A Preliminary Study on the Cardioprotection of Acupuncture Pretreatment in Rats with Ischemia and Reperfusion: Involvement of Cardiac β-Adrenoceptors

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Abstract: The purpose of this study was to determine the cardioprotective effects of the repetitive pretreatment of acupuncture in rats with myocardial ischemia and reperfusion (MIR). Experimental MIR was produced by ligating and reperfusing the left anterior descending coronary artery in the rats. The elevated ST segments of electrocardiogram (ECG), cardiac arrhythmias, and ratio of infarct size/risk zone were compared among the normal control (NC), ischemia and reperfusion (IR), electro-acupuncture (EA), electro-acupuncture plus propranolol (EAP), and EA at nonacupoint (EAN) groups. Before the experiment, EA was applied at bilateral Neiguan acupoints (PC6) in the forelimbs in EA and EAP groups for 30 min once a day for 3 consecutive days. In the EAN group, the same EA treatment was administered at bilateral nonacupoints in the hind limbs. In the EAP group, propranolol, a nonspecific antagonist of β-adrenoceptors, was administered intraperitoneally 15 min before each EA pretreatment. The results showed that the elevated ST segment of ECG, cardiac arrhythmia score, and ratio of infarct size/risk zone were significantly attenuated in the EA group when compared with those in the IR group (P < 0.05), indicating a cardioprotection of EA pretreatment. When propranolol was given before each EA pretreatment in the EAP group, the cardioprotective effect of EA pretreatment was abolished, showing an involvement of β-adrenoceptors in mediating the effect of EA pretreatment. There was no significant cardioprotective effect observed in the EAN group. The results suggest that pretreatment may be a better way to apply acupuncture in the prevention and treatment of coronary heart disease.

Key words: pretreatment, electro-acupuncture, ischemia and reperfusion, β-adrenoceptors.

The sympathetic activity affecting the heart [1] and cardiac responsiveness to the sympathetic influence [2] were known to be enhanced during ischemia and reperfusion, leading to an overload of intracellular calcium [3] and subsequent cardiac injury. It is well documented that ischemic preconditioning protects the cardiac cells from injury induced by subsequent ischemia and reperfusion. Pretreatment with norepinephrine [4], a neurotransmitter of sympathetic endings in the heart, was shown to effectively mimic the protective effect of ischemic preconditioning on the heart subjected to ischemia and reperfusion. The protective effect induced by this pretreatment was associated with the induction of cardiac tolerance or adaptation to the subsequent overstimulation of β-adrenoceptors in cardiomyocytes [5] during the ischemia and reperfusion so as to attenuate the consequent cardiac impairment [6]. Acupuncture stimulation was reported to influence the activity of sympathetic nerves [7, 8]. It is quite likely that pretreatment with acupuncture prior to the ischemia and reperfusion may also attenuate the ischemic injury of the heart, as ischemic preconditioning or norepinephrine pretreatment does. The aim of the present study was to preliminarily determine the protective effect of the pretreatment of acupuncture in the hearts of rats with ischemia and reperfusion and to explore whether cardiac β-adrenoceptors are involved in the mediation of the cardiac protection induced by acupuncture pretreatment.

METHODS

Animal model of myocardial ischemia and reperfusion. The present study was approved by the Committee on the Use of Live Animals in Research of the China Academy of Chinese Medical Sciences. Male Sprague-Dawley rats weighing 266–320 g were divided into five groups, namely, normal control (NC) group, ischemia-reperfusion (IR) group, electro-acupuncture (EA) group, electro-acupuncture plus propranolol (EAP) group, and EA at nonacupoints (EAN) group. Before the myocardial ischemia and reperfusion experiment, the animals in both EA and EAP groups were pretreated with EA applied at bilateral Neiguan acupoints (PC6) for 30 min once a day on 3 consecutive days. Two needles...
were applied at each acupoint 2–3 mm apart and were connected to positive and negative poles of the acupuncture apparatus, respectively. The Neiguan acupoints were shown clinically and experimentally to be effective in the treatment of myocardial injury induced by ischemia and reperfusion [9, 10]. The acupoints were located in the forelimbs according to the textbook of experimental acupuncture in animals and stimulated with an intensity of 5 mA and a frequency of 20 Hz in the present study. