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Effects of late exercise on cardiac remodeling and myocardial calcium handling proteins in rats with moderate and large size myocardial infarction☆

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ABSTRACT

Background: Physical exercise attenuates myocardial infarction (MI)-induced cardiac remodeling. However, it is unsettled whether late exercise modulates post-infarction cardiac remodeling differentially according to infarct size. We investigated the effects of exercise started at late stage heart failure on cardiac remodeling in rats with moderate and large sized MI.

Methods: Three months after MI, rats were assigned into sedentary and exercise groups. Exercise rats underwent treadmill for three months. After assessing infarct size by histological analysis, rats were subdivided into four groups: moderate MI sedentary (Mod MI-Sed; n = 7), Mod MI exercised (Mod MI-Ex; n = 7), Large MI-Sed (n = 11), and Large MI-Ex (n = 10).

Results: Before exercise, MI-induced cardiac changes were demonstrated by comparing results to a Sham group; alterations were more intense in rats with large than moderate MI size. Systolic function, evaluated by echocardiogram using the variation in LV fractional area change between after and before exercise, was improved in exercise than sedentary groups. Calsequestrin expression increased in exercised compared to sedentary groups. L-type calcium channel was higher in Mod MI-Ex than Mod MI-Sed. SERCA2a, phospholamban, and Na^+/Ca^{2+} exchanger expression did not differ between groups.

Conclusion: Late exercise improves systolic function and modulates intracellular calcium signaling proteins in rats with moderate and large MI.

1. Introduction

Heart failure is a major worldwide cause of disability and death [1]. Animal myocardial infarction (MI) models are relevant in pathophysiology and treatment studies as myocardial ischemia and infarction are common causes of cardiac remodeling and heart failure in humans [1]. After MI, cardiac remodeling is associated with progressive ventricular dysfunction, decompensated heart failure, and cardiovascular death. Despite progressive improvement in pharmacological treatment, heart failure prognosis is still poor [2]. Efforts have therefore been directed towards non-pharmacological treatment, and physical exercise is recommended as an important strategy in attenuating post-infarction cardiac remodeling and heart failure development [2].

Ligation of the left anterior descending coronary artery in rats leads to a wide range of infarction size, which modulates the ensuing cardiac remodeling and left ventricular (LV) dysfunction [3,4]. The effects of physical exercise have been assessed in rats with different myocardial infarction sizes, ranging from 30% to 40% [5–10] and over 40% total LV area [6,9,11–14]. Aerobic exercise in rats with moderate size myocardial infarct has been shown to improve survival, systolic and mitochondrial function, and inflammatory profile [5,7–9]. Furthermore, high-intensity sprint training [10] attenuated myocyte hypertrophy and normalized Ca^{2+} transient and sarcoplasmic reticulum function in myocytes. Exercise in rats with large MI improved cardiac remodeling and ventricular function, which was associated with restored contractile function and intracellular Ca^{2+} handling, and decreased myocardial fibrosis and oxidative stress [11–14]. In contrast, swim training in rats with large MI decreased survival and impaired LV remodeling [6]. Only a

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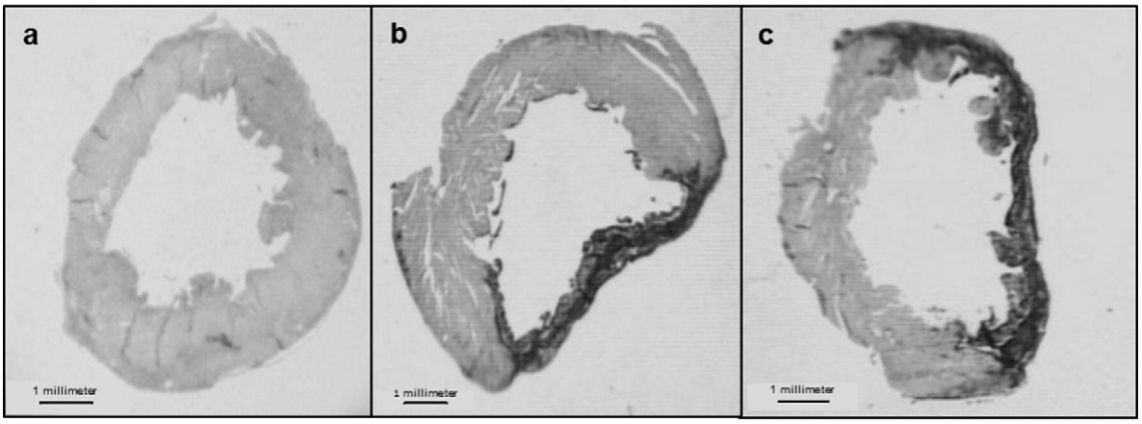

