

Post-Myocardial Infarction Exercise Training Induces Angiogenesis in the heart

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Abstract

Cardiac angiogenesis after myocardial infarction (MI) is critical to the development of compensatory hypertrophy in the viable myocardium; however, compelling evidence suggests that post-MI cardiac angiogenesis is inadequate, and may promote the transition from adaptive cardiac hypertrophy to left ventricular (LV) dilation and dysfunction. Exercise, through increased vascular shear stress, potentiates a powerful antigenic stimulus. We investigated whether exercise could promote cardiac angiogenesis in post-MI rats. MI was surgically induced on 7-wk-old Sprague-Dawley rats by ligation of the coronary artery. The survivors were assigned to 3 groups (n=10/group): Sham (no MI, no exercise), MISed (MI, no exercise), and MIEx (MI + exercise). Treadmill exercise training began 1-wk post-MI and lasted for 8-wks. Cardiac tissue was harvested at the end of the experiment and capillary density was assessed using CD-31 staining. Our results indicated that left ventricular (LV) capillary density (counts/mm²) of Sham and MIEx groups were significantly higher compared to the MISed group (1280 ± 66 counts/mm², 950 ± 47, 610 ± 44, respectively, p < 0.05). In the septum, capillary density was also higher in the MIEx group compared to MISed (1220 ± 54 vs. 890 ± 43 counts/mm²). Conclusion: Our data indicate that post-MI exercise training at a moderate intensity significantly increases cardiac angiogenesis, which in turn may attribute to the improvement in morbidity and mortality produced by exercise training in patients with MI.

Keywords: myocardial infarction; exercise training; angiogenesis; animal model;

Introduction

Cardiovascular disease remains the leading cause of morbidity and premature mortality despite advances in medical therapy, such as percutaneous coronary interventions and surgical revascularization [14]. After myocardial infarction (MI), the adequate growth of new capillaries and arterioles, or angiogenesis, represents a critical process in the development of compensatory hypertrophy in the remaining non-infarcted myocardium [2]. Although compensatory angiogenesis can be observed in both the ischemic and infarcted heart, previous studies have demonstrated that angiogenesis may be inadequate [12]; in fact, recent evidence suggests that impaired angiogenesis may lead to maladaptive left ventricular (LV) remodeling, promoting the transition from adaptive cardiac hypertrophy to LV dilation and dysfunction [18].

Exercise, through increased vascular shear stress, potentiates a powerful angiogenic stimulus [5]. In this regard, a number of studies clearly demonstrate that exercise activates vascular endothelial growth factor (VEGF) dependent antigenic pathways [17, 3, 10, 11] which represent critical molecular mechanisms by which exercise triggers angiogenesis [11]. Previous studies by our group and others have demonstrated that exercise training positively influences cardiac function and attenuates myocardial remodeling in rats with MI or congestive heart failure [11, 7, 1, 19] in addition, exercise-induced up regulation of VEGF in patients with heart failure has also been documented [9]. To date, however, few studies have examined the effects of exercise-induced angiogenesis in the infarcted myocardium; therefore, the purpose of this study was to investigate the effects of exercise training on cardiac angiogenesis in post-MI rats.

Methods and Materials

Animal Preparation

Seven-week-old (185-200 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) were treated in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (ILAR XXXX), and study protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas at San Antonio. To ensure the rats were accustomed to running, they were trained on a rodent treadmill at 10 to 16 m/min, 5 min/day for one week prior to surgery. MI was surgically induced by ligation of the left anterior descending coronary artery as described previously [25].

One week after surgery, the surviving rats were matched with cardiac function (Fractional Shortening) determined by echocardiography measurement and randomly assigned to three experimental groups (n=10/group): a sham-operated control (Sham), a sedentary group with MI (MI-Sed) and an exercise group with MI (MI-Ex). The MI-Ex group started exercising one week post-MI using a motorized rodent treadmill, while the Sham and MI-Sed groups remained sedentary throughout the entire experiment. To allow gradual adaptation to exercise stress, training was initiated at 10 m/min; 5° incline for 10 min per session. The speed and duration were gradually increased to 16 m/min and 50 min per session (including a 5-min warm-up at 10 m/min) and maintained constant throughout the experiment.

The exercise training was performed 5 days per week for 8 weeks. The determination of treadmill speed and exercise duration was based on the previous studies [20, 4, 25]. This exercise regimen was well tolerated by rats with MI. There were no mortalities during the 8 weeks of exercise training.

