

Pathophysiological Characteristics of the Post-Myocardial Infarction Heart Failure Model in Rats

Paulo J. F. Tucci

Escola Paulista de Medicina - Unifesp

Abstract

Congestive heart failure (CHF) caused by myocardial infarction in rats is the most commonly used experimental model to study the congestive heart failure syndrome. Following myocardial necrosis, the manifestations observed in rats very satisfactorily reproduce the findings in humans with cardiac decompensation and substantiate the study of CHF pathogenesis, pathophysiology, and treatment. Two features are inadequate in the model: the high mortality rate in the first 24 hours after coronary occlusion, and the considerable diversity of myocardial infarct sizes. In this review we described methodological and pathophysiological aspects of the model, concluding with a reference to an alternative technique, which uses radio frequency electric current to produce myocardial necrosis, and evolves with low mortality rates and homogeneous infarct sizes.

For years, congestive heart failure (CHF) has been among the most investigated topics in cardiology. Besides the information obtained in humans, contribution made by the research conducted in laboratory animals is also noteworthy. Among the several experimental models available, the most used is that which promotes a congestive heart syndrome by the induction of myocardial necrosis in rats. Its current popularity and the foreseen increased interest in this model stress the importance of describing the pathophysiological characteristics after myocardial infarction (MI) in rats. Recently, a valuable literature review on the rat myocardial infarction model was published in the *Arquivos Brasileiros de Cardiologia*¹.

Although useful for the assessment of the congestive syndrome and the myocardial remodeling that occurs in the remaining cardiac muscle, the pathophysiological process established in rats after coronary artery ligation is not analogous to what happens in human coronary syndromes. The model lacks the arterial lesions that determine the distinctive clinical

profile of human coronary artery disease: arrhythmias, transient ischemia, and recurrence of coronary occlusion, which are absent in laboratory animals.

Occlusion of the anterior interventricular coronary artery is the strategy most frequently used for the induction of MI.

The electrocardiogram² and the echocardiogram³⁻¹⁵ are noninvasive methods capable of identifying the area of necrosis or scarring. Although it identifies the presence of an MI-dependent electrically inactive area, the ECG is not a method that can quantify the infarction area². The echocardiogram can determine the presence and the size of the MI with very good sensitivity³⁻¹⁵, even in periods as early as two days after necrosis, and it also provides information about deviant atrial and ventricular function. The best method for post-mortem diagnosis of MI depends on the stage in which the autopsy occurs. In the first days after myocardial necrosis, the histopathologic exam may be inconclusive as to the limits of the necrotic area. For this phase, the triphenyl-tetrazolium (TTZ) method is more accurate. In contact with living cells, this dye is reduced by dehydrogenases and takes on a dark red color. Dead cells, which no longer have the reduced forms of dehydrogenases, are not stained. The immersion of the myocardium in hydrogen peroxide then removes the red color of the hemoglobin present in the necrotic region hematoma, clearing it (Figure 1A). The TTZ permits the identification of necrosis immediately after cell death.

Two peculiarities are noteworthy in this model: the great variability in the size of the infarction and high mortality in the first 24 hours after coronary occlusion. Immediately after coronary occlusion the mortality rates described in literature range from a minimum of 13%¹⁶ to a maximum of 65%¹⁷. In our laboratory, the immediate mortality was about 44% for male rats, and 17% for female rats. The reason for this discrepancy between genders is not original, but its cause is unknown. There is compelling evidence that arrhythmias are the main cause of death in the 48-hour period after coronary occlusion¹⁷. A noticeable peculiarity of the model is the large range of variation in the size of the infarction after a standardized anterior interventricular coronary artery occlusion. In our last series, conducted to determine the size of the infarctions resulting from coronary occlusion, we observed a mean size of $40 \pm 19\%$ ($x \pm SD$). This great variability is confirmed by the elevated value of the standard deviation: 48% of the mean value. Large infarcts ($\geq 40\%$ of LV) - which are most relevant for the follow-up of the pathophysiology of CHF - occurred in 60% of survivors.

As similarly described for human beings, the size of the infarction is a critical determinant of the functional

Keywords

Heart failure/physiopathology; myocardial infarction; rats.

Mailing address: Paulo José Ferreira Tucci •

Rua Estado de Israel, 181/94 - Vila Clementino - 04022-000

São Paulo, SP - Brazil

E-mail: ptucci@cardiol.br, paulotucci@terra.com.br

Manuscript received October 29, 2009; revised manuscript received January 05, 2010; accepted February 23.

