Effect of Moxibustion on Cardiac Remodeling and Myocardial Function in Rats with Exercise-Induced Fatigue

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Abstract

Objective: To explore the effect of moxibustion at Shenque(CV8) on myocardial structure and function in rats with exercise-induced fatigue.

Methods: A 12-week treadmill training program was used to establish a rat model of exercise-induced fatigue. Fifty-six male SD rats removed six rats that did not reach the molding condition. Remaining rats were randomly divided into the following five groups: a normal group (n=10) that did not under go the exercise routine and were not treated, a control group (n=10) that did not under go the exercise routine, and received a mild dose of moxibustion at “Shenque” (CV 8) for 15 min, an untreated group (n=10) that received no treatment after exercise, a CV 8 group (n=10) that received a mild dose of moxibustion at “Shenque” (CV 8) for 15 min after exercise, and a non-acupoint(tail) group(n=10) that received a mild dose of moxibustion at “non-acupoint” for 15 min after exercise. At one hour after the end of the 12-week training program, the left ventricular diastolic volume (LVDV), left ventricular systolic volume (LVSV), peak early diastolic mitral blood flow velocity (E), and peak late diastolic mitral blood flow velocity (A) were measured, and the E/A ratio were calculated. The serum myoglobin (Mb), creatine kinase-muscle/brain (CK-MB), and cardiac troponin-I (cTnI) levels were detected using an automatic biochemical analyzer.

Results: When the values obtained before and after treatment were compared within the same groups, the LVDV, LVSV, E, and A were increased (P<0.05 or P<0.01), and the E/A were decreased (P<0.01) in the untreated group and the tail group. Compared to the untreated group and the tail group, the serum Mb, CK-MB, and cTnI levels were increased (P<0.05 or P<0.01), and the E/A were decreased (P<0.01) in the untreated group and the tail group compared to the normal group and control group. Compared to the untreated group and the tail group, the LVDV, LVSV, E, and A were decreased (P<0.05) in the untreated group and the tail group compared to the normal group and control group. Compared to the untreated group and the tail group, the serum Mb, CK-MB, and cTnI levels were increased (P<0.01) in the untreated group and the tail group, and the serum Mb and CK-MB levels were also increased (P<0.01) in the CV 8 group. Compared to the untreated group and the tail group the serum Mb, CK-MB, and cTnI levels in the CV 8 group were decreased (P<0.01). Conclusions: Moxibustion at Shenque(CV8) can effectively prevent cardiac structural changes caused by exercise-induced fatigue and enhance heart function. This treatment does not have side effects in healthy rats and is a safe and effective technique.

Keywords: Cardiac remodeling, exercise-induced fatigue, moxibustion, myocardial function, rats, Shenque (CV8)

Introduction

Cardiac remodeling is defined as abnormal changes in molecules, cells, and the extracellular matrix in myocardial tissue that cause changes in the size and function of the left ventricle. Changes in the myocardial structure are the pathological basis of arrhythmia.[1] Exercise-induced arrhythmia is an important issue in the fields of sports medicine and sports science and affects the stamina, health, regular training, and competition of athletes. It can restrict the performance and improvement of some elite athletes and even force them to withdraw from the competition or retire. The incidence of exercise-induced arrhythmias in endurance athletes is as high as 100%. [2] The occurrence of exercise-induced arrhythmia is related to pathological changes...
in the structure of the myocardium caused by long-term and repeated high-intensity exercise\cite{31} therefore, measures that effectively prevent the occurrence of cardiac remodeling may reduce the incidence of exercise-induced arrhythmia in athletes and sports enthusiasts. Due to the irreversibility of cardiac remodeling, symptomatic treatment with drugs is the mainstay of clinical management, but most athletes are reluctant to receive treatment because of the fear of drug-induced excitability. Previous experiments have confirmed that moxibustion at the Shenque (CV 8) acupoint inhibits exercise-induced fatigue by regulating the central and peripheral nervous systems\cite{4-7}. Additionally, moxibustion at Shenque (CV 8) regulates myocardial oxygen free radical production and the antioxidant capacity in rats with exercise-induced fatigue and reduces myocardial damage\cite{8}. This study will evaluate the therapeutic efficacy of moxibustion at Shenque (CV 8) on myocardial structure and function in a model of exercise-induced fatigue by measuring serum myoglobin, creatine kinase-muscle/brain (CK-MB), and cardiac troponin-I (cTnI) levels, left ventricular diastolic volume (LVDv), left ventricular systolic volume (LVSv), peak early diastolic mitral blood flow velocity (E), peak late diastolic mitral blood flow velocity (A) and E/A. Moxibustion at Shenque (CV 8) is expected to be a method of physical therapy that not only effectively relieves exercise-induced fatigue but also prevents exercise-induced cardiac remodeling.

