

## EXPERIMENTAL STUDY

**Effect of electronic stimulation at Neiguan (PC 6) acupoint on gene expression of adenosine triphosphate-sensitive potassium channel and protein kinases in rats with myocardial ischemia**

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**Wang Wei, Li Jiquan, Meng Xiangyu, Chen Yiguo**, College of Acupuncture and Massage, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China**Supported by** National Basic Research Program of China (973 Program), Study on Biological Foundation of Response in Target Organ to Meridian Specificity of Acupoint Effect (No. 2012CB518503)**Correspondence to: Prof. Chen Yiguo**, College of Acupuncture and Massage, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China. [cyg@lnutcm.edu.cn](mailto:cyg@lnutcm.edu.cn); **Prof. Wang Wei**, College of Acupuncture and Massage, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China. [wangwei425@126.com](mailto:wangwei425@126.com)**Telephone:** +86-15804090562**Accepted:** February 12, 2015**Abstract****OBJECTIVE:** To investigate the effects of electronic stimulation at acupoints Neiguan (PC 6) and Lieque (LU 7) on the gene expression of the adenosine triphosphate (ATP)-Sensitive potassium channel (KATP: Kir6.1, Kir6.2, SUR2A, and SUR2B) and protein kinases (PKA, PKG, and PKC $\beta$ 2) in myocardial cells of rats with myocardial ischemia (MI) induced by isoproterenol (ISO).**METHODS:** Rats were randomly divided into a control, model, Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups. The MI model was established by injecting rats with ISO. Electro-acupuncture treatment was given to the acupuncture groups, once a day for 7 days. Gene expression was analyzed with real-time PCR.**RESULTS:** The gene expression of KATP and proteinkinases in the model group was higher than those in the control group ( $P < 0.05$ ). After acupuncture treatment, the KATP and protein kinase expression levels were significantly lower in the Neiguan (PC 6) and Lieque (LU 7) groups compared with the model group ( $P < 0.05$ ). The Neiguan (PC 6) group lowered these levels significantly more than that of the Lieque (LU 7) group ( $P < 0.05$ ). No significant differences were observed between the model and non-acupoint groups ( $P > 0.05$ ).**CONCLUSION:** Our findings suggest that electronic needling of Neiguan (PC 6) can both reduce the gene expression of KATP and protein kinases in rats with ISO-induced MI.

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**Key words:** Point PC6 (Neiguan); Acupuncture; Electric stimulation; KATP Channels; Protein Kinases; Myocardial ischemia**INTRODUCTION**According to Traditional Chinese Medicine (TCM) theory, the meridians are the passages in which energy flows around the human body. Acupoints are the sensitive points on the meridians. Acupoints, known as "the fifteen Luo-connecting points," link exterior-interior meridians. Neiguan (PC 6), one of "the fifteen Luo-connecting Points," is located in the hand and belongs to the Jueyin pericardium meridian. TCM theorizes that the pericardium is the first to be attacked when pathogenic factors invade the heart. Therefore, heart disease is mainly caused by pericardium disorders.<sup>1</sup>

Allies *et al.*<sup>2</sup> injected two fluorescent dyes, true blue and diamidino yellow, into the pericardial sac, the medial brachial cutaneous nerve, or subcutaneously into the medial side of the brachium. Double-labeling was observed in the ipsilateral dorsal root ganglia neurons of spinal cord segments C8 (the eighth cervical vertebrae), T1 (the first thoracic vertebrae), and T2 (the second thoracic vertebrae), indicating that dichotomizing afferent fibers supply both the pericardium and the brachium.<sup>2</sup> This study provides a possible morphological explanation for referred cardiac pain. Lin Yang *et al.*<sup>3</sup> and Qiang Liu *et al.*<sup>4</sup> found that some nerve fibers in the heart and Neiguan (PC 6) originate from one same neuron in the spinal ganglia or the inferior ganglion of the vagus nerve by injecting peroxidase and choleragenoid  $\beta$ .<sup>3,4</sup>

Because of the relationship between Neiguan (PC 6) and the heart, stimulating Neiguan (PC 6) is essential to treating some heart diseases, such as MI.<sup>5,6</sup> Studies have shown that the mechanisms of the stimulation mainly involve blood rheology, bioactive substances, antioxidants, monoamines, intracellular signaling, myocardial enzymes, and energy metabolism.<sup>7,8</sup>