Cardiac Tissue Collection

Rats were anesthetized forty-eight hours after the last exercise session. The hearts were quickly harvested and rinsed in cold saline. The myocardial tissue of the non-infarcted left ventricle (LV) was collected and immediately frozen in is pentane with dry ice. Tissues and serum were stored at -80° C until use.

Infarct size determination

Six-micrometer thick sections of the heart were cut and stained with Masson’s trichrome. Infarct size was calculated by dividing the sum of the plan metered endocardial and epicardial circumferences of the infarcted area by the sum of the total epicardial and endocardial circumferences of the LV [25]. Total epicardial and endocardial lengths occupied by the infarct as identified by Masson’s trichrome staining was measured using Image Pro Plus program (Media Cybernetics, Silver Spring, MD).

Capillary Density

In brief, the protocol for determining capillary density was a two-stage immunohistochemical process involving marking for CD-31 and light microscopy quantification. After fixation, 6µm transverse cross-sections were incubated at 4° C overnight with primary antibody (1:20 dilution) anti-CD-31 antibody (BD #550300) in a humid chamber. After rinsing off the primary anti-body, a secondary anti-body enhancement kit (DAKO #K0609) with a biotinylated link and streptavidin-HRP was applied according to manufactures’ instructions. Stains were then developed via diaminobenzidine (DAB) table sets (Sigma D-4293). After dehydration and mounting of the slides, images were obtained using an inverted Olympus Q-Fire side mounted digital camera. The capillary density was determined by counting capillaries from 3 equally spaced images of the LV wall (remote area of the LV for the MISed and MIEx groups), 3 equally spaced images of the intraventricular septum, and 3 equally spaced images of the right ventricle of the myocardium cross-section using Image Pro Plus software (ver. 5, Media Cybernetics). Using a point to count technique with Photoshop (Adobe ver. 10), results was expressed as counts/mm² of myocardium. Capillaries were defined as < 10µm in diameter. A stage micrometer was used to standardize the microscope, the imaging software, and the measurement area within the counting software as adapted from previous studies.

Statistical Analysis

Values are expressed as mean ± standard error of mean (SEM). The group means were compared with one-way analysis of variance (ANOVA) and significant F ratios was followed by Student-Newman-Keuls post hoc comparisons. A P value of less than 0.05 was considered statistically significant.

Results

General Characteristics

Following ligation, there was a ~50% mortality rate with all the deaths occurring after the procedure. The exercise protocol was well tolerated and no deaths were observed throughout the treatment period of the study. The general characteristics for the study population are summarized in table 1. Infarct percentages among the MIEx and MISed groups were comparable. Total heart weight was significantly higher in the MIEx and MISed groups compared to the Sham group (1.46 ± 0.04 g, 1.42 ± 0.05 vs. 1.14 ± 0.04, respectively, p < 0.05). Similarly, heart to body weight ratios were also significantly higher in the MIEx and MISed groups compared to the Sham group (3.65 ± 0.07 g, 3.58 ± 0.2 vs. 3.24 ± 0.1).

Unsuccessful PCI procedure related complications included

Table 1: General Characteristics

	Infarct Size (%)	BW (g)	Ht Wt (g)	Ht Wt (g)/ BW (kg)
Sham	±	352 ± 3.4*	1.14 ± 0.04*	3.24 ± 0.1*
MI-Ex	37.5 ± 2.6	400 ± 9.3	1.46 ± 0.04	3.65 ± 0.07
MI-Sed	33.7 ± 2.4	398 ± 7.8	1.42 ± 0.05	3.58 ± 0.2

Values are expressed as mean + SEM. *P < 0.05 Sham versus the MI-Ex and MI-Sed groups.

Capillary Density

Figure 4 shows the representative images of the immunohistochemical CD-31 staining of LV capillaries, which were visualized using a light microscope at 20 x magnifications and appear brown in color. Our results indicated that capillary density in the septum (Figure 1) was significantly higher in the MIEx group compared to MISed (1220 ± 54 vs. 890 ± 43 counts/mm², p < 0.05). Furthermore, LV capillary density (Figure 3) of Sham and MIEx groups were significantly higher compared to the MISed group (1280 ± 66 counts/mm², 950 ± 47, 610 ± 44, respectively, p < 0.05), thus indicating that exercise significantly enhanced cardiac angiogenesis in the septum and LV nine weeks after MI. Such effects were not observed in the right ventricle (Figure 2).