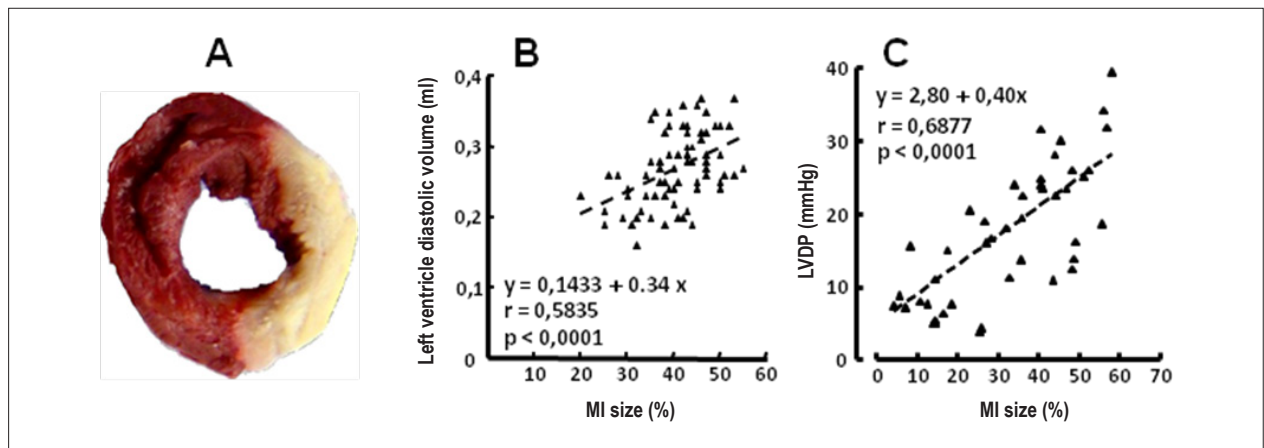


Figure 1 - Panel A: Cross section of heart subjected to 24 hours of coronary occlusion, stained with triphenyltetrazolium. The area of necrosis appears in white, contrasting sharply with the remaining myocardium. The figure also shows the linear correlations found between the size of the Myocardial Infarction and the left ventricular diastolic volume (Panel B), as well as between the size of the Myocardial Infarction and the left ventricular end diastolic pressure (LVDP) (Panel C), in HF rats, a few weeks after infarction. It is shown that these two variables increase in proportion to infarct size.

impact of MI on the heart. Figure 1B shows the correlation observed in the fifth day after coronary occlusion⁶ between the left ventricle diastolic volume (LV) and the size of the infarction. And figure 1C shows the correlation between the size of the infarction and the left ventricle final diastolic pressure (LVDP), six weeks after the induction of myocardial necrosis. The linear association between the volume and the LVDP with the size of the infarction in the LV is discernible. Curiously, the diastolic ventricular pressure had no correlation with the time elapsed after MI induction. The left ventricle fractional area shortening,* analyzed six weeks after the induction of MI, presented an inverse correlation with the size of the scar (Figure 2A), with an increasing reduction in the ejection capacity as the MI size increased. In this same post-coronary occlusion period, the most noticeable correlations between MI size and functional impact (Figure 2) were those between MI size and indicators of atrial emptying (AE ratio) and pulmonary congestion (lung weight/body weight ratio). These ratios increased exponentially with MI size (Figures 2B and 2C), and the absence or paucity of impact, for both variables, in infarcts covering less than 25% of the ventricular circumference is notable. There are no evident changes in these indicators when the MI encompasses less than 25% of the LV circumference, which indicates that, in the period analyzed, infarcts of less than 25% have reduced impact on atrial emptying and, therefore, do not induce pulmonary congestion.

* We no longer use ventricular volumes to analyze ventricular function in rats, considering that: the incidence of the echocardiogram, the implications of the geometry and the small size of the camera - especially the end-systolic volume - compromise the accuracy of the estimated values. Currently, we prefer LV cross-sectional area measurements. For similar reasons, we no longer use the echocardiogram to determine ventricular mass, due to an additional inconvenience: the very small size of the cavity wall (slightly thicker than 1 mm), and the uncertainty of its measurement. As to LV mass, the distrust was based on comparisons (Bland-Altman) with contemporary assessments of LV weight (unpublished data).

