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ORIGINAL ARTICLE

Effects of miR-29a and miR-101a Expression on Myocardial Interstitial Collagen Generation After Aerobic Exercise in Myocardial-infarcted Rats

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Background and Aims. Myocardial infarction (MI) is accompanied by increased collagen deposition, cell necrosis and angiogenesis in cardiac tissue, which results in reduced ventricular compliance. Both microRNA-29a (miR-29a) and microRNA-101a (miR-101a) target the mRNAs encoding collagens and other proteins involved in fibrosis.

Methods. We assessed the effects of intermittent aerobic exercise on the expression of cardiac miR-29a and miR-101a and following effects on the TGFβ, *fos*, Smad2/3, COL1A1 and COL3A1 in MI model of rats. Intermittent aerobic exercise for MI rats was begun from the second week and ended at the ninth week postsurgery. Expressions of microRNAs (miRNAs) and fibrosis-associated genes were detected from the infarction adjacent region located in the left ventricle. The heart coefficient (HC = heart weight/body weight) and hemodynamics assay were used to evaluate cardiac function level.

Results. Intermittent aerobic exercise inhibited myocardial interstitial collagen deposition and significantly improved cardiac function of MI rats. The results of real-time PCR and Western blot indicate that intermittent aerobic exercise enhanced the expression of miR-29a and miR-101a and inhibited TGFβ pathway in the MI rats.

Conclusions. Our results suggest that controlled intermittent aerobic exercise can inhibit TGFβ pathway via up-regulation to the expression of miR-29a and miR-101a and finally cause a reduced fibrosis and scar formation in cardiac tissue. We believe that controlled intermittent aerobic exercise is beneficial to the healing and discovery of damaged cardiac tissues and their function after MI. © 2017 IMSS. Published by Elsevier Inc.

Key Words: Myocardial infarction, Aerobic exercise, miRNA, Collagen, Myocardial fibrosis.

Introduction

Myocardial infarction (MI) caused by coronary artery occlusion is a major cause of morbidity and mortality worldwide. Although most patients survive the acute phase of MI because of intense therapy, it may gradually develop into chronic heart failure as a consequence of post-infarct cardiac remodeling. Interstitial fibrosis occurring in both infarcted and adjacent myocardium represents a characteristic pathological alteration of post-infarct remodeling and is regarded as a major determinant of the progressive deterioration of ventricular function and decreased ventricular compliance after MI (1–3). Fibrosis is characterized by the excessive deposition of the extracellular matrix (ECM) proteins such as collagen I, collagen III, fibronectins, fibrins, and elastin. Cardiac fibroblasts (CFs), accounting for 60–70% of human heart cells, are the main source of ECM production (4). During MI, CFs are excessively activated and produce excessive interstitial fibrosis (5). Therefore, great efforts have been devoted to this disease with the goal of exploring the molecular mechanisms and

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developing novel antifibrotic strategies to improve its therapy.

MicroRNAs (miRNAs) are endogenous small noncoding RNAs that anneal inexactly to complementary sequences in the 3′-untranslated regions (UTR) of target mRNAs to down-regulate protein expression. Growing evidence shows that miRNAs play critical roles in the pathogenesis of cardiovascular diseases, including cardiac hypertrophy (6–9), heart failure (10), arrhythmia (11), and cardiac injury (12,13). Notably, the regulation of several miRNAs was regarded to effect the development and progression of cardiac fibrosis based on the regulation to the excessive expression of some fibrosis-related proteins (14–19). Up-regulation of miR-21 provoked the development of fibrosis (16,19), whereas *in vitro* the overexpression of miR-29 (17), 133 (14,15,18), 30c (14) and 590 (15) suppressed fibrotic responses of CFs.

