Res Cardiovasc Med. 2014 November; 3(4): e22941. DOI: 10.5812/cardiovascmed.22941

Published online 2014 November 1. **Research Article**

Echocardiographic Evaluation of Cardiac Function in Ischemic Rats: Value of M-Mode Echocardiography

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Received: August 20, 2014; **Accepted:** October 8, 2014

Background: Echocardiography is a well-established diagnostic tool for a safe, reproducible and accurate evaluation of cardiac anatomy, hemodynamics and function in clinical practice.
Objectives: We sought to demonstrate the efficacy and feasibility of M-mode echocardiography to evaluate cardiac structure and function in normal and MI-induced adult rats.
Materials and Methods: All animal procedures were approved by the ethics committee of Tehran University of Medical Sciences and the investigation conformed to the "Guide for the Care and Use of Laboratory Animals" published by the United States National Institutes of Health. Forty-eight male Wistar rats weighing 280-300 grams were obtained from a single breeding colony. The statistical analyses were performed using SPSS 20.0.
Results: Echocardiographic measurements were possible in all rats before and after the operation. In our survey, we studied echocardiographic alterations in rats after MI induction. Changes can be seen in all echocardiographic mean values after myocardial infarction (MI), but significant decrease ($P < 0.01$) of Fractional shortening and Ejection Fraction as well as significant increase ($P < 0.05$) of end systolic diameter and systolic volume after left anterior descending coronary artery (LAD) ligation can be good signs of MI induction.
Conclusions: In light of our results, it can be concluded that we succeeded in establishing a precise echocardiographic method to confidently assess the success of LAD ligation surgery in rats. It is feasible to thoroughly monitor the functional efficiency of regional therapeutic interventions such as intra-myocardial stem cell injection.

Keywords: Echocardiography; Rat; M-mode Evaluation

1. Background

Echocardiography is a well-established diagnostic tool for a safe, reproducible and accurate evaluation of cardiac anatomy, hemodynamics and function in clinical practice. Therefore, there is increasing interest to use echocardiography as a basic research tool according to the current standards with its newer methods in research dealing with standard laboratory animals (1, 2). To better understand the pathophysiology and develop new treatment regimens for heart diseases, investigators developed animal models to assess human diseases (3). Rats are commonly used in cardiovascular researches and the rat model of myocardial infarction (MI) has been widely used as an experimental model because of its convenience in handling and housing as well as its lower costs. The most common procedure used for the induction of MI in these animals is ligation of the left anterior descending coronary artery (LAD) (4). Chronic ischemic heart failure after experimental MI in rat is a very popular model of cardiovascular research and used recently to study the therapeutic potential of the latest myocardial repair strategies such as stem cell transplantation or tissue engineering (1). This model requires accurate, serial in vivo assessment of the time course of changes during the disease progression or therapeutic intervention via noninvasive diagnostic measurements. However, reproducible echocardiography in small laboratory animals like rats is challenging due to technical problems, in particular the small size of rat heart and fast heart rates relative to human heart (5). Knowledge of baseline normal values in commonly used rat species would be worthwhile. M-mode echocardiography complements 2D echocardiography by recording detailed motions of cardiac structures. It is best derived with guidance from a 2D echocardiographic image by placing a cursor through the desired structure. M-mode is used for the measurement of dimensions and essential for display of subtle motion abnormalities of specific cardiac structures (6). Advantages

of M-mode interrogation include high temporal resolution (1000-3000 Hz compared to 20-60 Hz for traditional 2D echocardiography) and high spatial resolution (7).

2. Objectives

We sought to demonstrate the utility and feasibility of M-mode echocardiography to evaluate cardiac structure and function in normal and MI-induced adult rats.

3. Materials and Methods

3.1. Animal Preparation

All animal procedures were approved by the ethics committee of Tehran University of Medical Sciences and the investigation conformed to the "Guide for the Care and Use of Laboratory Animals" published by the United States National Institutes of Health (8). Forty-eight male Wistar rats weighing 280-300 grams were obtained from a single breeding colony (Tehran University of Medical Sciences, Faculty of Pharmacy). The animals were housed 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.

