NEW ECHOCARDIOGRAPHIC PROTOCOL FOR THE ASSESSMENT OF EXPERIMENTAL MYOCARDIAL INFARCTION IN RATS

Ruxandra DRAGOI GALRINHOa,b,c; Andrea Olivia CIOBANUa,c; Roxana Cristina RIMBASa,c; Catalin Gabriel MANOLEa,d; Bogdan MARINESCUd; Dragos VINEREANUa,c

a “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
b University of Economic Studies, Bucharest, Romania
c Emergency University Hospital of Bucharest, Romania
d “Victor Babeș” National Institute of Pathology, Bucharest, Romania

ABSTRACT

Background: The rat infarct model was used extensively to study the pathophysiology of myocardial infarction and to evaluate different therapies. Transthoracic echocardiography is used in rats in order to assess cardiac anatomy and function, being a safe and reliable non-invasive technique. However, studies combining conventional with new echo techniques, such as tissue Doppler imaging (TDI) and speckle-tracking echocardiography (STE), are lacking.

Objectives: To validate a protocol using the available conventional and new echocardiographic techniques (TDI and STE) for a comprehensive assessment of cardiac remodelling and function, after myocardial infarction in rats.

Methods: Ten Wistar (W) and five Sprague Dawley (SD) male rats (aged 21±2 weeks, mean weight 355±43 g) were evaluated by echocardiography, before and 24 hours post-ligation of the left coronary artery, with previous anaesthesia. Left ventricular (LV) structure was assessed by end-diastolic and end-systolic anterior wall thickness and LV diameters (from the SAX view), while LV function by fractional shortening (FS) and ejection fraction (EF) (by area-length formula), septal mitral annular plane systolic excursion (MAPSE), cardiac output (CO), myocardial performance index (MPI), septal mitral annular systolic velocity (S’, by TDI), and global circumferential and radial systolic strain (GCS, GRS) and strain rate (GCSr, GRSr) by STE, from the SAX view at the level of papillary muscles.

Results: Feasibility of measuring the above mentioned parameters was 100%. Twenty-four hours after myocardial infarction, rats had lower heart rate (373±44 vs. 351±32 bpm, p<0.05) and thinner LV anterior wall, while LV diameters and volumes were significantly higher. FS (54±7 vs. 33±9%), EF (72±9vs. 47±10%), septal MAPSE (2.02±0.17 vs. 1.44±0.22 mm), CO (76±15 vs. 48±12 ml/min), MPI (0.33±0.11 vs. 0.50±0.14), S’ (5.58±1.20 vs. 3.84±1.06 cm/s), and LV strain and strain rate...
INTRODUCTION

Since the first report in 1946 by Heimburger, the rat infarct model has been used extensively to study the pathophysiology of myocardial infarction (MI) and to evaluate different therapies, such as the effect of regenerative therapy on the ischemic myocardium (1,2). The anatomy of rat coronaries and the area perfused by each artery were described many years ago (3,4). Meanwhile, transthoracic echocardiography was widely used as an evaluation tool in cardiovascular research, being demonstrated as feasible in small laboratory animals (5). It is a non-invasive, accurate, and highly reproducible tool for the serial assessment of the left ventricular (LV) geometry and function, and it can potentially replace highly invasive or terminal procedures, such as the hemodynamic studies (6). Moreover, the novel echo techniques, tissue Doppler imaging (TDI) and speckle tracking echocardiography (STE) have also good feasibility and reproducibility, already demonstrated for mice and rats (7-9). TDI quantifies regional myocardial velocities, while STE assesses myocardial deformation (10). STE might be superior to TDI, since it evaluates the intrinsic properties of the myocardium; furthermore, it is angle independent and not affected by the motion of the adjacent segments. To the best of our knowledge, there is no echocardiographic protocol, combining conventional, TDI, and STE techniques, in order to evaluate the impact of the myocardial infarction in rats.

OBJECTIVES

The aim of our study was to validate a protocol using the available conventional and new echocardiographic techniques (TDI and STE) for a comprehensive assessment of cardiac remodelling and function, after myocardial infarction in Wistar (W) and Sprague Dawley (SD) rats.

MATERIAL AND METHODS

Animals

The study conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH Publication no. 85–23, revised 1996), and was approved by the Institutional Ethical Committee of “Victor Babes” National Institute of Pathology from Bucharest. Animals were housed in individual cages, under controlled environment with a temperature of 22ºC and a 12-hours light/dark cycle. They were fed standard chow and water ad libitum, and proper analgesia after the surgical procedure was provided. The echocardiographic data for this protocol was gathered from animals belonging to a larger study, investigating the new therapies in experimental myocardial infarction. We showed previously that there are only minor differences in LV wall thickness between normal W and SD male rats, while more detailed parameters of LV and RV structure and function are similar (11). Therefore, we studied a mixed population of fifteen male rats (10 W and 5 SD) (aged 21±2 weeks, mean weight 355±43 g).