Fig. 1. Representative histological photos of Sirius red F3BA-stained left ventricles from Sham (A), and infarcted rats with moderate (B) and large (C) myocardial infarction sizes.

few authors have examined the effects of aerobic exercise on late stage cardiac remodeling after MI induction, when heart failure is more prevalent. At this stage, beneficial [15] effects were observed in cardiac remodeling.

It is therefore still not clear whether late exercise modulates post-infarction cardiac remodeling differentially according to infarct size. In this study we investigated the effects of a treadmill exercise protocol started at a late stage heart failure on cardiac remodeling and myocardial calcium handling protein expression in rats with moderate and large size myocardial infarction.

2. Materials and methods

2.1. Experimental design

Male Wistar rats (200–250 g, 50–60 days old) were purchased from the Central Animal House, Botucatu Medical School, UNESP. All animals were housed in a room under temperature control at 24 °C and kept on a 12-hour light/dark cycle. Food and water were supplied ad libitum. All experiments and procedures were performed in accordance with the Canadian Council on Animal Care guidelines and were approved by Botucatu Medical School Ethics Committee, UNESP, SP, Brazil (protocol number 808).

MI was induced according to a previously described method (n = 60) [16,17]. Briefly, rats were anesthetized with ketamine (70 mg/kg) and xylazine (1 mg/kg) and subjected to left lateral thoracotomy. After heart exteriorization, the left atrium was retracted to facilitate ligation of the left anterior descending coronary artery with 5–0 mononylon suture between the pulmonary outflow tract and the left atrium. The heart was then replaced into the thorax, the lungs were inflated with positive pressure, and the thoracotomy was closed. To demonstrate infarction-induced cardiac injury before the exercise protocol, results of infarcted rats were compared with those from sham-operated rats (n = 12).

Three months after surgery, survival rats were subjected to echocardiographic evaluation and assigned into sedentary (n = 22) and exercised groups (n = 22). Physical exercise protocol consisted of 40 min/day treadmill running at 16 m/min, 5 days/week, for three months [15]. During an adaptive period of two weeks, exercise velocity and duration were slowly increased. In the first two weeks of training, the animals were subjected to low-voltage electrical stimulation to start exercise. At the end of the exercise protocol, rats were subjected to echocardiographic evaluation and euthanized the next day for tissue collection. After assessment of myocardial infarction size by histological analyses, rats were then subdivided into four groups: sedentary rats with moderate MI size (Mod IM-Sed, n = 7), sedentary rats with large MI (Large MI-Sed, n = 11), exercised rats with moderate MI (Mod MI-Ex, n = 7) and exercised rats with large MI (Large MI-Ex, n = 10). Moderate MI groups contained rats with MI size <40% sum of total (infarcted and viable myocardium) endocardial and epicardial ventricular circumferences, and large MI groups contained rats with MI size ≥40% total LV circumferences [18].

