presented in this field in inbred Lewis and outbred Wistar and represented the echocardiographic parameter before and after MI in these strains.

Acknowledgement

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Conflict of interest

The authors declare no potential conflicts of interest.

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