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Basic Study

Effect of electroacupuncture pretreatment on adenine nucleotides in myocardial tissues of rats with myocardial ischemia-reperfusion injury detected by high performance liquid chromatography (HPLC)

基于HPLC观察电针预处理对心肌缺血再灌注损伤大鼠心肌组织中腺苷酸的影响

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Abstract

Objective: To observe the effect of electroacupuncture (EA) pretreatment on adenine nucleotides in the myocardial tissues of the myocardial ischemia-reperfusion injury (MIRI) rats, and to explore the mechanism of EA pretreatment on myocardial prevention and protection in MIRI rats.

Methods: Forty SPF male Sprague-Dawley (SD) rats were randomly divided into 5 groups: a blank group, a sham operation group, a model group, an EA at Neiguan (PC 6) group and an EA at Hegu (LI 4) group, with 8 rats in each group. Rats in the blank group only received binding to the rat plate, 30 min/time, once a day for 7 d; on the 7th day, rats in the sham operation group were subjected to threading for 40 min at the left anterior descending coronary artery without ligation, and then the rats were allowed to stand for 60 min before collection of the specimens; on the 7th day, rats in the model group were subjected to threading at the left anterior descending coronary artery with ligation, for 40 min before the blood flow was restored, and then the rats were allowed to stand for 60 min before collection of the specimens; on the 7th day of pretreatment with EA at Neiguan (PC 6) or Hegu (LI 4) for 30 min per day (once a day for 7 d), rats in the EA at Neiguan (PC 6) group and EA at Hegu (LI 4) group were subjected to modeling and sample collection same as in the model group. The left ventricular myocardium of the lower left anterior descending coronary artery was collected from rats in all 5 groups. Hematoxylin-eosin (HE) staining and transmission electron microscope (TEM) were used to observe the changes in myocardial pathological morphology. The change in the adenine nucleotide level of myocardial tissue was measured by high performance liquid chromatography (HPLC).

Results: The HE staining and ultrastructure showed that the myocardial injury was severer in the model group compared with the sham operation group. Compared with the model group, the myocardial injury in the EA at Neiguan (PC 6) and the EA at Hegu (LI 4) groups was mild or hardly any. The adenine nucleotide levels in the sham operation group and the model group were all decreased compared with the blank group (all $P<0.05$); compared with the sham operation group, the adenine nucleotide level of the model group was also decreased, but the difference was not statistically significant ($P>0.05$); compared with the model group, the adenine nucleotide level in the EA at Neiguan (PC 6) group was increased ($P<0.05$), and the adenine nucleotide level in the EA at Hegu (LI 4) group was significantly increased ($P<0.01$). The adenine nucleotide level in the EA at Hegu (LI 4) group was higher than that in the EA at Neiguan (PC 6) group, but the difference was not statistically significant ($P>0.05$). Compared with the EA at Neiguan (PC 6) group, the levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in the EA at Hegu (LI 4) group were significantly increased (all $P<0.01$).

Conclusion: Both EA at Neiguan (PC 6) and Hegu (LI 4) can alleviate the pathological damage to myocardium in MIRI rats, and increase the adenine nucleotide level in myocardial tissues, and thus protect MIRI rats. EA at Hegu (LI 4) has a better protective effect than Neiguan (PC 6).

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Keywords: Acupuncture Therapy; Electroacupuncture; Myocardial Ischemia; Myocardial Reperfusion Injury; Adenine Nucleotides; Adenosine Diphosphate ; Adenosine Triphosphate; Rats