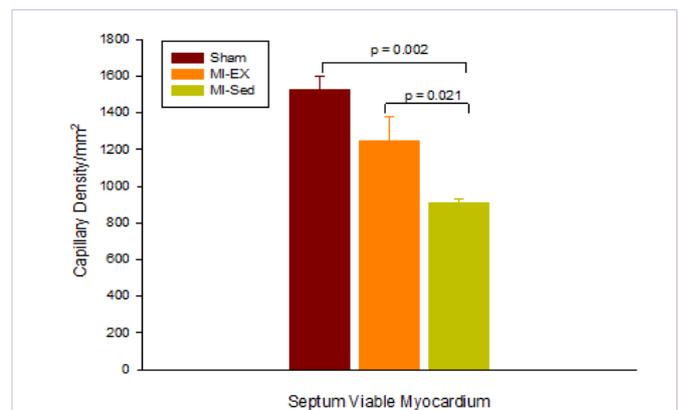


Figure 1: Immunohistochemical staining (CD 31) results of septum capillary density.

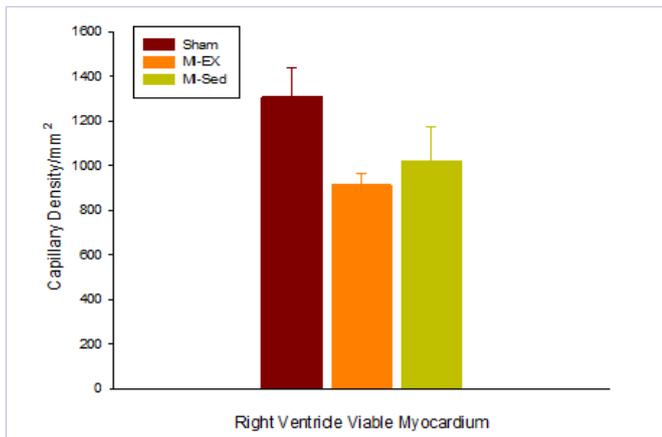


Figure 2: Immunohistochemical (CD 31) results of right ventricle capillary density

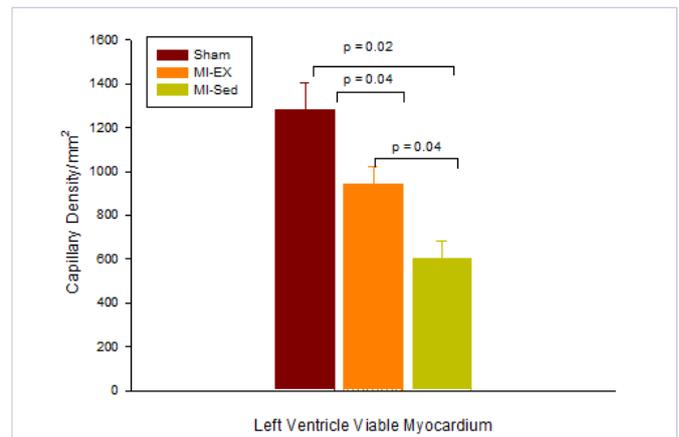


Figure 3: Immunohistochemical (CD 31) results of left ventricle capillary density