Anaesthesia and myocardial infarction protocol

Animals were anesthetized before echocardiography and surgical procedure with intraperitoneal acepromazine maleate (Neurotraneq; Alfasan, Woerden, Holland) 2 mg/kg and ketamine hydrochloride (Ketamine HCL; Kepro, Deventer, Holland) 100 mg/kg. After the anaesthesia, the animal was intubated and me-
Mechanically ventilated (CWE SAR-830/P Ardmore, PA, USA). The rat was placed on a heated plate (Harvard Apparatus, Holliston, MA, USA), with permanent monitoring of rectal temperature, in order to maintain it at 37°C. The ECG was monitored throughout the experiment, using a dedicated veterinary monitor (VET-420f, Goldway US Inc., NY, USA). A left thoracotomy was performed between the third and fourth ribs, the pericardium was opened, and left coronary artery (LCA) was surrounded with a surgical knot of Vicryl 5-0 (SMI, Hünningen, Belgium), immediately below the lower margin of the left appendage (12). The occlusion of LCA was verified by ST elevation on the ECG trace and the pallor of the myocardium. The chest was closed, the animal was disconnected from the ventilator, and the endotracheal catheter was removed after the restoration of spontaneous respiration. The surgical wound was covered with oxytetracycline (Oxyvet, Veterin, Attiki, Greece), a bacteriostatic fluid.

### Echocardiographic protocol

The echocardiography was performed before, and 24 hours after the induction of the myocardial infarction. The anterior chest hair was shaved, rats were positioned in left lateral decubitus, ECG was monitored throughout the experiment, and temperature was kept at 37°C on the heated plate. The examination was carried out under spontaneous ventilation by a single operator with a Vivid i echocardiography machine (General Electric, Milwaukee, Wisconsin), using an 11 MHz phased array probe (12S-RS).

#### Conventional parameters.

Left ventricular (LV) structure and function were assessed from the parasternal, long (LAX) and short (SAX) axis views (at the level of the papillary muscles), and from the 4- and 5- chambers apical views. Mitral inflow and aortic outflow were recorded by Doppler. Measurements were obtained from three consecutive cardiac cycles by off-line analysis (EchoPAC BT12, General Electric). Chamber diameters, wall-thickness, and fractional shortening (FS) were measured by M-mode, in the SAX view, according to the “leading edge to leading edge” convention. Ejection fraction (EF) was calculated by area-length formula from the apical 4-chambers view. Cardiac output (CO) was calculated based on pulsed-wave Doppler velocity recordings at the aortic annulus from the 5-chambers apical view, and on aortic annulus diameter measured from the LAX view (13). Septal mitral annular plane systolic excursion (MAPSE) was measured by M-mode. Myocardial performance index (MPI) was derived from mitral and aortic flow tracings (14).

#### TDI and STE parameters.

LV systolic myocardial velocity (S') was obtained from on-line TDI recordings at the level of the septal mitral annulus. STE was performed in the SAX view at the level of the papillary muscles by manual tracing of endocardial borders at the onset of systole. The adequacy of tracking was confirmed and manually corrected when necessary. Images were acquired at a frame rate of 116 frames/sec, with optimal sector depth and width. Global circumferential and radial systolic strain (GCS, GRS) and strain rate (GCSr, GRSr) were calculated. All measurements were performed by the same single observer.

#### Statistical analysis.

Statistical analysis was performed with SPSS version 19 (SPSS, Inc., Chicago, IL). Results are presented as mean ± standard deviation. Comparisons were performed using the paired t-test.

### Table 1. Comparison of heart rate and conventional echocardiographic parameters, before and after MI.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>373 ± 44</td>
<td>351 ± 32</td>
<td>0.03</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td>2.67 ± 0.52</td>
<td>4.87 ± 0.62</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>5.85 ± 0.54</td>
<td>7.37 ± 1.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LV anterior wall in systole (mm)</td>
<td>2.99 ± 0.36</td>
<td>2.16 ± 0.63</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LV anterior wall in diastole (mm)</td>
<td>1.72 ± 0.19</td>
<td>1.46 ± 0.32</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LV end-systolic volume (cm³)</td>
<td>0.06 ± 0.02</td>
<td>0.16 ± 0.05</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LV end-diastolic volume (cm³)</td>
<td>0.20 ± 0.04</td>
<td>0.31 ± 0.06</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>54 ± 7</td>
<td>33 ± 9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>72 ± 9</td>
<td>47 ± 10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Septal MAPSE (mm)</td>
<td>2.02 ± 0.17</td>
<td>1.44 ± 0.22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>76 ± 15</td>
<td>50 ± 0.14</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Myocardial performance index</td>
<td>0.33 ±0.11</td>
<td>0.50 ±0.14</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*TABLE 1. Comparison of heart rate and conventional echocardiographic parameters, before and after MI.*
formed by paired Student’s t-test. Two-tailed p<0.05 was considered significant. For intra-observer variability, the same observer repeated the measurements one week later. Bland Altman analysis was performed (15).