【摘要】目的：观察电针(EA)预处理对心肌缺血再灌注损伤(MIRI)大鼠心肌组织中腺苷酸的影响，探讨EA预处理对MIRI大鼠心肌预防保护的作用机制。**方法：**将40只SPF级雄性Sprague-Dawley (SD)大鼠随机分为5组，空白组、假手术组、模型组、EA内关组和EA合谷组，每组8只。空白组仅用鼠板束缚大鼠，每次30 min，每天1次,连续7 d；假手术组于第7天在左冠状动脉前降支穿线不结扎，穿线40 min后取线，静置60 min后取材；模型组于第7天在左冠状动脉前降支结扎，40 min后恢复血流，60 min后取材；EA内关组和EA合谷组每天EA内关或合谷30 min，每天1次，连续7 d，于第7天进行造模和取材，方法同模型组。收集5组大鼠的左冠状动脉前降支下段左心室心肌组织。采用苏木精-伊红染色法(HE)及透射电子显微镜(TEM)检测心肌病理形态学的变化，用高效液相色谱法(HPLC)测定心肌组织中腺苷酸的含量变化。**结果：**在心肌HE染色及超微结构方面，与假手术组比较，模型组心肌损伤严重；与模型组比较，EA内关组心肌损伤较轻微，EA合谷组心肌损伤亦较轻微，且趋于正常。在腺苷酸含量方面，与空白组比较，假手术组和模型组腺苷酸含量均降低(均$P<0.05$)；与假手术组比较，模型组腺苷酸含量亦降低，但差异无统计学意义($P>0.05$)；与模型组比较，EA内关组腺苷酸含量升高($P<0.05$)，EA合谷组腺苷酸含量有明显升高($P<0.01$)；与EA内关组比较，EA合谷组腺苷酸含量高于EA内关组，但差异无统计学意义($P>0.05$)；与EA内关组比较，EA合谷组中三磷酸腺苷(ATP)、二磷酸腺苷(ADP)和一磷酸腺苷(AMP)含量明显升高(均$P<0.01$)。**结论：**EA内关和合谷均能减轻MIRI大鼠心肌的病理损伤，升高心肌组织中腺苷酸含量，对MIRI大鼠具有预防保护作用，且EA合谷比EA内关的预防保护效果更佳。

【关键词】针刺疗法；电针；心肌缺血；心肌再灌注损伤；腺苷酸；二磷酸腺苷；三磷酸腺苷；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Clinical and animal studies have confirmed that electroacupuncture (EA) pretreatment has protective effects on ischemic myocardium[1-2]. Adenine nucleotides are chemical syntheses of adenine, ribose and phosphoric acid and widely found in animal cells. Adenine nucleotides are not only important components of ribose acid, but also have the function of energy transfer in animal body. Adenine nucleotides mainly include adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). As the most direct energy source in the organism, ATP is known as 'energy currency' when cells acquire energy[3]. ATP also promotes the repair and regeneration of various cells in the body, enhances the metabolic activity of cells, and plays a pivotal role in the cellular metabolic activities of organisms[4]. Disorder of the ATP energy metabolism (including energy production, transformation and utilization) will lead to the destruction of its function. ADP, AMP and ATP can transform into each other during the energy metabolism, that is, ADP is produced after ATP breaks a high-energy phosphate bond and releases the hydrolysis energy; AMP is produced after ADP breaks a high-energy phosphate bond, or ATP breaks two high-energy phosphate bonds and then releases the hydrolysis energy, which will change the levels of the three in the body[5]. In animal myocardial tissues, changes in ATP, ADP and AMP levels are intuitively reflected by their energy metabolism level[6]. ATP, ADP and AMP are the main biochemical observation indicators of abnormal energy metabolism. However, the sensitivity and stability of the chemical and spectrophotometry measures are poor due to the low level of such substances in the living body; though the enzymatic method and nuclear magnetic resonance technique are effective, they are expensive and difficult to be spread. The high performance liquid chromatography (HPLC) analysis method has the advantages of the above methods, and is fast, simple and highly specific. Therefore, in recent years, HPLC has been used to detect the adenine nucleotide level of tissues. Our previous studies have shown that EA pretreatment has protective effects on myocardial cells in rats with ischemia-reperfusion; however, its mechanism of the energy metabolism is still unclear. The aim of this study was to determine the adenine nucleotide level in myocardial tissues of rats with myocardial ischemia-reperfusion injury (MIRI) by HPLC; to observe the effect of EA pretreatment on adenine nucleotides in myocardial tissues of MIRI rats, thus to explore the preventive and protective mechanism of EA pretreatment in energy metabolism, and whether EA at Hegu (LI 4) and EA at Neiguan (PC 6) have the same preventive and protective effects.

