Abstract

Objective: To evaluate the diastolic left ventricular function by echocardiography one and three weeks after acute myocardial infarction (AMI).

Method: Nineteen Wistar rats (mean weight 209 g) were utilized. After anesthesia with ketamine (50 mg/kg) and xylazine (10 mg/kg), the left coronary artery was ligated after left thoracotomy to cause myocardial infarction. The animals were divided into two groups: group A (control, n=7) and group B (n=9). Echocardiographic evaluation was undertaken in the control group and one week (B1, n=9) and three weeks (B3, n=8) post-AMI in group B animals. The cardiac function was evaluated using a 21275 A HP Sonos 1500 Echocardiography equipped with a 7.5/5.5 MHz transducer. Diastolic function was evaluated by transmitral flow, by analysis of the A wave, E wave and atrial left volume (LA V). Histological specimens were evaluated on third week.

Results: There were no differences on E wave analyses (A = 62 cm/s, B1 = 65 cm/s, B3 = 69 cm/s) or A wave analyses (A = 43 cm/s, B1 = 40 cm/s, B3 = 41 cm/s) between the groups. There was an increase in LA V: A vs. B1 and A vs. B3 (A = 0.05 mL vs. B1 = 0.15 mL, p = 0.04 and A vs. B3 = 0.14 mL, p=0.01). Histological examination confirmed AMI in all animals.

Conclusions: The LA V may be useful to assess the diastolic function in rats with AMI. LA V could reflect increases in left ventricular end-diastolic pressure secondary to systolic or diastolic dysfunction.

INFARCTED (n=9), submitted to ECO after one week (group B1, n=9) and three weeks (group B3, n=8) of IAM. Three animals died during the observation period and one after the first ECO. It was confirmed by histopathological analysis in the third week.

Atrial volume (V AE) was assessed by Doppler transmitral with evaluation of the waves A and E and volume with a transducer of 7.5/5.5 MHz and diastolic function by ECO model 21275A HP Sonos 1500 after left thoracotomy. The evaluation was performed with water and feed ad lib. There was no mortality during the postoperative period. The animals were maintained in individual cages with water and feed ad lib. There was no mortality during the postoperative period.
descending artery (DA) located in the anterior wall between the left auricula and the pulmonary artery. This was ligated in the middle third using 7-0 polypropylene line (Ethicon ®, Inc. Somerville, NJ) with the aim of inducing AMI of the LV antero-lateral wall. The ischemic region was confirmed by the rapid change in color of the myocardium and an akinetic area was seen. The heart was replaced in its correct position and thoracotomy closure was performed using 2-0 silk line (Ethicon ® Inc., Somerville, NJ).

The echocardiographic evaluation of the animals in Group A (control) was denominated ECO-0. The infarcted animals (Group B) were evaluated one week (B1) and three weeks (B3) after inducing AMI. The first echocardiogram after AMI (ECO-1) was performed a week after the surgery (n=9). The second examination (ECO-2) was performed three weeks post-AMI in eight rats, because one of them had died during the anesthesia which is necessary to make the examination.

All echocardiographic examinations were performed according to the following protocol:

- Intrapерitoneal anesthesia using ketamine (50 mg/kg/weight) and xylazine (10 mg/kg/weight)
- Removal of hair of the anterior and lateral regions of the thorax.
- The animals were placed in the dorsal decubitus position
- The transducer was positioned on the left sternal border, apex, supra-sternal and subxiphoid regions. The first referential image was obtained with the bi-dimensional analysis and the measurements were achieved in M-mode and Doppler echocardiography

All measurements were performed twice by the same examiner and after this the means of each parameter were calculated. Transthoracic echocardiogram was utilized with a multi-frequency transducer of 7.5/5.5 MHz (Hewlett-Packard sons 1500 model 21275A, USA). The obtained images were registered on Panasonic cassette tape AG 7350, with videoprinter photography using Sony model UP 870-MD.

Analysis of the Doppler echocardiography was performed apically using four chambers and it was possible to obtain the Doppler spectrum of the mitral valve. Sample cuts were made with 3 to 4 micrometers of thickness, stained using hematoxylin and eosin and analysed using optic microscopy always by the same pathologist.

Histopathologic analysis

Three fragments of the animal hearts from the B3 group (n=8) were taken after euthanasia performed by bleeding after sectioning the ascending aorta. The areas with macroscopically evident damage, supposedly linked to the infarcted areas distal to the ligation of the DA artery were identified. The fragments were fixed in 10% formaldehyde solution, processed and embedded in paraffin. Histologic cuts were made with 3 to 4 micrometers of thickness, stained using hematoxylin and eosin and analysed using optic microscopy always by the same pathologist.

