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Effects of non-invasive remote ischemic conditioning on rehabilitation after myocardial infarction

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ABSTRACT

Recent studies have demonstrated that remote ischemic conditioning (RIC) creates cardioprotection against ischemia/reperfusion injury and myocardial infarction (MI); however, the effects of non-invasive remote ischemic conditioning (nRIC) on prognosis and rehabilitation after MI (post-MI) remain unknown. We successfully established MI models involving healthy adult male Sprague-Dawley rats. The nRIC group repeatedly underwent 5 min of ischemia and 5 min of reperfusion in the left hind limb for three cycles every other day until weeks 4, 6, and 8 after MI. nRIC improved cardiac hemodynamic function and mitochondrial respiratory function through increasing myocardial levels of mitochondrial respiratory chain complexes I, II, III, IV, and adenosine triphosphate (ATP) and decreasing the activity of nitric oxide synthase (NOS). nRIC could inhibit cardiomyocytes apoptosis and reduce myocardium injury through raising the expression of Bcl-2 and reduced the content of creatine kinase-MB, cardiac troponin I and Bax. The results indicated that long-term nRIC could accelerate recovery and improve prognosis and rehabilitation in post-MI rats.

1. Introduction

According to the World Health Organization, cardiovascular disease remains the leading cause of death and disability worldwide, and myocardial infarction (MI) is a major contributor [1]. Rupture of coronary atherosclerosis plaque and thromboembolism resulting in a large area of myocardial ischemia and hypoxia are the main causes of MI [2]. The degree of ischemia-reperfusion (I/R) injury and infarct size, which are the primary causes of cardiac dysfunction, determine the prognosis and rehabilitation for post-MI patients.

In 1986, Mury et al. [3] first described the concept of ischemic preconditioning (IPrC) as multiple brief ischemic episodes that might actually protect the heart from a subsequent sustained ischemic insult. After that, it was confirmed that tissues or organs outside of the heart could protect the heart against sustained ischemic injury. This phenomenon was described as remote ischemic preconditioning (RIPrC) [4]. After studying invasive limb RIPrC, our preliminary research affirmed that non-invasive limb ischemic preconditioning (nLIPrC) could significantly reduce the infarct size in the myocardium, decrease the apoptosis rate, protect the heart against myocardial injury, and improve the cardiac function, which could achieve the equivalent level of protection of IPrC for the local cardiac coronary artery [5].

The inability of IPrC to predict the onset of ischemia limited its use as a clinical cardioprotective strategy to attenuate I/R injury in arrhythmia and infarction. Therefore, the methods of ischemic postconditioning (IPoC) [6] and remote ischemic postconditioning (RIPoC) [7] were created; these are repetitively applied during early reperfusion, i.e., postconditioning, and were cardioprotective by attenuating reperfusion injury. In 2007, Schmidt et al. proposed the term “remote ischemic perconditioning” (IPeC) [8].

Although IPrC, IPeC, IPoC, or RIC have shown potential for protecting the heart and other organs from injury due to lethal I/R injury, short durations of protection (first window of protection is only approximately 2–3 h) and insufficient effects (during the second window of protection) limited their potential clinical application. Long-term ischemic conditioning has become a popular research topic due to its long duration of protection and wide application compared to the short duration of ischemic conditioning or RIC. Inspired by ischemic conditioning in skeletal muscle

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and blood pressure (BP) measurements, we explored long-term non-invasive remote ischemic preconditioning (nRIC) in our laboratory [10]. Our preliminary study demonstrated that nRIC could protect the heart against I/R injury, preserve cardiac function, and prevent heart injury from MI. This experimental protocol was the most effective method for achieving maximal cardioprotection (13 different regimens were compared) [9]. nRIC was designed to extend the conditioning duration and to provide long-term cardioprotection; therefore, it is much more practical and has more clinical value.

The cardioprotection of ischemic conditioning or RIC against I/R injury have been confirmed by our study and other many studies. However, the effects of nRIC on the prognosis and rehabilitation for post-MI were unclear. Based on the theory of ischemic conditioning cardioprotection, we hypothesized that nRIC could improve the prognosis and accelerate the recovery period and rehabilitation after MI In this study we aimed to investigate whether nRIC accelerate recovery and improve prognosis and rehabilitation in post-MI rats, and explored the possible mechanism. Our study may provide a reference to improve clinical outcomes after MI.

2. Materials and methods

2.1. Animals

Healthy adult male Sprague-Dawley rats weighing 250 ± 10 g were purchased from the Experimental Animals Research Institute of the Military Medical Science Academy of China (SPF grade, SCXK-2012-0004). Rats were fed in a controlled laboratory environment. The experimental protocol was approved by the Laboratory Animal Care and Use Ethical Committee of Tianjin Medical University (Tianjin, China). Care for the animals was performed as required according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals in China.

2.2. Experimental models

2.2.1. Model of MI

General surgical preparation was performed according to the methods of Li et al. [5] and Gao et al. [9]. Rats were administered pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 45 mg/kg (intraperitoneal). Each animal's trachea was intubated and ventilated with an HX-300 animal respirator (Chengdu Technology and Market Co., Ltd., Chengdu, China). After left thoracotomy and pericardiotomy, the left anterior descending (LAD) artery was wrapped with a braided silk suture at the junction of the left atrial appendage (LAA) and pulmonary arterial cone at the lower edge of the 2-mm level of the LAA. When the ST-segment in the ECG was elevated, myocardial ischemia was confirmed. All rats had wound infections that were healed with penicillin sodium (20 U, intramuscular) after surgery. The model was successful after 2 weeks.

2.2.2. Model of nRIC

During this procedure, the rats were anesthetized with pentobarbital sodium (45 mg/kg, intraperitoneal). A non-invasive BP tester for animals (BL-420E biology functional experimental system; Taimeng Technology Co., Chengdu, China) was tied to the thigh of the left hind limb of the rat. A pulse sensor was placed on the dorsal artery of the foot. nRIC was repeated using 5 min of inflation in the left femoral artery and 5 min of deflation for 3 cycles every other day until weeks 4, 6, and 8 of the study [9].

2.3. Experimental protocol

The model rats were randomly divided into the following three groups (Fig. 1): MI group, nRIC group, and sham group.

The MI group comprised 24 rats. The LAD coronary artery was ligated under spontaneous breathing conditions. The model was successful 2 weeks after it began. Thereafter, the rats were fed routinely until weeks 4, 6, and 8 (8 rats per session).

The nRIC group comprised 24 rats. After the LAD was ligated 2 weeks later, rats were treated with 3 cycles of 5 min of ischemia and 5 min of reperfusion in the left hind limb every other day until weeks 4, 6, and 8 (8 rats per session).

The sham group (sham-operated group) comprised 24 rats. Threading was performed under the LAD, but the coronary artery was not ligated. After sham operation, rats were normally bred until weeks 4, 6, and 8 of the study (8 rats per session).

2.4. Hemodynamic measurements

At the end of weeks 4, 6, and 8 of the study, all rats were anesthetized and the right carotid artery was cannulated with a heparin-filled polyethylene catheter for BP monitoring. Each animal's trachea was intubated. After the heart rate and BP were stable, a catheter was continuously inserted toward the heart while observing the pressure curve. After stabilization for 10 min, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), +dp/dtmax, and -dp/dtmax were measured and electrocardiogram (ECG) was performed.

2.5. Assessment of mitochondrial respiratory chain complexes I, II, III, and IV and determination of adenosine triphosphate (ATP) and nitric oxide synthase (NOS) activity in the myocardium

Myocardial tissues from the left ventricular anterior wall were sampled at the end of weeks 4, 6, and 8. They were homogenized and centrifuged (4 °C, 3000 rpm, 10 min) to extract supernatants that were used to determine the content of the mitochondrial respiratory chain complexes I, II, III, and IV, NOS, and ATP. These were measured using an ELISA method that caused the supernatants to react with tested reagents in the detection kits (Hermes Criterion Biotechnology, Vancouver, Canada; performed according to the manufacturer's directions).

2.6. Assessment of apoptosis

Apoptosis was examined using a TUNEL assay (Apoptosis Detection Kit; Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. Myocardia were sampled from each group at the end of weeks 4, 6, and 8. Two slices from each rat were observed and five visual fields were selected randomly from the ischemic penumbra of each slice at ×400 magnification. The apoptosis index was expressed by the ratio of positive apoptotic cells and total cells.

2.7. Assay for B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated protein content (Bax) and levels of creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) in the serum

All rats were euthanized and whole blood was collected from the abdominal aorta. Serum was separated to measure the amount of Bcl-2, Bax, CK-MB, and cTnI. It was then centrifuged (3000 rpm, 15 min, 4 °C) and tested with an ELISA kit (Hermes Criterion Biotechnology) according to the manufacturer's instructions.

2.8. Statistical analysis

Values were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to determine the

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