Determining the presence of CHF is a crucial issue in the follow-up of HF rats because, in rodents, the symptoms and clinical signs of the congestive state are subtle and infrequent¹⁸. In laboratory series, all HF animals that died in a follow-up of more than one week had CHF, with lung and liver congestion; the clinical manifestations of CHF remained unnoticed¹⁸. Left ventricular failure in HF rats may be determined by non-invasive methods (Doppler echocardiogram - DE), invasive methods (left ventricle diastolic pressure - LVDP), or post-mortem (lung water content or lung weight/body weight ratio). The DE assessment offers valuable information for the serial follow-up of the animals, because it shows a reduction in atrial emptying due to an increased LVDP. Both AE ratio and tissue Doppler analysis (E/Em ratio) can identify an increased LVDP¹⁵. Curiously, in rats, the initial pattern of impaired atrial emptying analyzed by mitral valve flow (AE ratio reduction) is not observed, whereas the restrictive pattern of DE is identified (AE ratio increase). In many rats of our series, AE ratio mean values \pm SD oscillated around 2.0. In the group of HF rats analyzed, AE ratio values higher than 2.7 identified an LVDP higher than 12 mmHg, with 90% of sensibility, and 80% of specificity¹⁵. In more recent series, the study showed AE ratio values of 2.15 ± 0.44 , in 44 normal rats. In animals with MI $\geq 40\%$ of the LV, it is common to observe mean values higher than 4.0. This seems to be the most dependable non-invasive indicator that the LVDP is high¹⁵ and, therefore, the subject has CHF. It should be emphasized that DE can reveal clinically hidden ventricular dysfunctions by determining the ratios between systolic and diastolic ventricular measurements (diameter, area, volume)¹¹. It is also said that the TEI index can assess the ventricular systolic function^{3,19}. DE can also offer information that helps detect pulmonary hypertension⁷ and forecast survival¹⁴ in rats. The detection of hemodynamic ventricular dysfunction is characterized by a slight reduction in systolic pressure and a noticeable increase in LVDP (Figure 2D). In anesthetized animals breathing spontaneously without mechanical ventilation, we identified the limiting value of 12 mmHg as the highest permissible level for LVDP¹⁵. Often, the diastolic pressure curve has a morphology very