3.2. Induction of Myocardial Infarction

To induce MI via ligation of the LAD, animals were anesthetized with an intraperitoneal injection of a mixture of 75 mg/kg Ketamine and 5 mg/kg Xylazine. After anesthesia, animals were orally intubated with a 16-gaugeintravenouscatheter, placed in the supine position on a temperature control pad and ventilated with room air using a Harvard rodent ventilator (model 683). Left-sided thoracotomy was performed by cutting the fourth and fifth ribs. The incision was expanded by a small blunt-ended retractor to explore the heart in such a manner that the lungs were avoided in the area of retraction. The pericardial sac surrounding the heart was opened carefully to avoid any injury to the heart. Once the site of ligation of the LAD was determined, it was permanently ligated from the left border of the pulmonary conus to the right border of the left atrial appendage using a 6-0 Prolene (Ethicon Inc., USA) suture and tied with two knots. Proper ligation of the LAD was confirmed by observing blanching of the myocardial tissue distal to the suture as well as dyskinesia of the anterior wall. The muscle layers were sutured with 4-0 Prolene sutures and skin incision with 2-0 nylon sutures. At the time of closure, penicillin powder and betadine were applied onto the muscle and skin stitch sites, respectively. At the end of operation, Cefazolin (25 mg/kg IM), Flunixin (1-2 mg/kg IM) and warm sterile saline (0.5-1 mL, SC) were injected and the rats were left on the heating pad until recovering from anesthesia. Once rhythmic, rapid and shallow breathing was verified, rats were extubated and removed from the ventilator. The dosage of Flunixin was repeated every six to twelve hours. Postoperative care included daily monitoring for the first week to verify adequate mobility, grooming and eating habits (9-11).

3.3. Echocardiographic Study

All 48 male Wistar rats underwent baseline echocardiography before the commencement of experimental protocols. Echocardiographic studies were repeated two days after the induction of MI using a VIVID 7 dimension system (General Electric-Vingmed Ultrasound, Horton Norway), equipped with a 12-MHz electronic transducer. Under the same anesthesia as for the infarct surgery (75 mg/kg Ketamine and 5 mg/kg Xylazine IP), which was maintained throughout the echocardiographic examination, echocardiographic parameters were recorded in accordance with the American Society of Echocardiography guidelines (12). After shaving the chest of animals, rats were placed in supine position and transducer was placed directly on the shaved chest wall. Images were obtained from the left parasternal short-axis views of the left ventricle (LV) at the level of papillary muscles to define wall thicknesses and internal diameters during systole and diastole and regional wall motion abnormality in the LAD territory as well (Figure 1 and 2). Exact dimensions were calculated using MATLAB R2011b software. The left ventricular posterior wall (LVPW) and the interventricular septum (IVS) thicknesses were measured. The left ventricular end-diastolic diameter and left ventricular end-systolic diameter are abbreviated as LVEDD and LVESD, respectively. To derive further parameters from echocardiographic data, following equations were used (Table 1).

3.4. Statistical analysis

The statistical analyses were conducted using IBM SPSS Statistics 20 for Windows (IBM Corp., Armonk, NY). The values are presented as mean ± standard deviation (SD), except for the graphs, where the values expressed as mean ± standard error (SE). To compare two paired groups, student t-test was used. Differences were considered significant if P value < 0.05.

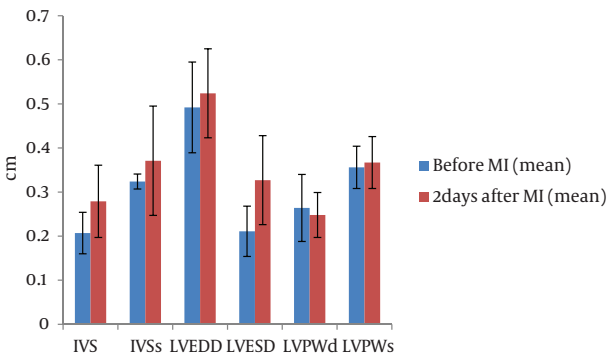
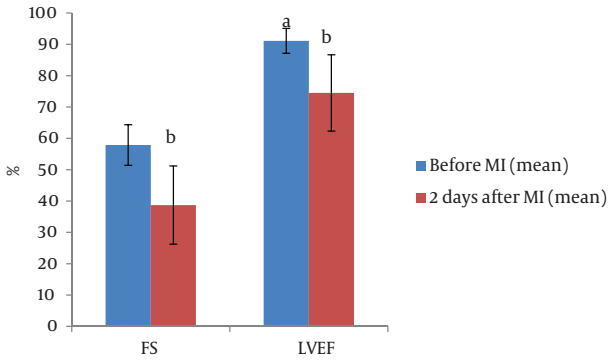


Figure 1. Changes in Echocardiographic Data of Study Animals Before and After Myocardial Infarction (MI), (P < 0.05)

Figure 2. Changes in FS and LVEF of Study Animals Before and After Myocardial Infarction (MI)



[a] $P < 0.05$; [b] $P < 0.01$.