Exercise training is the most promised lifestyle that can be beneficial to cardiac function. Physiological adaptations to aerobic training consist of a set of morphological and functional adaptations to metabolism, circulation, and cardiac function (20–23). However, the mechanisms responsible for these beneficial effects of exercise training on myocardial remodeling and function improvement after MI remain to be explored and elucidated. In this study, we report that in the hearts of the MI rats with a controlled intermittent aerobic exercise, the expression of miR-29a and miR-101a were up-regulated and the expression of fibrotic proteins down-regulated. The alteration of miR-29a and miR-101a can attenuate cardiac interstitial fibrosis and improve left ventricular compliance of MI rats.

Materials and Methods

Animals

Male Sprague Dawley rats (180–230 g, 3 months old, $n = 52$) were provided by the Laboratory Animal Centre of Xi'an Jiaotong University. These studies were performed in accordance with the ''Guiding principles for research involving animals and human beings'' (24). All experimental protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Shaanxi Normal University.

Surgical Procedure

MI was induced by ligation of the left descending coronary artery under anesthesia (pentobarbital 30 mg/kg) (25). The coronary artery was ligated ~2.0 mm from its origin using a 6.0 silk suture (MI rats, $n = 28$). MI was confirmed by apparent S-T segment elevation in ECG and cyanosis of the myocardium. Sham-operated rats (SC rats, $n = 24$) that underwent operation without coronary artery ligation served as control group. Three rats died during the surgery and one died 12 h after surgery.

Aerobic Exercise Protocol

Seven days after surgery, rats were randomly assigned to either a sedentary or training group as follows: myocardial infarct-sedentary (MI, $n = 12$), sham-sedentary (S, $n = 12$), myocardial infarct-exercise (ME, $n = 12$) and sham-exercise (SE, $n = 12$). Rats in the ME and SE groups were submitted to 8 weeks of intermittent aerobic exercise (23) using a motorized rodent treadmill (DSPT-202, Li Tai Technology, Hangzhou, China). This exercise regimen was well tolerated by MI rats. There were no mortalities during the 8 weeks of exercise (Figure 1).

Hemodynamics Assay

At the end of the 8 weeks of training or sedentary behavior, rats were fasted for 24 h and anesthetized as mentioned above. A pressure transducer was inserted retrograde from the right common carotid artery to the LV cavity, and traditional intraventricular catheter recordings (Powerlab 8/30, ML 870, AD Instruments, Castle Hill, Australia) were performed to evaluate cardiac function. The following hemodynamic parameters were measured: LV systolic pressure (LVSP, mmHg), LV end-diastolic pressure (LVEDP, mmHg), and maximal positive and negative first derivative of LV pressure ($\pm dp/dt_{max}$). All rats were decapitated after hemodynamic measurements.

Cardiac Morphometry

Heart samples taken from the LV infarct border area were fixed in 4% paraformaldehyde for 24–48 h, embedded in paraffin and sectioned (5 μm thick) for histopathologic examination. The slices were stained with Masson's trichrome and were used to observe the construction of cardiac tissue in the infarct area of the LV. To evaluate the degree of fibrosis, the collagen volume fraction (CVF) was measured in ten fields for each LV section of Masson's trichrome staining. CVF (fibrosis area/total area of myocardium) values were calculated using IMAGE-PRO PLUS 6.0 (IPP 6.0, Media Cybernetics, Bethesda, MD).

Real-time PCR

After the experimental treatment, total RNA from cardiac tissues was isolated using Trizol reagent (Aidlab

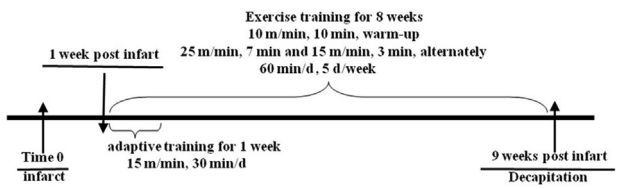

Figure 1. Aerobic exercise protocol. Heart coefficient measurement. The heart coefficient (HC) was calculated using the following formula: HC = heart weight (mg)/body weight (g).