Effect of acupuncture at Neiguan (PC 6) on cardiac function using echocardiography in myocardial ischemia rats induced by isoproterenol

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OBJECTIVE: To investigate the effect of acupuncture at Neiguan (PC 6) on cardiac function using echocardiography in rat models of myocardial ischemia (MI) induced by isoproterenol (ISO).

METHODS: Twenty-seven Sprague-Dawley rats were randomly assigned to normal, model, and acupuncture groups. The model and acupuncture groups were given injections of ISO (85 mg/kg) to establish the MI model. After model establishment, the acupuncture group was treated with acupuncture at Neiguan (PC 6) for 30 min. Echocardiography was used to monitor diastolic and systolic function for 30 min starting from the time after the acupuncture needles were removed. Changes in the length of left ventricular internal diameter at end-diastole (LVIDd), length of left ventricular internal diameter at end-systole (LVIDs), the ratio of mitral peak velocity at early diastole and atrial contraction (E/A), ejection fraction (EF), fractional shortening (FS), and stroke volume (SV) were measured.

RESULTS: Compared with the model group at 0 and 15 min after needles were removed, the means of LVIDd and LVIDs were significantly lower ($P < 0.01$) and E/A, EF, FS, and SV significantly higher ($P < 0.01$) in the acupuncture group. In the acupuncture group, the means of LVIDd and LVIDs 15 min after the needles were removed were significantly higher ($P < 0.01$) than those at 0 min. The means of E/A, EF, FS, and SV significantly decreased ($P < 0.01$) from 0 to 15 min in the acupuncture group.
CONCLUSION: These findings indicate that acupuncture at Neiguan (PC 6) can affect cardiac function by increasing left ventricular diastolic and systolic function in MI rat models, but the effect only lasts for 15 min.

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Key words: Acupuncture; Point PC 6 (Neiguan); Myocardial ischemia; Heart function tests; Echocardiography; Isoproterenol

INTRODUCTION
Myocardial ischemia (MI) is a condition commonly caused by some heart diseases. MI occurs when the myocardial blood perfusion is partially or completely obstructed. Reductions in myocardial blood perfusion can lead to hypoxia in the heart or myocardial energy metabolism disorders. Therefore, MI can cause myocardial cell dysfunction and myocardial cell necrosis. Some studies have shown that acupuncture treatment can improve cardiovascular and nervous system function. Echocardiography is a non-invasive diagnostic technique that provides information on cardiac function and hemodynamics. It is essential for assessments of left ventricular systolic and diastolic function and myocardial and coronary perfusion.

Studies on the mechanisms of acupuncture treatment for MI have investigated the central nervous system, cardiovascular active substances, anti-peroxidation compounds, monoamines, and cardiac enzymes. Previous studies on the mechanism of acupuncture in MI were focused on biochemistry and the neuroendocrine system. No studies have focused on acupuncture’s effect on cardiac function in MI rat models. We aimed to observe the effect of acupuncture at Neiguan (PC 6) on cardiac function in MI rat models induced by isoproterenol (ISO). Acupuncture at Neiguan (PC 6), located on the pericardium meridian, is often used to relieve MI symptoms in traditional Chinese medicine. Echocardiography was continually used to monitor the cardiac function of MI rats after acupuncture treatment until no significant response was observed in the echocardiography.

MATERIALS AND METHODS

Animals
Twenty-seven adult male specific pathogen free grade Sprague-Dawley rats [4 months old, (250 ± 10) g weight] were provided by the Experimental Animal Center of Liaoning University of Traditional Chinese Medicine (License number: SCXK [Liao] 2010-0001). During the experiment, animals were treated in accordance with the Guidance of National Institutes for the Care and Use of Laboratory Animals. Rats were randomly divided into three groups using a random number table: normal group (n = 9), model group (n = 9), and acupuncture group (n = 9). Rats were given 3 days to acclimatize to the environment ([18 ± 1] °C, 40%-50% relative humidity), and were provided with enough food and water before the experiment. The experiment was approved by the Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine.

Establishment of MI model
Rats were fixed in the supine position and given a subcutaneous injection of ISO (Sigma-Aldrich, St. Louis, MO, USA) (85 mg/kg) on the limb roots and back. After 24 h, rats were given a second injection. The MI model was verified by serum creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH) levels.