The adenosine triphosphate (ATP)-sensitive potassium channel (KATP) plays an important role in cardiovascular diseases, such as MI, ischemic preconditioning, arrhythmia, and hypertension.<sup>9</sup> As a new target of anti-MI drugs, KATP has received widespread attention.<sup>10</sup> In this study, Neiguan (PC 6) and Lieque (LU 7) were acupunctured in rat models of MI induced by injecting isoproterenol (ISO). RT-PCR was used to analyze changes in Kir6.1, Kir6.2, SUR2A, SUR2B, PKA, PKG, and PKC $\beta$ 2 expression. This study aimed to reveal the relationship between acupuncturing Neiguan (PC 6) and gene expression of KATP.

## MATERIALS AND METHODS

### Animals

Healthy Sprague-dawley (SD) rats (specified pathogen free grade, 70 males) were obtained from Liaoning Chang Sheng Biotechnology (Benxi, China), production license No. SCXK [Liao] 2010-0001 and use license No. SYXK [Liao] 2010-0001. Rats received food and water ad libitum for 1 week before experiments [temperature at (24  $\pm$  1) °C and humidity at 50%  $\pm$  5%]. The ethics committee from the Experimental Animal Center of Liaoning University of TCM (Traditional Chinese Medicine) approved the trial from National Essence Basic Research and Development 973 program. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals in Liaoning University of TCM.

### Drugs and reagents

ISO was purchased from Sigma (St. Louis, MO, USA).

TRIzol was obtained from Invitrogen (Carlsbad, CA, USA). Synthetic primers were provided by Beijing Genomics Institute. RT-PCR kit was purchased from Takara (Dalian, China).

### Model establishment and grouping

Ten rats were selected randomly as the control group. Normal saline solution (85 mg/kg) was injected into the roots of the medial limbs of the control group, whereas ISO (isoproterenol, 85 mg/kg) was injected into the remaining groups.<sup>11,12</sup> The injection was performed on all the rats for a second time 24 h later, and then an ECG was recorded for each rat. The models were considered a success as previously described by Nandave *et al.*<sup>13</sup> and Coval *et al.*<sup>14</sup> Briefly, the model was established when the ECG T-wave changed from positive to negative or biphasic, accompanied by ST-segment elevation, QRS wave widening, sinus tachycardia, contractions, or other arrhythmias.<sup>13,14</sup> There were no deaths in the control group after modeling. Forty rats survived the ISO injections. The remaining rats were randomly divided into model, Neiguan (PC 6), Lieque (LU 7), and non-acupoint groups, with 10 rats in each group.

### Acupuncture methods

The electroacupuncture of Neiguan (PC 6) and Lieque (LU 7) was based on the rat acupuncture positioning method described in "Experimental Acupuncture Science."<sup>15</sup> The middle Tianshu (ST 25) and Shenque (RN 8) points were selected for the rats in the non-acupoint group. Needles (0.18 mm  $\times$  25 mm, Suzhou Hua Tuo Medical Instruments, Suzhou, China) were inserted into each corresponding point (bilateral). The needles were linked to a 6805-D EA (Shantou Medical Equipment Factory, Shantou, China) in sparse dense wave with the intensity of 2-20 Hz. The current intensity was considered adequate when a slight quiver of the forelimbs was observed, which is consistent with electroacupuncture in frequency. The same stimulation was applied to Neiguan (PC 6), Lieque (LU 7), and the non-acupoint groups. Acupuncture was maintained for 20 min per session, one time per day, for 7 days. The rats in the control and model groups did not receive needling.

### Tissue sample preparation

Rats were sacrificed and their cervical vertebra and left ventricle tissues collected. The tissue was immediately placed into tubes after the addition of 1 ml TRIzol. Then, tissue samples were stored at -80 °C until use.

### Real-time PCR analysis

Total RNA was separated from approximately 100 mg of myocardial tissue, and subjected to one-step real-time PCR. RNA samples were deemed to be high quality if the sample A260/280 ratios ranged from 1.8 to 2.0. Total RNA (1  $\mu$ L) was reverse transcribed into

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