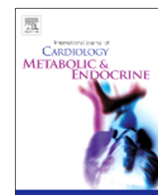
IJC Metabolic & Endocrine 13 (2016) 1–5

Contents lists available at ScienceDirect

IJC Metabolic & Endocrine

journal homepage: http://www.journals.elsevier.com/ijc-metabolic-and-endocrine



Aerobic exercise can ameliorate heart function in patients with myocardial infarction through up-regulating M3 receptor☆

Wang Chengji, Huang Shoujun *

College of Physical Education, Chaohu University, Hefei, Anhui Province,China

A R T I C L E I N F O

Article history:
Received 27 April 2016
Accepted 6 August 2016
Available online xxxx

Keywords:
M3 receptor
Myocardial infarction
Aerobic exercise
Cardiomyocyte apoptosis

A B S T R A C T

Objective cardioprotective effect and mechanism of exercise training up-regulating the expression of M3 receptor on myocardial infarction. Methods 48 male Sprague-Dawley rats were randomly assigned to three groups ($n = 16$, per group):control group (C), myocardial infarction group (MI), moderate-intensity aerobic exercise with myocardial infarction group (ME). Rats in C group were breed normally. MI was induced by ligation of the left anterior descending (LAD) coronary artery in MI group. Rats in ME group took treadmill exercise for 8 weeks. after 1 week post-operation. ME group running began at the speed of 10 m/min for 5 min, then accelerated from 3 m/min to 16 m/min. The total time of ME is 60 min, 5 d/week, for 8 weeks. LVSP, LVEDP, ±dp/dtmax and the cardiac function changes were measured after training. Myocardial collagen fibers were observed histological section and Masson staining. The expression of myocardial M3 R was observed and analyzed by immunofluorescence. The myocardial protein content of M3 R, $MEK_{1/2}$,P-$ERK_{1/2}$,$ERK_{1/2}$ and apoptosis related Bcl-2 and Bax was assayed by Western blot. Results Compared with the C group, MI increased CVF and LVEDP ($P < 0.01$), but decreased LVSP and −dp/dtmax ($P < 0.01$). After MI myocardial M3 positive staining, after MI M3 protein expression significantly higher compared with the C group ($P < 0.01$), $MEK_{1/2}$,P-$ERK_{1/2}$/$ERK_{1/2}$ protein expression were significantly increased compared with the C group ($P < 0.01$, $P < 0.01$), after the MI the Bcl-2/Bax expression significantly reduced compared with the C group ($P < 0.01$). ME group CVF%, LVEDP significantly reduced compared with the MI group ($P < 0.01$), but −dp/dtmax significantly increased ($P < 0.01$). ME group was identified myocardial M3, compared with the MI group, M3 protein expression significantly increased ($P < 0.01$), but Bcl-2/Bax expression significantly reduced ($P < 0.01$). Conclusions moderate-intensity aerobic exercise can up-regulated the M3 R-$MEK_{1/2}$-$ERK_{1/2}$ signaling pathway, thus inhibit the apoptosis of myocardial cells, reduced myocardial interstitial fibrosis and promote cardiac function after MI.

© 2016 Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In recent years, the research shows that aerobic exercise is one of the important methods to prevent and treat heart disease [1]. Continuous aerobic exercise can ameliorate heart function for patients with myocardial infarction (MI), improve the quality of life of patients with MI [2], but its mechanism is not fully understood.

One study found that high intensity intermittent exercise can improve the myocardial mitochondrial dysfunction in MI rats [3–4], and compared with the continuous aerobic exercise, it can also increase the VO_2max of heart patients more effectively [5], and can improve the muscle adaptive changes [6]. The two kinds of exercise can improve left ventricular ejection fraction [7]. Previous studies showed that distributed in the myocardial cell membrane of the Acetylcholine receptor (ACh R) was mainly M2 receptor subtype [8]. Recent studies have found that the M3 receptor (M3 R) subtype is also distributed in the myocardial cell membrane, and it plays a protective role in the development of chronic congestive heart failure, ischemic arrhythmia [9].

Some studies have reported that M3 R activation and through down-regulated I receptor of Angiotensin II (receptor-1 AT, 1R Angiotensin II) to reduce Ang II induced cardiac hypertrophy [10]. Up-regulation of M3 R can correct the cardiac hemodynamics dysfunction and inhibit myocardial cell apoptosis, and reduce the myocardial injury caused by ischemia. At present, there is no literature report about Characterization of M3 R and the protection effect on the heart in MI. This study intends

☆ These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author.
E-mail address: wcjch2008@126.com (H. Shoujun).

http://dx.doi.org/10.1016/j.ijcme.2016.08.001
2214-7624/© 2016 Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

to investigate whether exercise have protective effect on cardiac function in MI by up-regulated M3 R and its possible mechanism, and provide scientific basis for screening safe and effective rehabilitation program and therapeutic target of MI.

2. Materials and methods

2.1. Experimental animals and groups

Three-month-old thirty-six Sprague–Dawley rats (190–210 g) were purchased from the experimental animal centre of the Chinese People's Liberation Army (PLA) Military Academy of Medical Science (SCXK2007-004; Beijing, China). All animals were individually housed in a standard plastic cage using a 12:12-h reverse light cycle in a temperature- (18–23 °C) and humidity-controlled (50–60%) room and given food and water ad libitum. Animals were randomized into three groups: control group (C), myocardial infarction group (MI) and MI + aerobic exercise group (ME).