Acupuncture treatment
Rats in the acupuncture group were given acupuncture treatment on the second day after model establishment. According to the Animal Experimental Acupuncture Points Diagram designated by Association of Experimental Acupuncture Research (AEAR) of the National Association of Acupuncture (NAA), the Neiguan (PC 6) acupoint in rats was selected. The rats were fixed in the supine position and acupuncture needles (Suzhou Medical Appliance Co., Suzhou, China) 15 mm long and 0.35 mm in diameter were inserted 2-3 mm into the subcutis at an angle of 30 degrees. The needle was retained for 30 min.

Echocardiography determination
Rats were anesthetized with isoflurane inhalation. The hairs on their chest were removed with depilatory paste (Strep Co., Milan, Italy). Then, rats were fixed in the supine position on the scanning platform of a high-resolution ultrasound system with a 40 MHz transducer (S-Sharp Corporation, Taiwan). The chest of each rat was covered with a layer of ultrasonic coupling agent. M-mode echocardiography was performed to measure cardiac parameters. The measured cardiac parameters were: length of left ventricular internal diameter at end-diastole (LVIDd), length of left ventricular internal diameter at end-systole (LVIDs), ratio of mitral peak velocity during early diastole and atrial contraction (E/A), ratio of stroke volume at left ventricular end diastolic volume (ejection fraction, EF), (LVIDd - LVIDs) / LVIDs (fractional shortening, FS), and the ejection volume of a ventricle on one heart beat (stroke volume, SV). Rats in the acupuncture group were recorded before and after acupuncture at 0 min (exactly after the acupuncture needles were removed) and 15 min (after needles were removed).
**Measuring CK-MB and LDH contents**

After recording the echocardiography data, all rats were sacrificed with 10% chloral hydrate (Shanghai Enzyme Biotechnology Co., Shanghai, China). Blood samples were collected for further testing. CK-MB and LDH contents were assayed with an automatic biochemical analyzer.

**Statistical analysis**

Experimental data were analyzed with SPSS 21.0 (IBM Corp., Armonk, NY, USA). Data are expressed as mean ± standard deviation (\( \bar{x} \pm s \)). One-way analysis of variance was used to compare differences between the groups. \( P < 0.01 \) was considered statistically significant.

**RESULTS**

**CK-MB and LDH concentration in MI rats**

Compared with the rats in the normal group, the serum CK-MB and LDH levels were significantly higher after ISO injection indicating successful model establishment \( (P < 0.01, \text{Figure 1}) \).

**LVIDd and E/A**

In echocardiography, LVIDd and E/A are parameters that commonly represent the diastolic function of the left ventricle.\(^{11}\) Compared with the normal group, the LVIDd was significantly higher and E/A significantly lower in the model and acupuncture groups \( (P < 0.01) \). Compared with the model group, the LVIDd was significantly lower in the acupuncture group, and E/A was significantly higher at 0 and 15 min \( (P < 0.01) \) after acupuncture treatment. In the acupuncture group, LVIDd significantly increased while E/A significantly decreased from 0 to 15 min \( (P < 0.01, \text{Figure 2-3}) \). The findings suggest that acupuncture at Neiguan has a significant effect on left ventricular diastolic function immediately after acupuncture. This effect lasted for 15 min and decreased afterwards.

**LVIDs, EF, FS, and SV**

Compared with the normal group, LVIDs was significantly higher and EF, FS, and SV were significantly lower in the model and acupuncture groups \( (P < 0.01) \). Compared with the model group, LVIDs was significantly lower in the acupuncture group at 0 min \( (P < 0.01) \), and EF, FS, and SV were significantly higher in the acupuncture group 15 min \( (P < 0.01) \) after acupuncture. In the acupuncture group, LVIDs significantly increased and EF, FS, and SV significantly decreased 15 min after acupuncture \( (P < 0.01, \text{Figure 4}) \).