Statistical analysis

The descriptive measurements are presented in tables as mean, minimum and maximum values. The value of “n” refers to the size of the sample. The non-parametric Test (Wilcoxon) was applied for statistical analysis of two independent samples, that is, non-matched samples. The statistics programs Minitab 13 and SPSS 8.0 were used. A difference of 0.05 or 5% would lead to rejection of the null hypothesis.

RESULTS

Twelve rats were submitted to the surgery for AMI induction. Three animals died in the trans-operative period with left ventricular failure and respiratory insufficiency, giving a mortality rate of 25%.

The results of the echocardiograms performed were analysed in the animals of the control group (Group A; n=7) and in the animals of the infarcted group (Group B; n=9). The animals in Group B were evaluated by echocardiogram after one week (Group B1; n=9) and after three weeks of
infarction (Group B3; n= 8). One animal of the infarcted group died in this period during the anesthetic procedure to perform the ECG.

Fusion of the E and A waves were observed by transmitral Doppler at heart rates greater than 300 beats per minute in animals 4 and 5 of the Group A and in animal 1 of Group B1.

In the first postoperative week, seven (77.7%) animals demonstrated evident scaring of infarction on echocardiograms, defined as an area of high refringency in the anterior wall of the myocardium. In the third postoperative week, all animals presented echocardiographic signals of prior infarction.

In the analysis of the parameters measured by transmitral Doppler echocardiography, there was no significant difference when comparing the flow velocity of the groups. The values of the E and A waves are shown in Table 1.

The LA V was analyzed from the orthogonal plane of the four chambers, with the results shown in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=7)</th>
<th>Group B1 (n=9)</th>
<th>Group B3 (n=8)</th>
<th>A x B1 (p)</th>
<th>A x B3 (p)</th>
<th>B1 x B3 (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vel E(cm/s)</td>
<td>62.68(52.2-75.9)</td>
<td>65.01(58.2-77.3)</td>
<td>69.86(61.2-98.3)</td>
<td>0.648</td>
<td>0.358</td>
<td>0.562</td>
</tr>
<tr>
<td>Vel A(cm/s)</td>
<td>43.73(27.3-54.4)</td>
<td>39.31(26.6-54.8)</td>
<td>41.93(16.6-50.6)</td>
<td>0.648</td>
<td>0.897</td>
<td>0.475</td>
</tr>
<tr>
<td>E/A Ratio</td>
<td>1.44</td>
<td>1.73</td>
<td>1.82</td>
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</tr>
</tbody>
</table>

The numbers represent means and minimum and maximum values. N – number of animals per group; vel E – maximum velocity of the E wave; vel A – maximum velocity of the A wave; Wilcoxon Test *Significant differences p<0.05. AMI – Acute myocardial infarction

There was a significant increase in the LA V comparing Groups A and B1 and Groups A and B3 (p<0.05). There was no difference between the Groups B1 and B3.

**Histopathologic evaluation**

An area of evident necrosis was observed by macroscopic examination in the antero-lateral wall of each of the hearts removed. In one animal a dilated area compatible to a point aneurysm of the LV was clearly seen. The presence of myocardial infarction affecting almost all or all the thickness of the LV wall was observed in all cases. This was characterized by:

- Degeneration and death of heart muscle fibers, cardiomyocytes, evidenced by loss of striations and nuclei.
- Replacement of the heart muscle fibers by young conjunctive (conjunctive-vascular tissue) and fibrotic tissue (dense conjunctive tissue).

**COMMENTS**

There is no single method that can totally evaluate the diastolic function. Currently the echocardiogram is the main method.
examination utilized for ventricular diastolic evaluation and this allows indirect assessment of the LV diastolic function by analysis of the mitral valve flow and of the pulmonary veins. Analysis using Doppler at the mitral valve with the transducer placed in the precordial region, enables visualization of the LV apex and demonstrates the normal pattern of biphasic flow, with the first wave (E wave), which occurs in the protodiastole, followed by a second wave (A wave) which corresponds to the atrial systole. The E/A ratio, although it is commonly used to evaluate the diastolic function, suffers alterations depending on the pre-load and does not present a linear relation to the ventricular dysfunction. One of the most complete measurements can be performed in humans by an analysis of the pulmonary venous flow and the tissue Doppler. However, in rats, due to technical difficulties in attaining good images to analyze the Doppler effect and due to the high heart rate that fuse E and A waves together, it is difficult to measure of the diastolic function [10].