Table 1. Equations for for Deriving Echocardiographic Parameters in Experimental Groups (5) [a,b,c]

Parameter	Equation
Diastolic volume, mL	1.047 (LVEDD)^3
Systolic volume, mL	1.047 (LVESD)^3
Stroke volume, mL	diastolic volume-systolic volume
Fractional shortening, FS %	(LVEDD-LVESD)/LVEDD × 100
Ejection fraction, %	stroke volume / diastolic volume×100
Cardiac output, mL/min	stroke volume × heart rate
Left ventricular mass, g	1.04 [(LVIDd + PW + IVS) 3-LVIDd3] × 0.8+0.6

[a] Abbreviations: LVEDD, left ventricular end diastolic diameter; LVESD, Left ventricular end systolic diameter; IVS, interventricular septum thicknesses
[b] IVS, LVEDD, LVESD and LVPW are all in cm.
[c] All the measurements were averaged on three consecutive cardiac cycles.

4. Results

Echocardiographic measurements were possible in all rats before and after the operation. The main echocardiographic parameters measured before as well as two days after LAD ligation are summarized in Table 2. In our survey, we studied echocardiographic alterations in rats after MI induction. All rats with MI were examined by an echocardiographist to confirm the operation results. M-mode echocardiography was feasible in all animals. IVSs, IVSd, LVESD, LVEDD, left ventricular posterior wall thickness in systole, left ventricular posterior wall thickness in diastole and heart rate were determined from the obtained M-mode pictures using MATLAB R2011b software. These parameters were used to calculate systolic volume, diastolic volume, stroke volume, FS, LVEF, cardiac output and LV mass according to the above-mentioned formulas.

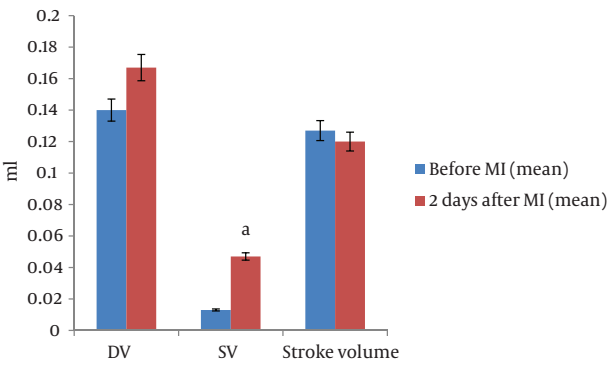
The results are summarized in Table 2. Ventricular dysfunction after MI can be indicated from these findings. Changes can be seen in all echocardiographic mean values after MI, but significant decrease ($P < 0.01$) of FS and LVEF as well as significant increase ($P < 0.05$) of LVESD and systolic volume after LAD ligation can be good signs of MI induction (Figure 3 and 4).

Table 2. Echocardiographic Data of Study Animals Before and After Myocardial Infarction (MI) [a,b]

Variable	Before MI	Two days after MI
IVSd, cm	0.207 ± 0.047	0.279 ± 0.082
IVSs, cm	0.324 ± 0.017	0.371 ± 0.124
LVEDD, cm	0.492 ± 0.103	0.524 ± 0.101
LVESD, cm	0.211 ± 0.057	0.327 ± 0.101[c]
LVPWd, cm	0.264 ± 0.076	0.248 ± 0.051
LVPWs, cm	0.356 ± 0.048	0.367 ± 0.059
DV-ml	0.140 ± 0.075	0.167 ± 0.083
SV-ml	0.013 ± 0.007	0.047 ± 0.034[c]
Stroke volume-mL	0.127 ± 0.069	0.120 ± 0.061
FS - %	57.885 ± 6.485	38.712 ± 12.467[d]
LVEF - %	91.125 ± 3.975	74.495 ± 12.163[d]
HR- beat/min	331.6 ± 19.242	259.222 ± 73.913
CO	38.758 ± 22.652	32.488 ± 24.227
LV mass-g	1.253 ± 0.233	1.526 ± 0.569

[a] Abbreviations: IVSd, interventricular septum thicknesses in diastole; IVSs, interventricular septum thicknesses in systole; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; LVPWd, left ventricular posterior wall thickness in diastole; LVPWs, left ventricular posterior wall thickness in systole; DV, diastolic volume; SV, systolic volume; FS, fractional shortening; LVEF, left ventricular ejection fraction; HR, heart rate; CO, cardiac output; LV mass, left ventricular mass.
[b] Data are presented as Mean ± SD.
[c] before MI vs. after MI: $P < 0.05$.
[d] before MI vs. after MI: $P<0.01$.

Figure 3. Changes in DV, SV and Stroke Volume of Study Animals Before and After Myocardial Infarction (MI)



$P < 0.05$.

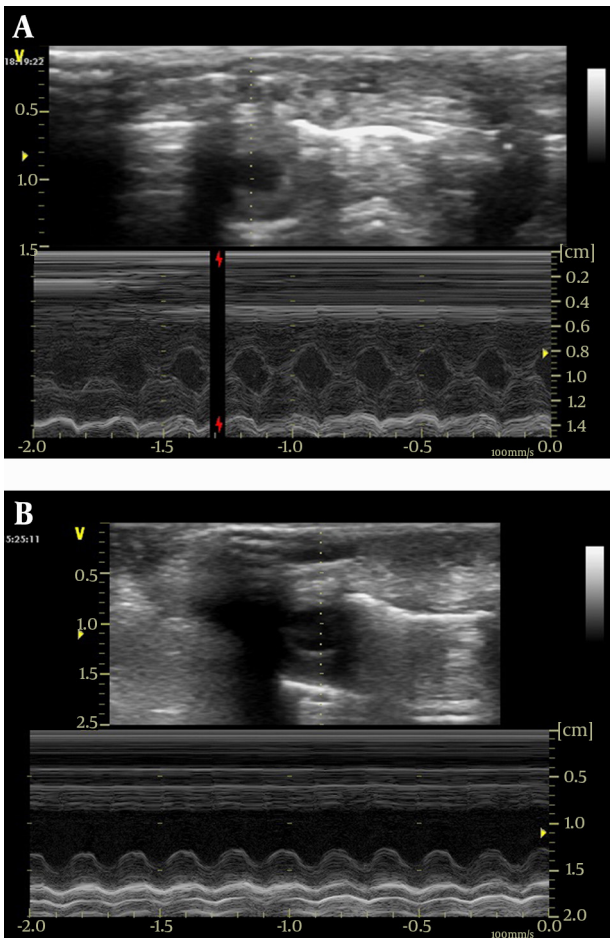


Figure 4. M-Mode Measurements of left Ventricular Structures Over Several Cycles Before (A) and After (B) MI Induction

5. Discussion

This study showed technical feasibility and reliability of M-mode echocardiography in evaluation of cardiac function in rats. Echocardiographic measurements were possible in all rats before and after the operation. We found typical ischemic changes, including a significant increase in LVESD as well as decrease in fractional shortening, LVEF and stroke volume following post-infarction LV remodeling in adult rats. Our data are comparable with other studies regarding the feasibility and reliability of M-mode echocardiography in rats (13, 14). Ligation of the LAD of rats is the most appropriate method for an experimental MI model and extensively used in cardiovascular research to study new therapy strategies for heart failure. Finding the exact position of LAD in rats is of great importance to achieve a precise LAD ligation and MI model. In most cases, it is very difficult to find the exact location of LAD, which forces researchers to use anatomical landmark standardization. Consequently, it is very important to evaluate cardiac function following surgery to ascertain whether the LAD ligation has been performed accurately for an MI model to be able to assess different treatment modalities like cell therapy. Invasive techniques are associated with relatively high morbidity and mortality rates and allow mainly a single evaluation when serial follow-ups are needed. On the other hand, echocardiography a well-established diagnostic tool in clinical practice, has been accepted as a useful noninvasive tool for the evaluation of cardiac function and morphology changes. It has been suggested that new features of echocardiography, namely tissue Doppler imaging, 2D strain and strain rate imaging, provide precise information, especially of regional myocardial function. Nonetheless, in small rats with fast heart rates, high temporal resolution of the imaging modality is vitally important. Strain rate imaging requires high temporal resolution (>100 Hz) to avoid underestimation due to under sampling. Doppler, because of its high temporal resolution, is therefore superior to speckle tracking for strain rate imaging. However, tissue Doppler-derived strain variables have come under criticism recently, particularly in relation to angle dependency, noise interference and substantial intraobserver and interobserver variability (15, 16). Although M-mode echocardiography yields a one-dimensional view of cardiac structures moving over time, it has a high temporal resolution allowing the echocardiographer to obtain more accurate measurements of cardiac dimensions and more critically evaluate cardiac motion and subtle changes. In this study, morphological changes such as increase in LVESD, LVEDD and LV mass as well as functional changes like decrease in FS and LVEF were clearly observed at two days post infarction. Our data are comparable, although not strictly, with those of other reports (1-3, 5, 17). It seems that differences in age, weight and echocardiographic transducers or techniques can yield slightly different values in different laboratories. In light of our results, it can be concluded that we succeeded in establishing a precise echocardiographic method to confidently assess the success of LAD ligation surgery in rats. It is feasible to thoroughly monitor the functional efficiency of regional therapeutic interventions such as intra-myocardial stem cell injection.

Acknowledgements

The authors hereby sincerely thank the efforts of Mr. Haider Syed Ghasemi who helped in this project.

Authors' Contributions

Original idea of investigation, management and veterinarian work: Amir Darbandi Azar. Analysis of data: Fateme Tavakoli. Revision of the manuscript and echocardiography: Anita Sadeghpour.

Funding/Support

This project was financially supported by the Department of Pharmacology and Toxicology, Faculty of Tehran

University. Rajaie Cardiovascular, Medical and Research Center helped use in performing the investigation.

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