Original article

Short-term swimming therapy is not benefical in an experimental animal model of myocardial infarction

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Abstract

Myocardial infarction is a major cause of mortality worldwide. Physical exercise appears to reduce complications associated with myocardial infarction by contributing to cardiac remodeling. The aim of this study was to investigate the relation between cardiac remodeling and short-term physical activity. We segregated 9 male Wistar rats into 3 groups: non-operated control, sham-operated (subjected to open chest surgery), and operated (subjected to open chest surgery and ligation of the left anterior descending coronary artery). Operated and sham-operated animals were enrolled in a physical activity program shortly after surgical recovery. Non-operated control rats did not receive physical activity stimulation. The heart size and heart wet weight/body weight ratio in operated rats significantly increased compared with that in control or sham-operated rats. Moreover, operated rats showed histological evidence of myocardial cell swelling and disorganization of myocyte architecture. These changes were not observed in control or sham-operated rats. Performing short-term physical activity at the acute phase of myocardial injury does not mitigate the effects of myocardial infarction, as evidenced by increased heart size and altered histological features. Longer training sessions might be required for obtaining any benefit.

Keywords: myocardial infarction, physical exercise.

1 Introduction

Myocardial infarction – a major cause of mortality worldwide – is an occlusive atherosclerotic disease of the coronary artery, with complex mechanisms at the biochemical, electrical, and functional levels. The larger the area of myocardial injury, the greater the degree of disease. Unfortunately, approximately 30.3% of the patients who suffer a heart attack develop heart failure (BRASIL, 2002; LATADO, PASSOS, BRAGA et al., 2006). The socioeconomic character of this disease affects the entire health system, which has limited resources. Moreover, patients need allocation prioritization. Therefore, it is important to encourage investment in basic biomedical research and increase the life expectancy of patients who experience a heart infarction (ALBANESI-FILHO, 2005; PIMENTEL, 2006).

Ischemic heart failure can be induced experimentally in animal models (JOHNS and OLSON, 1954); the injured area is easily identified by histological staining with hematoxylin and eosin (HE) and triphenyltetrazolium (TFT) (VIVALDI, KLONER and SCHOEN, 1985).

Exercise may attenuate the consequences of ischemic heart failure. Pulmonary congestion was found to be reduced and diastolic function improved in hearts isolated from infarcted rats treated with captopril and coenzyme Q10 when subjected to a 30-day chronic treadmill exercise (BECH, SORENSEN, JENSEN et al., 1990; LIBONATI, 2003).

Other studies have evaluated the relationship between swimming exercise, myocardial infarction, and the water content of the lungs, and found that these type of exercise is beneficial in the prevention or alleviation of pulmonary congestion and heart failure. These effects were observed in rats subjected to swimming exercise every day (60 min/day) for a period of 2-13 months starting 21 days after induction of myocardial infarction (PORTES and TUCCI, 2006).

This study was aimed at evaluating the effects of a 1-week swimming exercise program, performed every day for 10 minutes (LEITÃO, CASTRO, BASILE et al., 2003) and initiated 1 week after occlusion of the anterior descending coronary artery, on the pathophysiology of the rat heart.

2 Materials and methods

The experimental protocol was approved by the Ethics Committee for Animal Research, Sergipe Federal University (CEPA Case No. 49/2007) in accordance with guidelines of the Brazilian College of Animal Experimentation (COBEA).

Nine male Wistar rats (Central Animal UFS; weight 210 ± 40 g; age, 60-120 days) were divided into 3 groups: non-operated (NO) control, sham-operated (SO: negative control subjected to open chest surgery), and operated (OP: subjected to open chest surgery to induce myocardial infarction).