2.2. Preparation of myocardial infarction mode

The rats were anesthetized using 5% sodium (30 mg/kg, intraperitoneally). Small animal ventilator connected self-made assisted breathing mask (respiratory frequency was 66 times/min, tidal volume 10 mL, respiratory ratio 1:2). the electrocardiogram (ECG) was recorded by PowerLab/8 s. Open chest to expose the heart, at 2 mm the junction of the left atrial appendage lower edge and pulmonary arterial cone left edge, ligated the left anterior descending coronary artery (LAD) by 5/0 ligature, after ligation visible the left ventricular free wall near the apex of the myocardial color becomes shallow or white, S-T segment elevation or T wave inversion in EGC. So it is concluded that the MI model is successful. The layer by layer suture and exhaust gas, closing the chest.

2.3. Treadmill exercise protocol

According to the research of Xu et al. [11], the rats in ME groups were forced to run on a motorized treadmill. Starting 1-wk. after modeled successfully. Rats had a 1-wk. adaptation period (10 m/min,10 min/day for 5 days). After adaptation, the starting load was 10 m/min for 5 min. Then, the speed of treadmill running was increased 3 m/min each min until 16 m/min, at 0 degree of inclination. The 3 m/min speed gradually increased to 16 m/min, 5 d/week for 8 weeks, 60 min/times, 1 times/d (60%–70% VO_2max).

2.4. Measurement of hemodynamic index

On the next day after 8 week. training, all rats were anesthetized, the electrocardiogram (ECG) was recorded by PowerLab/8 s, moreover, the right common carotid artery intubation so that measured indexes of cardiac function, included left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), Left ventricular pressure maximum rise rate (+dp/dtmax) and maximum decrease rate (−dp/dtmax). After the data collection was completed, thoracotomy quickly and removed the heart. Then, carry out subsequent experimental.

2.5. Tissue preparation

After measured indexes of cardiac function, randomly selected 6 rats in each group, the heart was quickly removed and fixed with 10% neutral formalin for 24 h, rinse, gradient ethanol dehydration, xylene transparent, paraffin embedded, Coronal sections of 10 μm thickness were made with a freezing microtome, conventional Masson staining, observe and photograph with a microscope. In addition, quickly removed the heart of another 6 rats and embed with aluminum foil paper, froze with nitrogen, then stored at −80 °C for Western blot. Sections were deparaffinized and washing with water and PBS, after microwave antigen repaired, for immunofluorescence experiment. In wet box with normal goat serum block out 30 min at 37 °C incubated overnight with primary antibody (rabbit anti mouse M3 R polyclonal antibody, 1:200) at 4 °C. Rewarming 45 min at room temperature, washing with PBS and Dropping TRITC-labeling second antibody (1:200), incubated 1 h at 37 °C, then washing with PBS. Dropping DAPI and incubated 2 h in dark, washing with PBS and mounted sections. Set up the blank control (replace primary antibody and second antibody with PBS) and negative control (replace primary antibody with PBS) at each staining. Observation using fluorescence microscope, selection the position under low power lens, 400 times under the microscope camera. 6 paraffin sections were selected from each group and selected 10 fields from each section, calculated mean optical density (MOD).

2.6. Western blot analysis

Total protein extraction with RIPA buffer, protein concentration determination by the Bradford method. Protein of equivalent was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane, stain membrane with Ponceau S, after shock block out 60 min at room temperature, dropping polyclonal antibody included rabbit-anti M3 R (1:500), $MEK_{1/2}$ (1:1000), $ERK_{1/2}$ (1:1000), p-$ERK_{1/2}$ (1:1000), Bcl_{-2} (1:500) and Bax (1:100), overnight at 4 °C. Washing membrane at room temperature, incubated 30 min with sheep anti rabbit IgG antibody, washing membrane at room temperature, visualized using the enhanced chemiluminescence (ECL). GAPDH as an internal reference, calculated band integral optical density (Integral Optical, IOD) of target protein and internal reference protein.

2.7. Data analysis

Tissue sections were observed through a light microscope, measurement and analysis using Image-Pro Plus software. Using Image QuantTLv 2005 software analysis and processing for films of Western blot, using GraphPad Prism 6.0 software conversion data and plotting. For all data, the mean and standard deviation were calculated using SPSS for Windows ver. 17.0 (SPSS, Chicago, IL, USA). All data are presented as means ± standard deviation and analyzed using one-way analysis of variance (ANOVA). Significance was accepted at the $P < 0.05$ level, extremely significance was accepted at the $P < 0.01$ level.

3. Results

3.1. The results of characterization for R M3 in myocardial cells

Immunofluorescence staining showed that R M3 positive staining was red fluorescent particles. The positive fluorescent particles of R M3 in C group were rare. After MI, the red fluorescent particles of R M3 with point distributed, and compared with C group, that of in MI group was significantly increased ($P < 0.01$). Compared with MI group, the red fluorescent particles of R M3 in ME significantly increased ($P < 0.01$) (Fig. 1).

3.2. $MEK_{1/2}$-$ERK_{1/2}$ pathway and Bcl_{-2}/Bax protein expression in myocardial cells

Western blot results show that, compared with C group, the expression of $MEK_{1/2}$-$ERK_{1/2}$ and $ERK_{1/2}$ in myocardial cells of MI group were significantly increased ($P < 0.01$). Compared with MI group, that of in ME group were significantly increased ($P < 0.01$) (Fig. 2). The Bcl_{-2}/Bax/ratio in myocardial cells of MI group was significantly lower than that of in C group ($P < 0.01$), compared with MI group, that of in ME group were significantly increased ($P < 0.05$) (Fig. 2). The results showed that the $MEK_{1/2}$-$ERK_{1/2}$ pathway in

Download English Version:

https://daneshyari.com/en/article/2927159

Download Persian Version:

https://daneshyari.com/article/2927159

Daneshyari.com