**METHODS**

**Animals and groups**

Fifty SPF male standard deviation (SD) rats, weighing 200 ± 20 g, were purchased from the Beijing Charles River Laboratories Animal Technology Co., Ltd., (License No. [SCXK (Beijing) 2012–0006], Beijing, China), and were randomly divided into 5 groups using the random number table method: a normal group, a control group, a untreated group, a Shenque (CV 8) group, and a non-acupoint (tail) group with 10 rats in each group. Rats were housed in sterile cages (5 rats per cage) at the Experimental Animal Center of Hebei University of Chinese Medicine. All animals had free diet access. All animal experiments followed the relevant regulations by the laboratory animal management at the Hebei University of Chinese Medicine (No. YXLL2018012).

**Main reagents and instruments**

The Mb kits (20190528, Nanjing Jiancheng Bioengineering Inc., China), CK-MB kits (20190527, Nanjing Jiancheng Bioengineering Inc., China), and cTnI kits (20190523, Nanjing Jiancheng Bioengineering Inc., China).

Moxa sticks (7 mm × 117 mm, Henan Nanyang Hanyi Moxibustion Technology Development Co., Ltd., China), VEVO 2100 small animal ultrasound system (VisualSonics Inc., Canada), and ChemI 530 automatic biochemical analyzer (Shenzhen Kubeier Biotechnology Co., Ltd., China).

**Model establishment**

After 7 days adaptive feeding, the exercise-induced fatigue model was established according to Literature.\cite{9} For all groups other than the normal group and the control group, rats in the other groups were trained for 12 weeks. Training 5 days per week and rest 2 days.

**Moxibustion treatment**

All rats were placed in a special moxibustion rat box\cite{10} (ZL201120193244.8, China) for 15 min.

Normal group: Rats did not undergo the exercise routine and were not treated.

Control group: Rats did not undergo the exercise routine but received a mild dose of moxibustion at “Shenque” (CV 8)\cite{11} for 15 min, with a total of 60 treatments.

Untreated group: Rats received no treatment after exercise, with a total of 60 times.

CV 8 group: Rats received a mild dose of moxibustion at “Shenque” (CV 8) for 15 min after exercise, with a total of 60 treatments.

Tail group: Rats received a mild dose of moxibustion at “nonacupoint”\cite{12} for 15 min after exercise, with a total of 60 treatments.

**Myocardial structure and function test**

Rats were lightly anesthetized with isoflurane and placed in a supine position on a heating plate (37°C) at 1 h after the end of the training sessions. After removing the hair on the left side of the chest, the animals were examined with small animal ultrasound system. The LVDv, LVSv, E, and A were measured, and the E/A ratio was calculated.

**Detection of serum MB, creatine kinase-muscle/brain, and troponin-I content**

Six hours after the end of the training sessions, the rats were anesthetized with 10% chloral hydrate (1 ml/100 g body weight). Immediately after anesthesia induction, 5 ml of blood were collected from the femoral artery and centrifuged at 3000 rpm for 10 min at a cold temperature. The supernatant was stored at −80°C until testing. Serum Mb, CK-MB, and cTnI levels were measured using an automatic biochemical analyzer.

All tests were determined by the Research Center of Hebei University of Chinese Medicine.

**Statistical analysis**

The SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measured data are presented as the mean ± SD, intergroup difference was analyzed through one-way analysis of variance, \( P < 0.05 \) showed a statistically significant difference.

**RESULTS**

Moxibustion at CV8 could improve left ventricular diastolic volume and left ventricular systolic volume in the heart of rats with exercise fatigue

As shown in Figure 1, when the values obtained before and
after treatment were compared with in each group, LVDv and LVSv were increased \((P < 0.01)\) in untreated group and tail group. In the inter-group comparison, LVDv and LVSv were increased \((P < 0.01)\) in untreated group and tail group compared to normal group and control group. Compared to untreated group and tail group, LVDv and LVSv were decreased \((P < 0.01)\) in CV 8 group.

**Moxibustion at CV8 could improve E, A, and E/A in the heart of rats with exercise fatigue**

As shown in Figure 2, when the values obtained before and after treatment were compared within each group, E and A were increased \((P<0.05\text{ or } P<0.01)\), and E/A were decreased \((P<0.01)\) in untreated group, tail group and CV 8 group. Regarding inter-group comparisons, E and A were increased \((P<0.01)\), and E/A were decreased \((P<0.05\text{ or } P<0.01)\) in untreated group and tail group compared to normal group and control group. Compared to untreated group and tail group, E and A were decreased \((P<0.01)\), and E/A were increased \((P<0.01)\) in CV 8 group.

**Moxibustion at CV8 could improve serum MB, CK-MB and cTnI in rats with exercise fatigue**

As shown in Figure 3, compared to normal group and control group, the serum MB, CK-MB, and cTnI levels were increased \((P<0.01)\) in untreated group and tail group, and the serum MB and CK-MB levels were also increased \((P<0.01)\) in CV 8 group. Compared to untreated group and tail group, the serum MB, CK-MB, and cTnI levels were decreased in CV 8 group \((P<0.01)\).

**Discussion**

A 12-week treadmill training program has been shown to lead to scattered, disordered, or even partially raptured myofibrils in the rat ventricular myocardium. These changes may promote the reactivation of fetal genes in parts of the myocardium, inactivation or mutation of genes in other parts of the myocardium, synthesis of regulatory proteins, and

![Figure 1: Comparison of cardiac morphology left ventricular diastolic volume and left ventricular systolic volume in each group. \(*P < 0.01\) compared to before treatment, \(\triangle P < 0.01\) compared to normal group, \(\blackloid P < 0.01\) compared to control group, \(\blacklozenge P < 0.01\) compared to untreated group, and \(\blacklozenge P < 0.01\) compared to tail group](image1)

![Figure 2: Comparison of cardiac function E, A and E/A in each group. \(*P < 0.01\) and \(** P < 0.05\) compared to before treatment, \(\triangle P < 0.01\) compared to normal group, \(\blackloid P < 0.01\) compared to control group, \(\blacklozenge P < 0.01\) compared to untreated group, and \(\blacklozenge P < 0.01\) compared to tail group](image2)

![Figure 3: Comparison of serum MB, CK-MB, and cTnI levels in each group. \(\triangle P < 0.01\) compared to normal group, \(\blackloid P < 0.01\) compared to control group, \(\blacklozenge P < 0.01\) compared to untreated group, and \(\blacklozenge P < 0.01\) compared to tail group](image3)
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The degree of myocardial damage caused by long-term exercise is relatively mild, and no large areas of myocardial necrosis in the early stage or characteristic electrocardiogram changes are observed. Currently, the dynamic detection of serum Mb and cTnI levels is the main method used to determine a clinical diagnosis of myocardial injury. Mb is an oxygen-binding heme protein that is present in the cytoplasm of the myocardium. After myocardial injury, Mb enters the blood and exhibits an early peak, but its specificity is poor. cTnI is a calcium-regulated protein that regulates the interaction between actin and myosin, and its myocardial specificity is much higher than that of other markers. Additionally, it is not affected by diseases of other organs, such as skeletal muscle. However, elevated serum cTnI levels are observed relatively late after myocardial injury. The combined measurement of Mb and cTnI levels is considered the "gold standard" for a clinical diagnosis of myocardial injury.

In the present study, serum MB, CK-MB, and cTnI levels in the model groups were increased compared to the blank groups, indicating that the 12-week treadmill training program resulted in cardiomyocyte injury in rats.

Compared to rats in the untreated group, the LVDv, LVSv, E, A, and serum MB, CK-MB, and cTnI levels were decreased and the E/A was increased in rats from the CV 8 group. Based on these results, moxibustion at CV 8 alleviates exercise-induced myocardial damage and effectively prevents cardiac remodeling. Additionally, no significant differences were observed between the rats from the tail group and the untreated group, indicating that moxibustion at nonacupoints does not exert a preventive effect. Furthermore, the control group did not exhibit any differences from the normal group, indicating that moxibustion at CV 8 does not exert obvious effects on the myocardial structure and function of healthy rats.

Conclusions
This study confirms that moxibustion at CV 8 effectively prevents changes in cardiac structure caused by exercise-induced fatigue and enhances heart function. This procedure does not affect healthy rats and is a safe and effective technique. Future studies will explore the possible mechanism of action of moxibustion at the Shenque acupoint from the perspective of the cardiac remodeling mechanism.

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Conflicts of interest
There are no conflicts of interest.

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