**RESULTS**

The comparison of heart rate and conventional echocardiographic parameters, before and 24 hours after MI, are presented in Table 1. Heart rate was significantly lower 24 hours after ligation of the LCA. LV anterior wall was thinner, and LV end-systolic and end-diastolic diameters and volumes were larger. Systolic function, evaluated by conventional echocardiography (FS, EF, MAPSE, CO and MPI), was significantly decreased.

By TDI, LV systolic function was significantly lower (p<0.01) (Figure 1). Meanwhile, by STE, global circumferential and radial systolic strain and strain rate decreased significantly (p<0.01) (Figure 2, 3).

Feasibility of measuring conventional, TDI, and STE parameters was 100%, while the intra-observer variability was very good (less than 5% for all parameters) (Table 2).

**DISCUSSION**

The pathological LV remodelling, with dilatation and impaired contractility, occurs in the first hours after myocardial infarction and leads to approximately two-thirds of cases of systolic heart failure, which still has a very poor prognosis, despite the new therapeutic methods (16).

The rat myocardial infarction model is used particularly for investigating cell therapies. M-mode, 2D, TDI, and STE were used separately in previous studies of rat models, but a standardised protocol of the assessment of myocardial infarction with all these combined echo methods has not been validated yet (7,8,10,17-19). Thus, we included in our model conventional and new echocardiographic parameters, in order to validate a comprehensive protocol for the assessment of cardiac function and remodelling, in the acute phase of myocardial infarction. Conventional M-mode and 2D echocardiography were used to assess the LV structure and function; meanwhile, MPI and CO were also measured, since they showed previously to correlate well with the results from the hemodynamic studies (13). TDI and STE were used to assess longitudinal function (by MAPSE and S’), circumferential, and radial function (by STE derived myocardial deformation parameters). We demonstrated that assessment of these parameters is feasible, and that all these parameters were altered significantly 24 hours after the ligation of the LCA. Our results are concordant with previous reports (7,19-23). Thus, similar results of LV remodelling after myocardial infarction in rats, such as chamber enlargement and thinning of the infarcted wall, as well as decreased LV systolic function, were reported in previous morphological or echocardiographic studies (6,20,21).

Meanwhile, decrease of cardiac output and myocardial performance index, parameters of global LV function, were also concordant with other previously published data (23).

Longitudinal myocardial function was assessed by M-mode and TDI, measuring the septal mitral annular plane systolic excursion and LV systolic myocardial velocity. This is new, since there are no previous data reported on these parameters after myocardial infarction in...
rats. Longitudinal myocardial function by STE was not assessed in our study, due to improper apical views in the experimental models in rats, needed for the accurate tracing of the endocardium. However, we did assess more specific functional parameters by STE, such as LV circumferential and radial strain and strain rate, which showed a significant decrease, similar with other reports (7,19,22).

TDI and STE are able to detect more subtle changes of myocardial function after MI than conventional parameters and, therefore, they might be useful in the evaluation of the effect of new cell therapies in reverse remodelling of myocardial dysfunction.

**Limitations.** The main limitations of this protocol are related to the difficulties of the ultrasound examination in small animals, either due to the high heart rate or to difficulties in obtaining accurate echocardiographic images because of the small size of the heart. Thus, 2- and 3- chambers apical views are technically impossible in rats, taking into consideration that the heart weights approximately 1 g. Similarly, for the majority of rats it was difficult to obtain an accurate image of the 4-chambers apical view, with an adequate visualization of the endocardium, necessary to quantify the longitudinal myocardial deformation. Meanwhile, A and E waves of the trans-mitral flow were merged, when heart rate was higher than 350 bpm.

**CONCLUSION**

The impact of myocardial infarction in rats could be assessed extensively using a comprehensive echocardiographic protocol of conventional and newer echocardiographic techniques, such as TDI and STE, in order to quantify the LV remodelling, global, and regional function (longitudinal, circumferential, and radial). We suggest this protocol might be used to assess the effect of different regenerative therapies in experimental myocardial infarction in rats.

**Conflict of interests:** none declared.

**Financial support:** This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/159/1.5/S/138907 “Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields -EXCELIS”, coordinator The Bucharest University of Economic Studies.

**REFERENCES**


16. McMurray JJ, Adamopoulos S, Anker SD, et al. – ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J 2012;33:1787-1847