2.2. Echocardiographic study

Echocardiography was performed using a commercially available echocardiograph (General Electric Medical Systems, Vivid S6, Tirat Carmel, Israel) equipped with a 5–11.5 MHz multifrequency probe. Rats were anesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg) and xylazine (0.5 mg/kg). A two-dimensional parasternal short-axis view of the LV was obtained at the level of the papillary muscles. M-mode tracings were obtained from short-axis views of the LV at or just below the tip of the mitral-valve leaflets, and at the level of the aortic valve and left atrium [19–21]. M-mode images of the LV were printed on a black-and-white thermal printer (Sony UP-890MD) at a sweep speed of 100 mm/s. All LV structures were manually measured by the same observer (KO), who was blind to the experimental groups. Measurements were the mean of at least five cardiac cycles on M-mode tracings. The following structural variables were evaluated: left atrium (LA) diameter, aorta diameter (AO), LV diastolic and systolic diameters (LVDD and LVSD, respectively), LV diastolic posterior wall thickness (PWT), and LV diastolic and systolic area. LV function was assessed by the following parameters: posterior wall shortening velocity (PWSV), fractional area change (FAC), early

Table 1
Cardiac structural parameters analyzed by transthoracic echocardiogram before exercise protocol.

	Sham (n = 12)	Moderate MI (n = 15)	Large MI (n = 29)
LVDD, mm	8.38 ± 0.35	10.25 ± 0.62*	10.78 ± 0.70*,**
LVDD/BW, mm/kg	18.78 ± 1.73	22.99 ± 1.79*	22.75 ± 1.87*
LVSD, mm	4.05 ± 0.44	7.72 ± 0.97*	8.57 ± 0.83*,**
PWT, mm	1.35 ± 0.07	1.53 ± 0.12*	1.60 ± 0.16*
AO, mm	3.70 ± 0.26	3.63 ± 0.22	3.59 ± 0.24
LA, mm	5.55 ± 0.54	6.73 ± 0.65*	7.69 ± 0.66*,**
LA/AO	1.50 ± 0.16	1.84 ± 0.17*	2.10 ± 0.53*
LA/BW, mm/kg	12.43 ± 1.44	15.19 ± 1.83*	16.06 ± 1.64*
LV diastolic area, cm^2	0.52 ± 0.07	0.79 ± 0.11*	0.81 ± 0.07*,**
LV systolic area, cm^2	0.16 ± 0.03	0.54 ± 0.07*	0.63 ± 0.08*,**

Data are expressed as mean ± SD. Moderate MI: rats with myocardial infarction (MI) size <40% total left ventricle (LV) endocardial and epicardial circumferences; Large MI: rats with MI size ≥40% total LV circumferences; LVDD and LVSD: LV diastolic and systolic diameters, respectively; PWT: LV diastolic posterior wall thickness; AO: aorta diameter; LA: left atrium diameter; BW: body weight. ANOVA and Student Newman–Keuls test.
* $P < 0.05$ vs Sham.
** $P < 0.05$ vs Moderate MI.

Table 2
Left ventricular functional parameters analyzed by transthoracic echocardiogram before exercise protocol.

	Sham (n = 12)	Moderate MI (n = 15)	Large MI (n = 29)
HR, Beats/min	262 ± 18	301 ± 45*	297 ± 38*
PWSV, mm/s	35.6 ± 4.50	25.3 ± 5.95*	22.8 ± 5.94*
FAC, %	69.6 ± 3.84	32.2 ± 6.93*	29.2 ± 5.85*
E-wave, cm/s	84.5 ± 8.78	97.0 ± 17.21	108.1 ± 21.44*
A wave, cm/s	49.1 ± 11.98	67.1 ± 26.49*	37.3 ± 18.19**
E/A	1.80 ± 0.40	1.90 ± 1.55	3.82 ± 2.19*,**
IVRT, ms	26.2 ± 4.79	33.0 ± 6.21*	26.9 ± 6.12**
EDT, ms	48.2 ± 5.84	44.3 ± 12.52	37.1 ± 5.57*,**

Data expressed as mean ± SD. Moderate MI: rats with myocardial infarction (MI) size <40% total left ventricle (LV) endocardial and epicardial circumferences; Large MI: rats with MI size ≥40% total LV circumferences; HR, heart rate; PWSV: posterior wall shortening velocity; FAC: fractional area change; E/A: early-to-late diastolic mitral inflow ratio; IVRT: isovolumetric relaxation time; EDT: E-wave deceleration time. ANOVA and Student Newman–Keuls test.
* $P < 0.05$ vs Sham.
** $P < 0.05$ vs Moderate MI.