After the rats were weighed, anesthesia was induced by intramuscular administration, of a solution containing ketamine (50 mg.kg^{-1} of body weight; Cristália®, São Paulo, SP, Brazil) and midazolam (5 mg.kg^{-1} of body weight; (Cristália®). Upon verification of loss of pedal withdrawal reflex, the animals underwent surgical exposure of the

trachea for endotracheal intubation; ventilatory support was provided at a frequency of approximately 50 cycles/min with a 2.5 mL volume. In the animals of the OP group, we performed thoracotomy in the left hemithorax at the intercostal space of the ictus cordis for, pericardiotomy and ligation of the left coronary artery by, using a mononylon suture (6-0; Ethicon®, São José dos Campos, SP, Brazil), to induce anterolateral wall myocardial infarction. In animals of the SO group, the chest was sutured immediately after pericardiotomy.

Rats were allowed to recover for 7 days, after which rats from the SO and the OP groups were enrolled in a swimming exercise protocol of 6 min/day for 7 days. Non-operated control rats did not receive physical activity stimulation. At day 15, the animals were killed and the hearts collected. The effectiveness of the surgical procedure was demonstrated by change in the color of the left ventricular wall (OP animals) after immersion of the hearts in a 1% solution of TFT in a water bath at 37 °C and pH = 7.4. The wet weight of the hearts was determinate, in absolute values and corrected by the body weight (OLIVEIRA JÚNIOR, OKOSHI, LEOPOLDO et al., 2009); in order to verify an increase in total body mass - an indication of clinical cardiac dysfunction.

The histological analyze of the myocardial involved a cross-sectional area of the ventricles. After fixation with 4% formaldehyde, the hearts were embedded in paraffin and cut into 5 µm-thick sections. The sections were stained with Hematoxylin-Eosin[9]. The aim was to identify areas of myocardial infarction and detect the emergence of or change in structures that are linked to fibrosis promoted by injury. The sections were photographed with the aid of a microscope at a magnification of 20× (Olympus®, Tokyo, Japan) coupled with a digital camera (12.1 mega pixels; Sony, São Paulo, SP, Brazil).

Statistical analysis was performed using one-way analysis of variance (ANOVA) with pos-hoc Bonferroni post-test. A p value of less than 0.05 was considered statistically significant.

3 Results

In the present study, short-term swimming exercise did not promote changes in body weight in the animals tested (Figure 1). The ratio of heart wet weight to body weight was assessed for all groups. This ratio was significantly higher (by 63%) in OP rats as compared to NO or SO rats (5.9 ± 0.6 g versus 3.6 ± 0.3 g or 3.0 ± 0.3 g, respectively (Figure 2).

Isolated hearts were immersed in TFT for 10 minutes. Hearts of OP animals exhibited areas of post-myocardial infarction healing (Figure 3c). In contrast, these changes were not observed in the hearts of NO or SO animals (Figure 3a, b). Since TFT staining revealed hearts hyperplasia and increased necrotic area, we histologically evaluated the area of myocardial damage (Figure 4). We found that OP animals showed morphological derangement of the injured tissue architecture, cell swelling, cardiomyocyte disorganization, increased intercellular space and presence of inflammatory cells in the infarcted zone (Figure 4c). These changes were not observed in the hearts from NO or SO animals (Figure 4a, b).

The experimental model of left anterior descending coronary artery ligation used in this study was effective

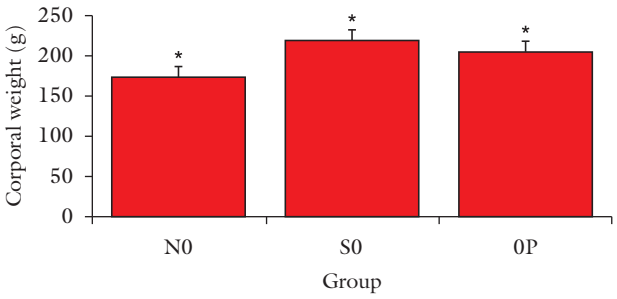

Figure 1. Body weight of non-operated (NO), sham-operated (SO), and operated (OP) rats. The data represent the average ± EPM of 3 animals per group. The asterisks indicate statistically significant difference (<0,05) between groups, as determinate by one-way analysis of variance (ANOVA) with post-hoc Bonferroni test.