1 Materials and Methods

1.1 Laboratory animal and grouping

Forty SPF male Sprague-Dawley (SD) rats, weighing 250-300 g, were provided by the Animal Experimental Center of Hunan University of Chinese Medicine [qualification number: SYXK (Xiang) 2013-0004/0005]. Feeding temperature was 20-25 ℃ with a humidity of 50%-70%. Rats were randomly divided into 5 groups according to the random number table method, a blank group, a sham operation group, a model group, an EA at Neiguan (PC 6) group and an EA at Hegu (LI 4) group, with 8 rats in each group. Animal experiments strictly adhered to the *Guiding Opinions on the Treatment of Experimental Animals* issued in 2006 by the Ministry of

Science and Technology and the ethics regulations of the animal experiment.

1.2 Main instruments and reagents

ECG-1106G digital six-channel electrocardiograph (Shenzhen Carewell Electronics Co., Ltd., China); ALC-V108 animal ventilator (Shanghai Alcott Biotech Co., Ltd., China); E2695 high performance liquid meter (Waters Company, USA); OptionR7 utra AN ultrapure water system (ELGA Company, UK); model 5424R high speed refrigerated centrifuge (Eppendorf AG Company, Germany); KQ5200DV type numerical control ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument Co., Ltd., China); vortex instrument (Henry Troemner, USA); eppendorf pipette (Eppendorf AG Company, Germany); Hwato Brand SDZ-V electronic acupuncture instrument (Suzhou Medical Products Factory Co., Ltd., China); Hanyi Brand disposable sterile acupuncture needle (specification: 0.25 mm in diameter, 13 mm in length, Changchun Aiquan Medical Devices Co., Ltd., China).

10% chloral hydrate and 4% paraformaldehyde (Shanghai Shanpu Chemical Co., Ltd., China); potassium dihydrogen phosphate and dipotassium hydrogen phosphate (ANPEL Company, USA); ATP reference substance (batch number: wkq16122902, HPLC ≥98%), ADP reference substance (batch number: wkq16122903, HPLC ≥98%) and AMP control substance (batch number: wkq16122904, HPLC ≥98%) (Sichuan Weikeqi Biological Technology Co., Ltd., China); methanol for chromatography (Merck AG, Germany); hematoxylin-eosin (HE) staining reagent (Sigma, USA); analytical grade perchloric acid and ultrapure water.

1.3 Modeling method

MIRI model preparation: Each rat in the model group, the EA at Neiguan (PC 6) group and the EA at Hegu (LI 4) group was fixed on the rat board in a supine position, after intraperitoneal injection with 10% chloral hydrate at a dose of 3.0 mL/(kg·bw) for general anesthesia. Preoperative electrocardiogram was performed after the electrode was punctured under the rat skin with the standard voltage of 20 mm/mV and the paper-feeding speed of 25 mm/s. Rats with abnormal electrocardiogram was excluded. The neck and the chest areas of rats were depilated, and the skin was disinfected. The animal artificial respirator was connected after the tracheal intubation with a breathing ratio of 1:2, tidal volume (VT) of 20-30 mL/(kg·bw) and respiratory rate of 70 times/min. The skin was opened along the midline of the sternum 0.5-1.0 cm away from the left side to expose the ribs. The chest was opened from the 3rd and 4th intercostal spaces to expose the heart. The pericardium was gently opened to expose the left atrial appendage using a cotton swab and to locate the left anterior descending coronary artery. At the upper and middle 1/3 of the left anterior descending coronary artery, a 4-0 medical suture was threaded with a small round curved needle on the needle holder. The blood vessels were ligated with a silicone tube at the threading point, and the changes in the T-wave and ST-segment of the electrocardiogram (ECG) Ⅱ lead were immediately monitored. Vascular bulging and cyanosis of the lower left coronary artery after ligation, cyanosis of the left ventricular anterior wall or ST-segment elevation with T-wave high-amplitude in ECG standard Ⅱ lead and AVF lead were taken as the signs of myocardial ischemia. After ligation for 40 min, the silicone tube was loosened, and the left anterior descending artery was perfused for 60 min. After loosening of the ligation of the blood vessel, the blood supply was recovered, thus the color of the lower left coronary artery changed from cyanosis to light dark or dark red and the T-wave descended by more than 0.2 mV, which indicated the successful reperfusion[7].

1.4 Intervention methods

1.4.1 Blank group

Rats only received binding to the rat plate, 30 min each time, once a day for 7 d.