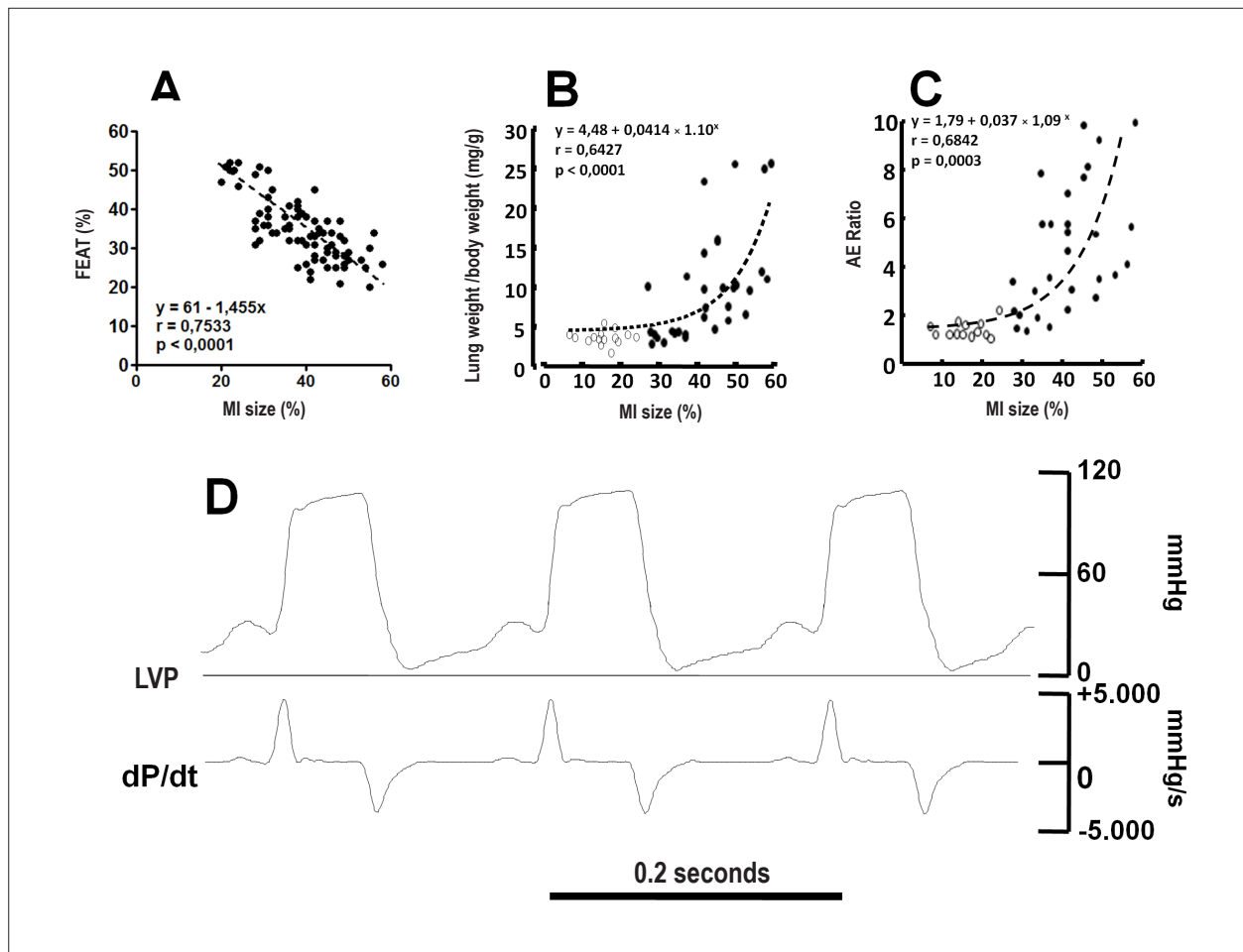


Figure 2 - Panel A: Inverse linear correlation between fractional area shortening of LV cross-sectional area and size of MI, showing that ventricular ejection capacity is reduced in proportion to the size of infarction. The exponential correlations between the size of infarction and the right lung weight/body weight ratio (Panel B), as well as between the size of infarction and the AE ratio (Panel C) indicate that infarcts of less than 25% of the ventricular circumference do not evoke significant ventricular dysfunction in rats. Exponential increase occurs after these values. Panel D: Graphic curve representing left ventricular pressure (LVP) and its temporal derivative (dP/dt), in rats with Myocardial Infarction greater than 40% of the ventricular circumference, six weeks after coronary occlusion. Noteworthy is the prominent increase in LV diastolic pressure during rest, which is a characteristic of ventricular filling impairment. Typically, in normal mice, dP/dt maximum positive values are greater than 7000 mmHg/s. In the example, the observed values of less than 5000 mmHg/s indicate an impaired ability to generate pressure, i.e., the presence of systolic dysfunction.

suggestive of impairment of the ventricular filling (Figure 2D). The water content of organs [Water content (%) = (wet weight - dry weight/wet weight) x 100] is a variable strictly controlled within very narrow limits. For the lung, the normal mean value is 79%, with extremely low variability (standard deviation of 1%). These characteristics render the lung water content a very sensitive indicator to detect the presence of pulmonary congestion in a group of HF animals, because the low variability promotes the statistical significance of the differences between means. However, when considering individual values in HF animals, the strict limits imposed by the physiological control of water content hinder the identification of isolated abnormal values, because individual differences between normal and pathological values normally do not reach expressive levels. For individual analysis, the right lung weight/body weight ratio is an indicator of greater sensitivity, given the wider range (3.83 ± 0.63 mg/g) of the normal values of this ratio. In normal rats, this ratio rarely

exceeds the value of 5 mg/g, which is frequently observed in animals with congestive syndrome. Laboratory data indicate that, commonly, at the end of the first week after induction of myocardial necrosis, rats with MI > 40% have pulmonary congestion²⁰. The literature indicates that neurohumoral variables deviate from the normal range²¹ in rats with large infarctions since the first week post-MI.

In the acute phase of MI, the major determinant of ventricular dysfunction is the loss of cardiomyocytes. The inevitable impairment of the ejection function leads to increased left ventricular residual volume and, consequently, to ventricular dilation. We demonstrated that two days after the induction of coronary occlusion the ventricular dimensions were increased²². The increased volume of the ventricular cavity promotes myocardial remodeling in the chronic phase. According to Laplace's Law [The force (F) developed by the myocardium to generate a certain pressure (P) inside the cavity is directly proportional to the cavity radius (R) and inversely

proportional to the wall thickness (h): $F = P \times R/2h$], a cavity with a large radius increases the force required to generate a certain pressure, ie, increases the afterload. Ventricular cavity dilation is well recognized as a critical determinant of ventricular dysfunction and remodeling²³. In our laboratory we documented the influence of the increased ventricular volume on pressure-generating⁸ and pumping⁷ capacities. An impaired ejection is observed immediately after the induction of myocardial necrosis. Rats studied immediately after coronary occlusion had decreased left ventricular (LV) fractional shortening (FS) of the cross-sectional area (unpublished data). Two days later, the SF presented values that remained stable until the later stages of remodeling and CHF^{20,22}. In the early stages, the impaired contractility of the remaining myocardium does not contribute to ventricular dysfunction. Rats examined three weeks after arterial occlusion presented clear signs of ventricular dysfunction, although no impairment of inotropism was identified in the non-necrotic myocardium^{11,24}.