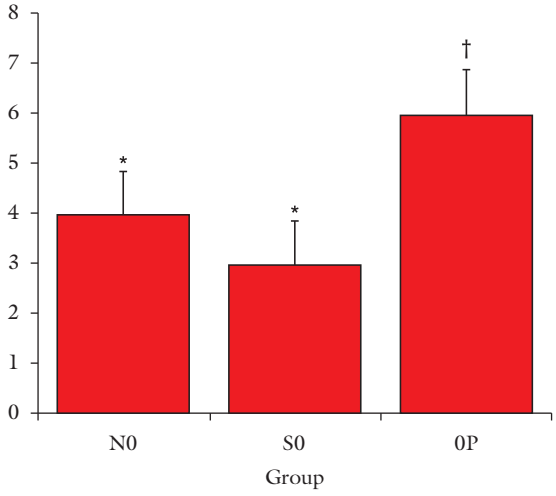

Figure 2. Relation between the heart wet weight and the body weight of non-operated (NO), sham-operated (SO) and operated (OP) rats. The data represent the mean of the heart wet weight/body weight ratio of 3 animals per group. Symbols (* and †) indicate statistically significant difference ($p < 0.05$) between groups (different symbols) or no significant difference (same symbols), as determined by one-way analysis of variance (ANOVA) with post-hoc Bonferroni test.

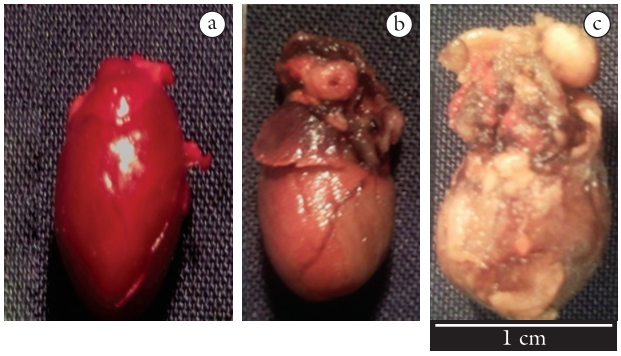

Figure 3. Representative images of: a) non-operated (NO); b) sham-operated (SO); and c) operated (OP) animals. Scale bar: 1 cm.