1.4.2 Sham operation group

Rats received binding to the rat plate for 30 min each time, once a day for 7 d; on the 7th day, rats were subjected to threading, at the left anterior descending coronary artery without ligation for 40 min, and then the rats were allowed to stand for 60 min before the specimens were collected.

1.4.3 Model group

Rats received binding to the rat plate for 30 min each time, once a day for 7 d. On the 7th day, MIRI model was prepared and the left anterior descending coronary artery was ligated for 40 min before the blood flow was restored, and the rats were allowed to stand for 60 min before the specimens were collected.

1.4.4 EA at Neiguan (PC 6) group

Model preparation: Model preparation and specimen collection were carried out on the 7th day, using the same method as that in the model group.

Points: Bilateral Neiguan (PC 6) (at the medial aspect of the forelimb and about 3 mm from the wrist joint, between the ulna and the radius)[8].

Methods: After routine disinfection, the needle was straightly punctured into the point for about 3 mm, and the needle maneuver of lifting, thrusting and twirling was performed (even reinforcing-reducing manipulation) for 1 min. The needle was then connected to the EA instrument (the point was connected to the negative electrode and the positive electrode was connected to the tail of the rat). The sparse-dense wave was used (the frequency of 10 Hz/50 Hz, the pulse width of 0.5 ms, the intensity of 1 mA) to achieve slight vibration of the upper limbs, 30 min each time, once a day for 7 d.

1.4.5 EA at Hegu (LI 4) group

Model preparation: Model preparation and specimen collection were carried out on the 7th day, using the same method as that in the model group.

Points: Bilateral Hegu (LI 4) (between the first and second metacarpal of the forelimb)[8].

Methods: Same as EA at Neiguan (PC 6) group.

1. 5 Observation items and detection methods

1.5.1 Specimen collection

The rat heart in each group was separated and the residual blood was washed with cold saline. The left ventricular myocardium from the left anterior descending coronary artery (about 3-5 mm from the apex) was removed using the ophthalmic scissors, divided into three pieces and quickly cut into 5 mm×1 mm×1 mm tissue blocks; one was immersed in 2.5% glutaraldehyde for 24 h (4 ℃) to observe the ultrastructure of the left ventricular ischemic tissue; one was quickly placed in 4% polymethanol fixative [containing 0.1% diethylpyrocarbonate (DEPC)] for HE staining; one was quickly placed in 4% polymethanol fixative (containing 0.1% DEPC) and stored at –80 ℃ for HPLC detection[9].

1.5.2 Morphological changes of cardiomyocytes in the left ventricular ischemic myocardium detected by HE staining and electron microscopy

Ultrastructural changes in the left ventricular ischemic area observed by electron microscope: The myocardial tissue was fixed with osmic acid in the refrigerator for 2 h after fixed in 2.5% glutaraldehyde for 24 h (4 ℃), dehydrated by conventional ethanol gradient, and embedded with 812 epoxy resin for 3 h and 2 times. Then the tissue was placed in an oven at 40 ℃ for 12 h and then at 60 ℃ for 48 h to make it harden by polymerization, forming a hard tissue block after natural cooling. After trimmed into the pyramid shape, semi-thin sectioning of the tissue blocks was performed. After positioning, ultra-thin section (500A) was sliced with a LKB ultra-thin slicer. Transmission electron microscopy specimens were prepared by double staining with uranium and lead. Each myocardial specimen was subjected to electron staining. Ultrastructure of myocardial fibrils, mitochondria and vascular endothelial cells were observed by transmission electron microscope (TEM).

HE staining was used to observe the histopathological changes of the left ventricular ischemic area: Paraffin sections of 5 μm thickness were placed on a clean glass slide and baked in an oven at 60 ℃ for 4 h. Dewaxed twice with xylene for 15 min each time. Rehydration was achieved with gradient ethanol solutions, 100% ethanol solution twice, 95% ethanol solution once, 80% ethanol solution once, and distilled water once. The nuclei were stained with hematoxylin for 10 min, differentiated with 10% hydrochloric acid alcohol, washed with tap water for 15 min (back to blue), and washed twice with distilled water. The cytosol was stained with eosin and dehydrated in gradient ethanol solutions. Transparency was performed with xylene. The slices were sealed with resin glue. Morphological changes of myocardial cells were observed under light microscope and photographed.