In recent decades the experimental model of CHF secondary to MI in rats has contributed markedly to the assessment of the pathophysiology and the treatment of congestive syndrome, despite its two major drawbacks: immediate mortality and high heterogeneity of MI size. Some years ago, aiming at solving these drawbacks, a novel model of cryoinjury-induced myocardial infarction was described²⁵. Our experience with this technique was unsatisfactory (unpublished data). The myocardium must be frozen several times to achieve necrosis, and the lesion is not always transmural, and often the maneuvers to induce myocardial hypothermia cause permanent asystole. More recently, we described²⁰ an original model for the induction of myocardial necrosis in the LV of rats, with radiofrequency ablation (RFA), using electrical current as the technique routinely used in

clinical practice to correct arrhythmias. The purpose was to find a technique that produced MI with less variability in size, and lower incidence of mortality. We observed infarcts measuring $45 \pm 8\%$ of the ventricular circumference. The lower variability of MI size obtained by RFA is associated with a significantly lower standard deviation than that observed for MI size obtained by occlusion of the anterior descending artery. The early mortality observed for the RFA technique was 7%. Concurrently, we documented that when infarcts of similar sizes were compared, the evolution of the myocardial and ventricular dysfunction and the pulmonary congestion found in the MI obtained by RFA were equivalent to those evoked by coronary occlusion. Furthermore, it is possible to control the infarct size by varying the size of the positive electrode used or the characteristics of RFA duration and RF energy released in the myocardium. More recently, we tested this new technique in mice, with equal success. The interest in using this model should now expand in the short term, given the current upsurge in studies evaluating post-myocardial infarction treatment with stem cells.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by FAPESP, CNPq and UNIFESP.

Study Association

This study is not associated with any post-graduation program.

References

1. Zornof LAM, Paiva SAR, Minicucci MF, Spadaro J. Infarto do miocárdio experimental em ratos: análise do método. *Arq Bras Cardiol.* 2009; 93: 434-40.
2. Bonilha AMM, Saraiva RM, Kanashiro RM, Portes LA, Antonio EL, Tucci PJF. A routine electrocardiogram should not be used to determine the size of myocardial infarction in the rat. *Braz J Med Biol Res.* 2005; 38: 615-9.
3. Cury AF, Bonilha AMM, Saraiva RM, Campos Fo O, Carvalho AC, de Paola AAV, et al. Myocardial performance index in female rats with myocardial infarction: relationship with ventricular function parameters by Doppler echocardiography. *J Am Soc Echocardiogr.* 2005; 18 (5): 454-60.
4. dos Santos L, Mello AF, Antonio EL, Tucci PJF. Determination of myocardial infarction size in rats by echocardiography and tetrazolium staining: correlating, agreements, and simplification. *Braz J Med Biol Res.* 2008; 41 (3): 199-201.
5. dos Santos L, Serra AJ, Antonio EL, Hull HF, Tucci PJF. Hiperbaric oxygenation applied immediately after coronary occlusion reduces myocardial necrosis and acute mortality in rats. *Clin Exp Pharmacol Physiol.* 2009; 36 (5-6): 594-8.
6. Helber I, Santos AA, Antonio EL, Flumignan RLC, Bocalini D, Piccolo C, et al. Digoxin prolongs survival of female rats with heart failure due to large myocardial infarction. *J Card Fail.* 2009; 15 (9): 798-804.
7. Kanashiro RM, Saraiva RM, Alberta A, Antonio EL, Moisés VA, Tucci PJF. Immediate functional effects of left ventricular reduction: a Doppler echocardiographic study in the rat. *J Card Fail.* 2006; 12 (2): 163-9.
8. Kanashiro RM, Nozawa E, Murad N, Gerola LR, Moisés VA, Tucci PJF. Myocardial infarction scar plication in the rat: cardiac mechanics in an animal model for surgical procedures. *Ann Thorac Surg.* 2002; 73 (5): 1507-13.
9. Nozawa E, Kanashiro R, Murad N, Carvalho ACC, Cravo SL, Campos O, et al. Performance of two-dimensional Doppler echocardiography for the assessment of infarct size and left ventricular function in rats. *Braz J Med Biol Res.* 2006; 39 (5): 687-95.
10. Moisés VA, Ferreira RL, Nozawa E, Kanashiro RM, Campos Fo O, Andrade JL, et al. Structural and functional characteristics of rat hearts with and without myocardial infarct: initial experience with Doppler echocardiography. *Arq Bras Cardiol.* 2000; 75 (2): 125-36.
11. Peron APON, Saraiva RM, Antonio EL, Andriolo A, Tucci PJF. Food restriction does not impair myocardial mechanics during the healing period of myocardial infarction in the rat. *Nutr Res.* 2005; 25: 1075-84.
12. Portes LA, Tucci PJF. O treinamento físico por natação atenua o remodelamento miocárdico e congestão pulmonar em ratas Wistar com insuficiência cardíaca secundária a infarto do miocárdio. *Arq Bras Cardiol.* 2006; 87: 54-9.
13. Portes LA, Saraiva RM, Santos AA, Tucci PJF. Swimming training attenuates remodeling, contractile dysfunction and congestive heart failure in rats with moderate and large myocardial infarction. *Clin Exp Pharmacol Physiol.* 2009; 36: 394-9.