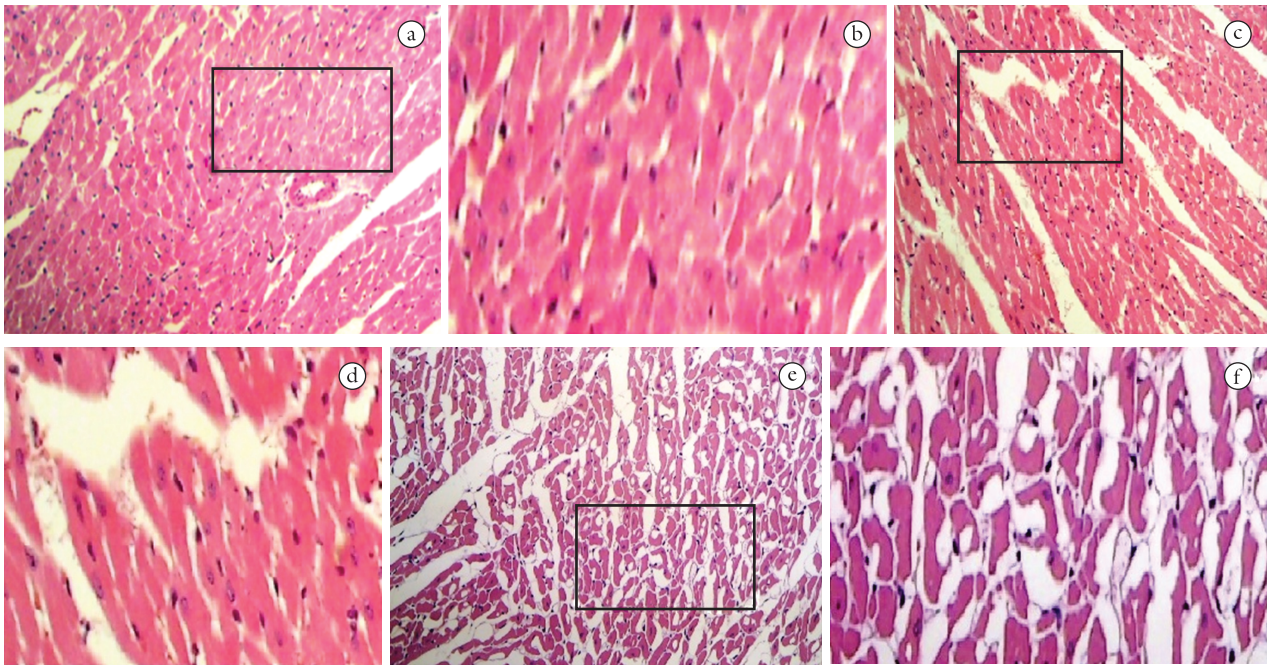

Figure 4. Histological sections of hearts from (a, b) non-operated (NO), (c, d) sham-operated (SO) and (e, f) operated (O) animals. Sections were stained with hematoxylin and eosin and observed at a magnification of 20× (a, c and e) or 100× (b, d and f).

in inducing cardiac injury. On the other hand, swimming exercise therapy was not beneficial to the adjacent tissues responsible for cardiac remodeling, but definite conclusions cannot be drawn because the observation time was short. In other experimental animal models, such as that reported by Doroshow, Locker, and Myers (1979), a pharmacological approach based on the administration of high doses of bupivacaine, adryamicin or isoproterenol was used. These cardiotoxic substances induce cardiac hypertrophy and congestive heart failure (DAVEL, KAWAMOTO, SCAVONE et al., 2006). Another procedure consists of the mechanical ligation of the anterior interventricular branch of the left coronary artery. In the present study, we used the latter method because it closely correlates with the events that provoke ischemic heart failure in humans. One of the limiting factors of this model, however, is the high cost involved in the maintenance of animals, including the costs for food, water, and cleaning (BUSCHE, GALLINAT, BOHLE et al., 2000).

Ventricular remodeling plays a major role in the pathophysiology of ventricular dysfunction after infarction. In response to a challenge, genetic, biochemical, and structural alterations result in long-term deterioration of the functional capacity of the heart, which ultimately leads to the emergence of necrotic areas and symptoms of heart failure and or sudden death. These changes culminate in myocardial failure (ZORNOFF, PAIVA, DUARTE et al., 2009). In this study, we showed a significant increase in the heart wet weight of infarcted animals as well as an increase in the necrotic area. Most of the cardiovascular diseases lead to heart failure, which is a significant cause of morbidity and mortality. This complication in post-infarction patients has socio-economic implications and decreases the quality of life (ALBANESI-FILHO, 2005).

Physical exercise appears to exhibit beneficial effects on infarcted animals in the chronic phase of disease. However, improvement in left ventricular function is observed only after long-term physical activity (9 months of treadmill training). Swimming also induces cardioprotective effects, in addition to ventricular remodeling, while reducing left ventricular hypertrophy (PORTES and TUCCI, 2006). Nevertheless, in the present study, swimming was ineffective in protecting the heart from cardiac damage. This result may be partly due to the short period of training. In conclusion, short-term exercise overload during the acute phase of injury does not contribute to myocardial remodeling.

4 Conclusion

In conclusion, we successfully induced myocardial damage by using an experimental model of left anterior descending coronary artery ligation, as evidenced by the presence of scar tissue in the injured area. Aerobic exercise, performed for a short period after myocardial infarction, was ineffective in promoting cardiac remodeling.

Acknowledgments: We acknowledge the support of the technicians at the Laboratory of Anatomy, Department of Morphology, Biological Health and Science Center, Sergipe Federal University (UFS). Potential conflict of interest: we declare no potential conflict of interest. Funding Sources: This study was funded by Assistance Program for Integration of Teachers, Administrative Technicians and New Doctors at Research Activities (PAIRD)/ 2007/ UFS. This study is not associated with a graduate program.

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Received October 4, 2010
Accepted May 25, 2011