The post-AMI diastolic function was evaluated by PRUNER et al. [12] by measuring the image of the mitral ring with a tissue Doppler in rats. These authors measured the velocity peak of the E and A waves and the isovolumetric relaxing time and correlated these data with the final LV diastolic pressure which was invasively checked. This study demonstrated a possible correlation between invasive and noninvasive measurements, but showed limitations which must be researched in the future in new studies.

Changes in the myocardial complacency can alter the transmirtal flow. The decrease of the heart complacency can cause a rapid increase in the ventricular pressure at the start of diastole resulting in the shortening of the deceleration time. This occurs because the gradient between the left atrium and the left ventricle decreases more rapidly and the atrial contraction velocity may be reduced [8].

Three patterns of transmitral flow can indicate diastolic dysfunction. First, “late relaxation” is characterized by a decrease of the E wave and an increase of the A wave with an E/A ratio < 1. This transmitral flow pattern is a consequence of an alteration in ventricular relaxation in which the early LV diastolic pressure is abnormally elevated. Second, the “pseudo-normal” filling pattern is characterized by an E/A ratio > 1. Finally, the “restrictive” pattern is characterized by an increase in the velocity of the E wave (rapid deceleration of the E wave due to a pressure elevation in the left atrium) and a decrease or disappearance of the A wave, resulting in an E/A ratio > 2 [6].

Notice that the analysis of the mitral flow velocity curves gives useful information to determine the ventricular filling pressures and the diastolic function. However, the mitral flow is dependent on multiple factors such as the velocity and length of ventricular relaxation, atrial and ventricular complacency and suction and left atrial pressure. In additional, the method to evaluate the diastolic function can be rather complex limiting its utilization, both in humans and in experimentation animals.

During ventricular diastole, the left atrium is directly exposed to the intraventricular pressure through the open mitral valve, suffering the influences of occasional elevations of filling pressures which alter its volume. MOLLER et al. [8] stress that the LAV may be a measurement better that the velocity indexes of the E and A waves to evaluate the diastolic function, as it suffers less interference from external factors thus, represents a measurement of the chronic diastolic function. The findings indicated a significant increase in the LAV in infarcted rats, which may signify post-AMI diastolic dysfunction in these animals. The increase of the LAV occurred in the first week after infarction and continued to the third week. The LAV has never been evaluated in the post-infarction period before, in experimental studies with rats. An unexpected aspect of the present study was that we did not observe significant alterations in the velocity of the A and B waves using Doppler echocardiography after infarction. This parameter has been utilized for the evaluation of the ventricular diastolic dysfunction in the post-infarction period of humans. Also the E/A ratio tended to increase, which may indicate an evolution to the pseudo-normal or restrictive pattern of diastolic dysfunction.

The results suggest that measurement of the LAV is a more reliable parameter in the evaluation of diastolic function when compared to the velocity of the A and E waves recorded by Doppler echocardiography in rats. Until now, this fact has not been reported in the literature.

The LV diastolic dysfunction is very much prevalent in the general population. It is a significant prognostic indicator of several heart diseases, including AMI. Diastolic dysfunction is the incapacity of the ventricle to accept blood flow or to do so without a compensatory increase in the left atrial pressure. The physiologic influences on these measurements are varied and must always be considered. TSANG et al. [9], in 2002, found a positive correlation among LAV, age, cardiovascular risk score and final LV diastolic and systolic sizes. From this work, the LAV has been utilized as an adjuvant measurement in the analysis of the diastolic function. PRICHERT et al. [10], in 2003, suggested that the LAV reflected the atrial transformation associated with cardiovascular disease.

There are factors that can evidently interfere in the results of the LAV measurements. The four-chamber image put the left atrium far from the ultrasound range, which can result in a loss of resolution and limitations in the visualization of the endocardium. Planimetry of the atrium requires a visual estimation of the posterior and lateral walls.
to exclude confluence of the pulmonary veins and of the left atrial appendix. Moreover, we did not observe technical difficulties in the attainment of four-chamber images to measure the LAV.

Transcutaneous echocardiograms in rats and mice are progressively being utilized in the evaluation of the systolic and diastolic function by many authors. The application of the echocardiogram associated with Doppler echocardiography in rats is a valuable cost-effective method of assessing heart function which can analyze the myocardial function after the use of new drugs and after surgical and genetic interventions of the cardiovascular system in a standardized reproducible manner, allowing the longitudinal study of these animals.

The E and A wave velocities of the mitral flow and their relation to diastolic dysfunction have already been duly studied, proving their great variability due to variables such as heart rate and the pre- and post-load conditions. Our work suggests that the LAV is useful in the definition of the LV diastolic dysfunction after AMI. The LAV can reflect an increase in the final LV diastolic pressure secondary to systolic or diastolic dysfunction.

BIBLIOGRAPHIC REFERENCES