1.5.3 Determination of adenine nucleotide levels in myocardial tissues by HPLC

About 0.1 g myocardial tissue was removed from the refrigerator at –80 ℃, accurately weighed and placed in a pre-cooled glass tissue grinder at a mass to volume ratio of 1:10. Pre-cooled 5% perchloric acid was added to precipitate the proteins and rapidly ground into a homogenate in an ice bath; centrifuged for 10 min at 4 ℃ and 15 000 r/min; the supernatant was collected and an equal volume of 1 mol/L potassium dihydrogen phosphate solution was added; vortexed after the pH was adjusted to 6.0, centrifuged for 5 min at 4 ℃ and 13 000 r/min; the supernatant was stored in a refrigerator at –20 ℃ for testing[10].

Chromatographic conditions: Venusil MP C18 chromatographic column (4.6 mm×250 mm, 5 μm); mobile phase A was phosphate buffer (pH 5.8), mobile phase B was methanol solution; detection wavelength was 254 nm; injection volume was 20 μL; column temperature was 26.5 ℃.

1.6 Statistical methods

Statistical processing was performed using SPSS version 17.0 software. All normally distributed measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$), and comparison among multiple groups was performed by one-way analysis of variance. The comparison between groups with homogeneity of variance was performed by the least significant difference (LSD) test; that with heterogeneity of variance was performed by Tamhane's T2 test. $P<0.05$ indicated a significantly statistical difference.

2 Results

2.1 Changes of myocardial histopathology in rats detected by HE staining and TEM

Compared with the sham operation group, the myocardial fibers were normal, the morphology of the myocardial cells was regular, neatly arranged with rich cytoplasm, and there was no interstitial hemorrhage, no necrosis and dissolution of myofilament, and no mitochondrial edema in the normal group; rats in the model group showed severe myocardial damage, myocardial cell structure disorder, cell swelling and necrosis, muscle fiber blur, partial myofilament necrosis, mitochondrial edema, and vacuolization.

Compared with the model group, rats in the EA at Neiguan (PC 6) group showed mild myocardial damage, slightly swollen cell, blurred borders of some myocardial cells, neat line of filaments, almost clear structure, abundant mitochondria, and partial sarcoplasmic reticulum edema; rats in the EA at Hegu (LI 4) group also showed mild myocardial damage, regular morphology of myocardial cells, neatly arranged muscle fibers, and normal mitochondrial structure (Figure 1 and Figure 2).