Review Article

14. Santos AA, Helber I, Flumignan RLC, Antonio EL, Carvalho AC, Paola AAV, et al. Doppler echocardiographic predictors of mortality in female rats after myocardial infarction. *J Card Fail.* 2009; 15 (2): 163-8.
15. Saraiva RM, Kanashiro RM, Antonio EL, Campos Fo O, Tucci PJF, Moisés VA. Rats with high left ventricular end-diastolic pressure can be identified by Doppler echocardiography one week after myocardial infarction. *Braz J Med Biol Res.* 2007; 40 (11): 1557-65.
16. Jain M, Liao R, Ngoy S, Whittaker P, Apstein CS, Eberli FR. Angiotensin II receptor blockade attenuates the deleterious effects of exercise training on post-MI ventricular remodeling in rats. *Cardiovasc Res.* 2000; 46 (1): 66-72.
17. Opitz CF, Mitchell GF, Pfeffer MA, Pfeffer JM. Arrhythmias and death after coronary artery occlusion in the rat: continuous telemetric ECG monitoring in conscious, untethered rats. *Circulation.* 1995; 92 (2): 253-61.
18. Flumignan RL, Kanashiro RM, Saraiva RM, Portes LA, Antonio EL, Ishiga MMS, et al. Incidence of heart failure in infarcted rats that die spontaneously. *Braz J Med Biol Res.* 2006; 39 (10): 1-6.
19. Barberato SH, Pecoits Filho R. Influência da redução da pré-carga sobre o índice de desempenho miocárdico (Índice de Tei) e outros parâmetros Doppler ecocardiográficos da função ventricular esquerda. *Arq Bras Cardiol.* 2006; 86: 425-31.
20. Antonio EL, Santos AA, Araujo SRR, Bocalini D, dos Santos L, Fenelon G, et al. Left ventricle radio-frequency ablation in the rat: a new model of heart failure due to myocardial infarction homogeneous in size and low in mortality. *J Card Fail.* 2009; 15 (6): 540-8.
21. Francis J, Weiss RM, Wei SG, Johnson AK, Felder RB. Progression of heart failure after myocardial infarction in the rat. *Am J Physiol Regul Integrat Comp Physiol.* 2001; 281 (4): R1734-R1745.
22. Santos L, Santos AA, Gonçalves GA, Krieger JE, Tucci PJF. Bone marrow cell therapy prevents infarct expansion and improves border zone remodeling after coronary occlusion in rats. *J Card Fail.* 2009 [ahead of print].
23. White HD, Norris RM, Brown MA, Brandt PWT, Whitlock RML, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation.* 1987; 76 (1): 44-51.
24. Peron APON, Saraiva RM, Antonio EL, Tucci PJF. A função mecânica do miocárdio remanescente a um infarto do miocárdio é normal durante o período de cicatrização, embora exista insuficiência cardíaca. *Arq Bras Cardiol.* 2006; 86: 105-12.
25. Huwer H, Winning J, Vollmar B, Welter C, Menger MD, Schafers HJ. Model of chronic systolic and diastolic dysfunction after cryothermia-induced myocardial necrosis in rats. *Comp Med.* 2000; 50 (4): 385-90.