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RESEARCH ARTICLE

Effect of electroacupuncture at Ximen (PC 4) and Hegu (LI 4) on expression of Akt in rats with myocardial ischemia-reperfusion injury

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Abstract

OBJECTIVE: To investigate the effect of electroacupuncture (EA) at acupoints on the pericardium meridian on the expression of phosphorylated Akt (p-Akt) protein in rat myocardium after ischemia and reperfusion.

METHODS: Seventy Wistar rats were evenly randomized into seven groups: the sham operation group (group A), ischemia-reperfusion model Ⅰ group (group B), ischemia-reperfusion model Ⅱ group (group C), EA at Neiguan (PC 6) group (group D), EA at Ximen (PC 4) group (group E), EA at Hegu (LI 4) group (group F), and LY294002 + EA at Neiguan (PC 6) group (group G). All processes were monitored by electrocardiography. In group A, the left anterior descending coronary artery was only threaded without ligation for 100 min. In group B, the left anterior descending coronary artery was ligated for 40 min and reperfused for 60 min. The left anterior descending coronary artery in group C was ligated for 40 min and reperfused for 100 min. Groups D, E, and F received EA for 20 min before undergoing ischemia for 40 min, and then received EA for 20 min before undergoing reperfusion for 60 min. Before modeling, group G was injected with LY294002 (0.3 mg/kg) into the tail vein, and then underwent the same intervention as the other EA groups. After reperfusion, myocardial tissue from the left cardiac ventricle was collected to enable Western blot analysis of the p-Akt level, and analysis of electrocardiographic changes.

RESULTS: In groups B and C, electrocardiography showed obvious elevation of the ST-segment Ⅱ lead (ECG-$ST_{Ⅱ}$), while the ECG-$ST_{Ⅱ}$ values were significantly lower in groups D, E, and G ($P < 0.01$). The p-Akt levels in groups D and E were significantly greater than those in groups B and C ($P < 0.01$). Compared with all other groups, group G showed a significantly different expression of p-Akt ($P < 0.01$).

CONCLUSION: The expression of p-Akt protein in cardiomyocytes was significantly greater in rats that were injected with LY294002 and received EA at Ximen (PC 4) compared with all other groups. This suggests that EA at Ximen (PC 4) resulted in activation of the phosphoinositide 3-kinase/Akt signaling pathway and phosphorylation of Akt.

Keywords: Reperfusion injury; Electroacupuncture; Point PC 4 (Ximen); Point LI 4 (Hegu); Oncogene protein v-akt

INTRODUCTION

Myocardial protection is one of the main aims in the management of cardiac disease. A variety of growth factors have a protective effect on the heart against the damage caused by ischemia and other injuries. Although the downstream transduction pathway of these growth factors is very complex, activation of v-Akt murine thymoma viral oncogene homolog (Akt) is generally a common feature. Akt plays an important role in the heart through regulation of cardiomyocyte survival, growth, proliferation, function, and metabolism. Akt activation reportedly reduces the mortality rate of myocardial cells in an *in vivo* ischemia-reperfusion model, and substantially improves the overall function of the myocardium.[1] Furthermore, measurement of cell contraction and calcium transport shows that Akt activation in myocardial cells protects cardiomyocytes from hypoxia-induced dysfunction *in vitro*.[2]

Akt is a serine-threonine kinase known as protein kinase B, with two phosphorylation sites.[3] Akt can only be fully activated when both sites are entirely phosphorylated.[4] The phosphorylated Akt (p-Akt) affects its targets downstream to improve mitochondrial energy production by reducing the opening of the mitochondrial permeability transition and maintaining the stability of the outer mitochondrial membrane.[5] p-Akt also plays a role in myocardial protection by reducing the activation of the apoptotic-promoting factor and thereby inhibiting apoptosis.[6] The extent of myocardial ischemia is reflected by the changes in the ST-segment Ⅱ lead ($ST_{Ⅱ}$) (electric potential value of the ST-segment after ligation of the left anterior descending coronary artery versus the ST-segment electric potential value before ligation) measured by electrocardiogram (ECG) evaluation. In the present study, we aimed to observe the effect of electroacupuncture (EA) at Neiguan (PC 6) and Ximen (PC 4) on the expression of p-Akt in rats with ischemia-reperfusion injury.

MATERIALS AND METHODS

The present study was commissioned by the experimental center of Shanxi Medical University, and was approved by the experimental animal ethics committee of Shanxi University of TCM.

Animals

Seventy Wistar rats (clean grade, 28-32 months old, bodyweight 250-280 g, 35 males and 35 females) were provided by the Center of Experimental Animals of Shanxi Medical University, Taiyuan [experimental animal certification No. SCXK (JIN) 2009-0001].

Instruments and reagents

Energy Conversion System, Electrophoresis and Transgenic Membrane System (Beijing Junyi-Dongfang Electrophoresis Equipment Co., Ltd., Beijing, China), JY96-Ⅱ Ultrasonic Cell Crusher (Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China), β-actin, p-Akt1 (Santa Cruz Biotechnology, Inc., Delaware Santa Cruz, CA. USA), Polyvinylidene Fluoride (PVDF) membrane (Merck Milipore, NJ, USA), Second Antibody (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China), Protein Extraction Kit (Applygen Technologies Inc., Beijing, China).

Grouping and treatments

Seventy Wistar rats were randomly divided into seven groups with 10 rats in each: the sham operation group (group A), ischemia-reperfusion group Ⅰ (group B), ischemia-reperfusion group Ⅱ (group C), EA at Neiguan (PC 6) group (group D), EA at Ximen (PC 4) group (group E), EA at Hegu (LI 4) group (group F), and LY294002 + EA at Neiguan (PC 6) group (group G). Rats were weighed and then anesthetized with 10% urethane (1 g/kg). The rats were ventilated with room air using a rodent ventilator (DH-150, Medical Instrument Factory of Zhejiang Medical University, Hangzhou, China), and the ECG values of lead Ⅱ were recorded before the surgery. The heart was then exposed via thoracotomy at the left third and fourth intercostal spaces, and myocardial ischemia was induced by ligation of the left anterior descending coronary artery with No. 0 medical suture ligature placed just proximal to the main branching point of the artery. The suture was tied using a shoestring knot over a 1-mm silicone tube that was left in place during the planned period of ischemia. Myocardial ischemia was confirmed by ST-segment changes on the ECG. The ischemic area was readily recognizable by a cyanotic appearance and the presence of a bulging region. The thoracic cavity was then closed, leaving one end of the coronary suture protruding from the chest. After the appropriate period of occlusion according to grouping, the coronary artery was reperfused. After the appropriate period of reperfusion according to grouping, the rats were euthanized and the hearts were extracted.[7] The treatments in each group were as follows (Figure 1):

Group A: the left anterior descending coronary artery of the rats were only threaded (without ligation). The ST-segment changes were then recorded for 100 min before extraction of the heart.

Group B: the left anterior descending coronary artery was ligated for 40 min, followed by reperfusion for 60 min, and then extraction of the heart.

Group C: the left anterior descending coronary artery was ligated for 60 min, followed by reperfusion for 60 min, and then extraction of the heart.

Group D: when the left anterior descending coronary artery was threaded, the ECG was stopped. EA was performed at bilateral Neiguan (PC 6) acupoints using a G6805-1 EA therapeutic apparatus with density waves and frequency of 30-100 Hz at an intensity of 1 mA for 20 min. The left anterior descending coronary artery was ligated for 40 min. The EA treatment was