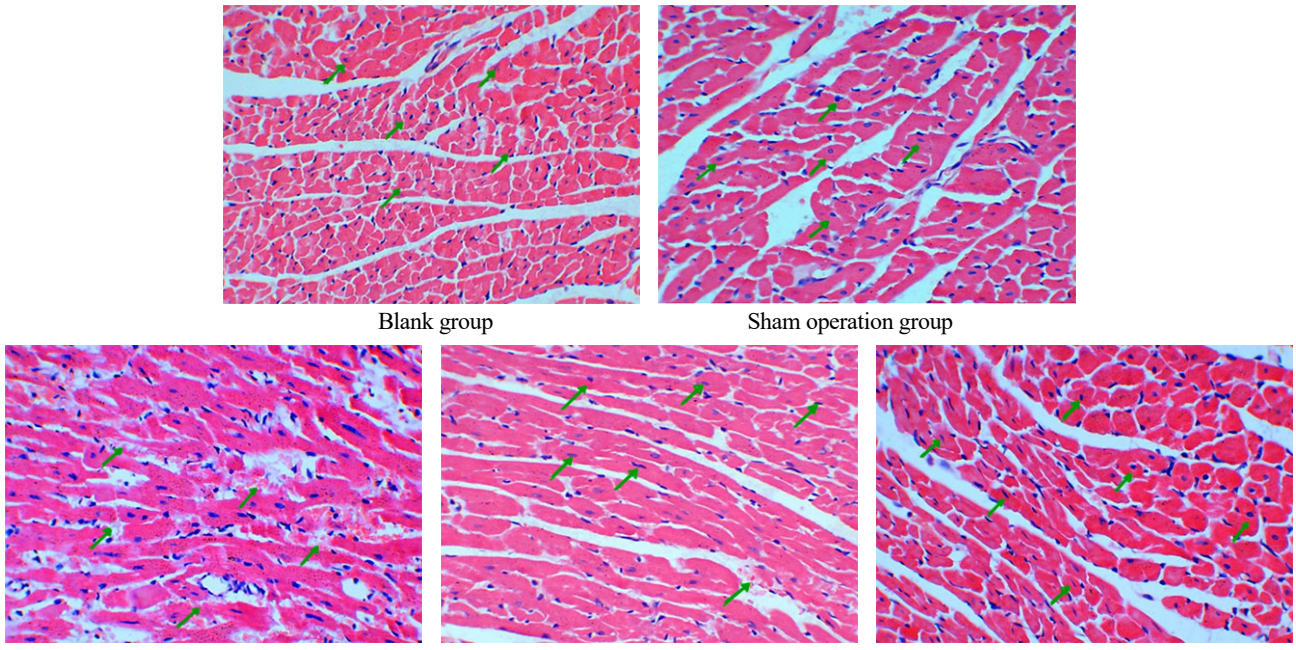

Figure 1. Pathological morphology of myocardial tissue in each group (HE, ×400)

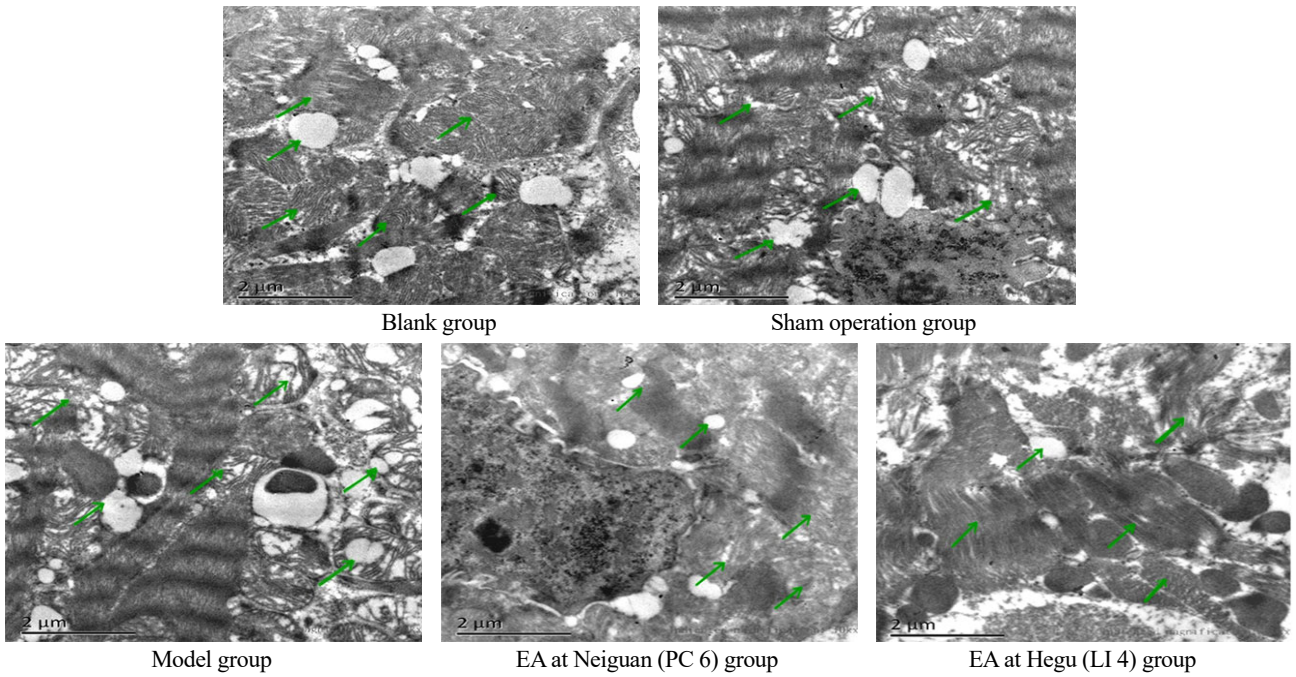

Figure 2. Myocardial ultrastructure of rats in each group (×10 000)