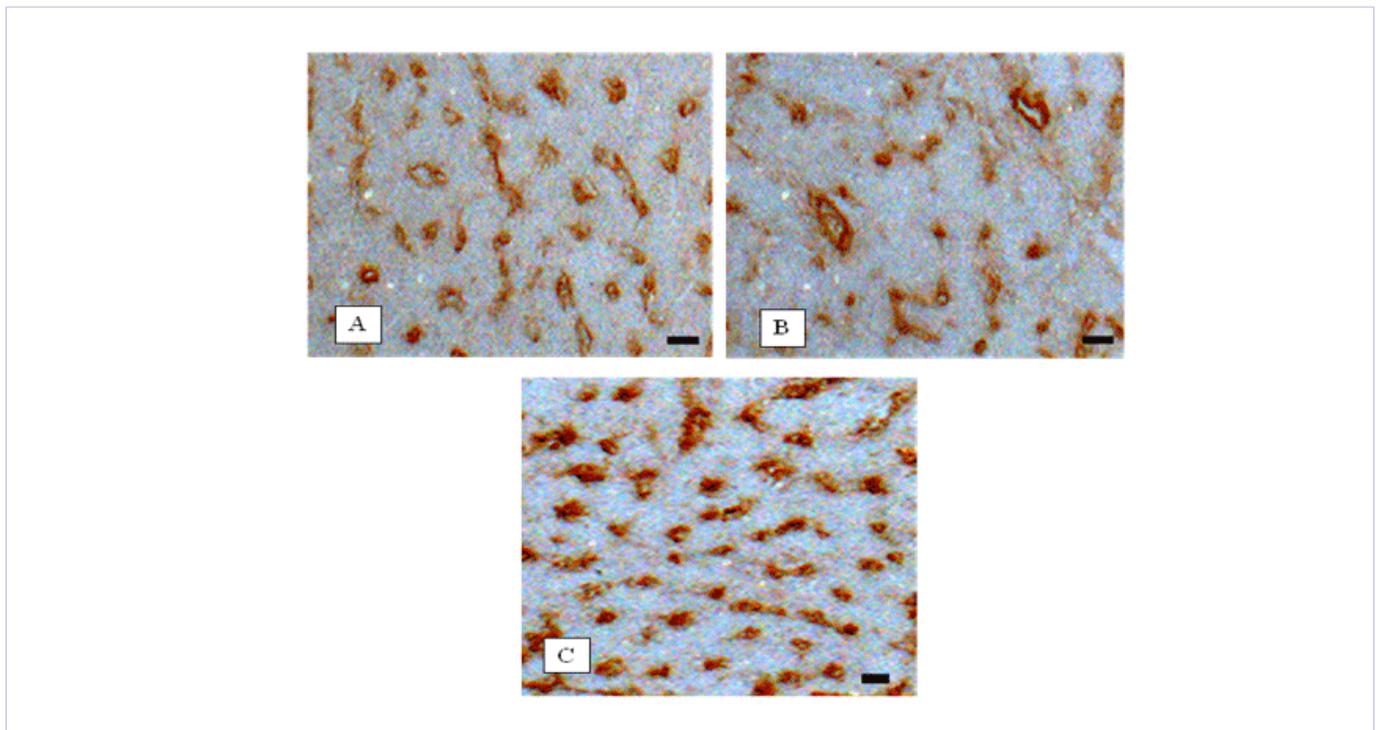


Figure 4: Representative image of the immunohistochemical CD 31 staining of LV capillaries, which appear brown in color: (A) MI-Ex (B) MI-Sed, and (C) Sham. Capillaries are visualized using a light microscope at 20x magnification. The bar in the photomicrographs indicates 10 μm.

Discussion

In a rat model of severe MI, we demonstrated that exercise training significantly increases capillary density in both the LV and septum of the viable myocardium. Exercise-induced angiogenesis such as that observed in our study may enhance myocardial blood perfusion and improve cardiac functioning in the infarcted heart. These data provide further insights into the mechanisms underlying the improvement in morbidity and mortality produced by exercise training in patients with MI.

Our results confirm previous evidence showing that low to moderate intensity treadmill exercise started late after MI

beneficially impacts cardiac function and attenuates adverse remodeling [16, 23]. Contrarily, training programs consisting of higher exercise intensities, and started early after MI have reported detrimental effects on LV geometry and mortality in rats [13, 6]. The use of endurance swimming, which elicits higher mental and hemodynamic stresses compared with treadmill exercise, in addition to increased training frequency (90 min/d, 6 d/wk) strongly differentiates these previous exercise protocols from that used in the present investigation, and may account for the negative effects of exercise in HF animals observed by these authors [6].

In the present study, our results illustrated that MI-Ex capillary density in the septum and left ventricle was 1.4-fold higher (Figure 1) and 1.6-fold higher (Figure 3) than in MI-Sed (1220 ± 54 vs. 890 ± 43 , and 950 ± 47 vs. 610 ± 44), suggesting that exercise promotes capillary growth in non-infarcted areas of severely decompensate hearts. Similarly, a study conducted by Leosco et al. reported that exercise induced a significant increase of capillary density in lateral border and remote zones of LV in HFEX hearts, but not in the LV anterior wall, which was largely involved in the infarcted area [15]. Although the pro-antigenic effect of exercise has previously been demonstrated in healthy swine hearts [22], data from the present study, in addition to the work of Leosco and colleagues, represents the only literature on exercise-dependent enhanced cardiac angiogenesis after MI. Our results showed that there was no significant difference in the ratio of heart weight to body weight regardless of the higher capillary density observed in the exercised trained LV. Although angiogenesis is contributed to hypertrophy of the heart [2]. Post-MI cardiac hypertrophy is mainly caused by angiotensin II mediated cell proliferation [8]. Our previous studies [21, 24] demonstrated that post-MI exercise training attenuated both plasma and cardiac tissue angiotensin II. This may explain why the heart weight to body weight ratio in the MI-EX group did not differ from their sedentary counterpart.

In conclusion, moderate intensity exercise training significantly increases capillary density in both the LV and septum of the viable myocardium. In this regard, exercise induced angiogenesis has the potential to impact numerous clinical conditions involving the ischemic and infarcted myocardium.

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