**DISCUSSION**

Some clinical studies have shown that acupuncture at Neiguan (PC 6) can alleviate MI symptoms. Neiguan acupuncture can decrease heart rate, lower blood pres-
Figure 3 LVIDd and E/A ratios in MI rats
A: LVIDd of each group; B: E/A ratios of each group. Normal group was given no treatment (n = 9); model group was subcutaneously injected isoproterenol (ISO) at a daily dose of 85 mg/kg for 2 days to establish the myocardial ischemia models (n = 9); 0 min: acupuncture group received subcutaneously injection of ISO at a daily dose of 85 mg/kg for 2 days and acupuncture treatment for 30 min, then was determined by echocardiography at 0 min after acupuncture treatment (exactly after the acupuncture needles were removed) (n = 9); 15 min: acupuncture group received subcutaneously injection of ISO at a daily dose of 85 mg/kg for 2 days and acupuncture treatment for 30 min, then was determined by echocardiography at 15 min after acupuncture treatment (at 15 min after needles were removed) (n = 9). LVIDd: length of left ventricular internal diameter at end-diastole; E/A: ratio of mitral peak velocity during early diastole and atrial contraction; MI: myocardial ischemia. *P < 0.01, compared with the normal group; **P < 0.01, compared with the model group; ***P < 0.01, compared with the 15 min group.

Figure 4 LVIDs, EF, FS, and SV in MI rats
A: LVIDs of each group; B: EF of each group; C: FS of each group; D: SV of each group. Normal group was given no treatment (n = 9); model group was subcutaneously injected isoproterenol (ISO) at a daily dose of 85 mg/kg for 2 days to establish the myocardial ischemia models (n = 9); 0 min: acupuncture group received subcutaneously injection of ISO at a daily dose of 85 mg/kg for 2 days and acupuncture treatment for 30 min, then was determined by echocardiography at 0 min after acupuncture treatment (exactly after the acupuncture needles were removed) (n = 9); 15 min: acupuncture group received subcutaneously injection of ISO at a daily dose of 85 mg/kg for 2 days and acupuncture treatment for 30 min, then was determined by echocardiography at 15 min after acupuncture treatment (at 15 min after needles were removed) (n = 9). LVIDs: length of left ventricular internal diameter at end-systole; EF: ejection fraction; SV: stroke volume; MI: myocardial ischemia. *P < 0.01, compared with the normal group; **P < 0.01, compared with the model group; ***P < 0.01, compared with the 15 min group.
sure, reduce oxygen consumption, increase coronary blood flow, and improve hemorrheology.15,16 Animal experiments found that electroacupuncture at Neiguan (PC 6) can regulate the median nerve, the spinal dorsal horn neurons of C3-T3, and the nucleus of the solitary tract in the brain stem. Electroacupuncture at Neiguan (PC 6) can also adjust vasoactive substances, such as thromboxane B2, prostacyclin I2, endothelin, and calcitonin gene-related peptide.16,17 The content of CK-MB and LDH in the blood can reflect myocardial cell injury and has been used to diagnose MI.18 This experiment showed that the contents of plasma CK-MB and LDH were significantly higher in the model group rats and acupuncture group rats compared with normal rats (P < 0.01). These higher levels indicate a successful MI model establishment.

After MI occurs, the contractile and diastolic forces of the heart, especially in the region of the MI, can significantly decrease. These changes are caused by ischemia in the ventricular walls, which limits movement.19 Previous studies showed that the prognosis of MI does not depend on the presence or absence of symptoms, but depends on lesion severity and left ventricular function.20 Therefore, left ventricular function is crucial to evaluating treating effect.

Echocardiography is a useful tool for measuring cardiac function. In this study, echocardiography was used to assess changes in left ventricular function after acupuncture at Neiguan (PC 6).

To evaluate the diastolic function of the left ventricle, LVIDd and E/A were measured. We found that acupuncture at Neiguan (PC 6) could effectively inhibit the elongation of LVIDd and increase E/A to improve left ventricular diastolic function. This effect is more significant immediately after treatment than 15 min after acupuncture.

To assess the systolic function of the left ventricle, LVIDs, EF, FS, and SV were measured. We found that acupuncture at Neiguan (PC 6) could effectively inhibit it the elongation of LVIDs and increase EF, FS, and SV to improve left ventricular systolic function. This effect is more significant immediately after treatment than at 15 min after acupuncture.

The Neiguan (PC 6) acupoint is located on the pericardium meridian. Since the pericardium meridian passes through the heart, PC6 is theorized to be connected to the heart. Acupuncture at Neiguan (PC 6) could exert its effect on the heart via the pericardium meridian, which reflects the meridian targeting effect of acupuncture treatment.

In conclusion, acupuncture at Neiguan (PC 6) could significantly improve echocardiogram parameters, including LVIDd, LVIDs, E/A, EF, FS, and SV. Our results suggest that acupuncture at Neiguan (PC 6) could significantly regulate cardiac function in MI rat models during treatment and 15 min after needles are removed.

REFERENCES

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