2.2 Determination of the adenine nucleotide level in myocardial tissues of each group

Compared with the blank group, the ATP levels in the model group and the EA at Neiguan (PC 6) group were decreased (P<0.05); the ADP levels in the sham operation, the model and the EA at Neiguan (PC 6) groups were all decreased (P<0.01); the AMP level in the model group was decreased (P<0.05); the adenine

nucleotide levels in the sham operation and the model groups were all decreased (P<0.05). Compared with the sham operation group, the ATP level in the EA at Hegu (LI 4) group was increased (P<0.05); the levels of ADP, AMP and adenine nucleotides in the EA at Hegu (LI 4) group were all increased (P<0.01); the ATP, AMP and adenine nucleotides levels in the model group were decreased, but the differences were not statistically significant (P>0.05); the ADP level in the model group was increased, but the difference was not statistically significant (P>0.05). Compared with the model group, the level of adenine nucleotides in the EA at Neiguan (PC 6) group was increased (P<0.05); the levels of ATP, ADP, AMP and adenine nucleotides in the EA at Hegu (LI 4) group were all significantly increased (P<0.01). Compared with the EA at Neiguan (PC 6) group, the levels of ATP, ADP and AMP in the EA at Hegu (LI 4) group were all increased (P<0.01); the adenine nucleotide level in the EA at Hegu (LI 4) group was higher than that in the EA at Neiguan (PC 6) group, but the difference was not statistically significant (P>0.05), (Table 1).

Table 1. Comparing the adenine nucleotide level in myocardial tissues of each group ($\bar{x}$ ±s, mg/g)

Group	n	ATP	ADP	AMP	Adenine nucleotides
Blank	8	3.854±2.206	8.442±1.157	8.154±4.585	20.096±7.363
Sham operation	8	1.052±0.286	6.847±0.901[2)]	2.217±0.898	8.902±2.789[1)]
Model	8	0.732±0.183[1)]	7.031±1.266[2)]	1.553±0.992[1)]	8.111±1.544[1)]
EA at Neiguan (PC 6)	8	0.690±0.177[1)]	7.002±0.760[2)]	3.129±1.088	11.549±2.085[5)]
EA at Hegu (LI 4)	8	1.874±0.566[3)6)7)]	8.543±0.670[4)6)7)]	9.742±2.109[4)6)7)]	16.422±3.669[4)6)]

Note: Compared with the blank group, 1) P<0.05, 2) P<0.01; compared with the sham operation group, 3) P<0.05, 4) P<0.01; compared with the model group, 5) P<0.05, 6) P<0.01; compared with the EA at Neiguan (PC 6) group, 7) P<0.01

3 Discussion

The main components of purine nucleotides are adenylates (ATP, ADP and AMP), in which ATP is the energy form necessary for the organism to maintain normal life activities. Studying the composition and level changes in adenine nucleotides of biological tissues or cells is one of the important means to elucidate the life phenomenon and its changing law[11]. The heart of a healthy person pumps blood about 5 L/min, more than 7 000 L/day and more than 260 000 L per year[3]. To complete the normal ejection, the myocardium needs to hydrolyze more than 6 kg of ATP per day[12]. The energy required for sustained contraction of the heart primarily comes from the breakdown of ATP. Ischemia-reperfusion injury is mainly caused by increased oxygen free radical production and calcium overload[13]. Li L, *et al*[14] showed that adenine nucleotide pretreatment could protect MIRI and affect the release of inflammatory cytokines. Li XJ, *et al*[15] showed that both AMP579 and adenine nucleotides protected the heart from ischemia-reperfusion injury by reducing myocardial infarction. In addition, the effect of AMP579 was better than that of adenine nucleotides. Yan J, *et al*[16] found that EA at Neiguan (PC 6) and Zusanli (ST 36) significantly improved the ATP reduction state, and its decomposition product AMP was increased; AMP produces a large amount of adenine nucleotides with enzyme catalysis, which escapes from the cell membrane into the blood circulation to expand small blood vessels, improve blood supply to the myocardium, to ensure energy supply during myocardial ischemia, thereby reducing myocardial damage. Wang C, *et al*[17] showed that EA at Neiguan (PC 6) pretreatment induced myocardial adenine nucleotide A1 receptor expression and increased serum adenine nucleotide levels, thereby initiating myocardial endogenous protection mechanism and enhancing myocardial tolerance to ischemia and hypoxia, reducing the pathological damage of the myocardium, and then played specific protective effect on MIRI.

Many clinical and experimental studies have demonstrated that EA at Neiguan (PC 6) has a significant protective effect on the myocardium[16-22], but EA at Hegu (LI 4) has relatively little effect on the myocardium. Hegu (LI 4) and Neiguan (PC 6) were compared in this experiment due to Hegu (LI 4) was also closely related to the heart system. Hegu (LI 4) is the Yuan-Primary point of the Large Intestine Meridian. Although no direct relationship is recorded between the Large Intestine Meridian and the heart in the ancient literatures, however, the holism is the characteristic of the traditional Chinese medicine. The heart is the master of the internal organs, and it governs the blood and vessels. Dysfunctions of the heart may cause disordered qi, blood, yin and yang.

There are not many documents of Hegu (LI 4) for cardio-thoracic diseases in the past, but studies have showed that Hegu (LI 4) can promote blood circulation and resolve blood stasis, restore yang and rescue collapse. It can be used for chest Bi-impediment or palpitations due to blood stasis. As an essential point to tonify qi and blood to restore yang, Hegu (LI 4) can also be used for acute chest pain. In addition, Hegu (LI 4) has

an overall regulation on the body when used in combination with other points. Therefore, Hegu (LI 4) was selected as the control of Neiguan (PC 6) to compare whether EA at Neiguan (PC 6) and EA at Hegu (LI 4) can produce a consistent preventive and protective effect.

This experimental study found that the adenine nucleotide level in the myocardium of model rats presented a significant change trend. Compared with the blank group, the adenine nucleotide levels of the sham operation group, the model group and the two groups of EA pretreatment were all decreased. Considering the pericardium damage during the threading and ligation, the pericardium is divided into fibrous pericardium and serosal pericardium; and the fibrous pericardium is a fibrous connective tissue with small stretch and toughness ability; the serosal pericardium is divided into a splanchnic wall and a parietal layer. The serosal pericardium is closely attached to the surface of the myocardium. The splanchnic wall is connected with the fibrous pericardium, and the pericardial cavity is between the splanchnic wall and a parietal layer. The surface of the serosa is mesothelial cells, which have the functions of secreting vasoactive substances, activating plasma plasminogen, and degrading fibrin, which are closely related to the maintenance of cardiac function[23-24]. The heart has a strong integrity, and even a small amount of cell apoptosis can reduce its overall function[25]. As a terminally differentiated cell, cardiomyocytes have limited self-renewal and repair ability. Wang XM, *et al*[26] showed that the pericardium tissue promoted self-repair after ischemic heart injury in the animal model with intact pericardium, and this protective effect may be related to the KDR^+ cells in the pericardium tissues. These cells have characteristic of cardiac stem cells. Therefore, the complete pericardium helps the heart to repair itself and plays an important role in the regeneration process of the heart muscle and blood vessels. In this experiment, except for the blank group, the rat's pericardium in the other groups was damaged due to the need of modeling, which affected the adenine nucleotide levels in the myocardial tissues. Compared with the sham operation group, the adenine nucleotide level was reduced; the myocardial tissue pathological changes observed by HE staining and transmission electron microscopy showed severe myocardial damage, myocardial cell structure disorder, cell swelling and necrosis, muscle fiber blur, partial myofilament necrosis, mitochondrial edema and vacuolization in the model group. It was suggested that MIRI rats had severe myocardial damage with many apoptotic cardiomyocytes[27], which also affected the adenine nucleotide level in myocardium. Compared with the model group, the adenine nucleotide levels in the EA at Neiguan (PC 6) group and the EA at Hegu (LI 4) group were significantly increased, and the results of the light microscopy and electron microscopy showed mild damage in the myocardium, regular morphology of myocardial cells, neatly arranged muscle fibers, almost normal mitochondrial structure. It was suggested that EA pretreatment partially prevented the pathological damage of myocardium and improved the energy metabolism of myocardium, indicating that EA at Neiguan (PC 6) and EA at Hegu (LI 4) had protective effects on myocardial tissues, and the protective mechanism against myocardial ischemia may be related to elevated adenine nucleotide level in myocardial tissues. The preventive and protective effect of EA at Hegu (LI 4) was better than Neiguan (PC 6).

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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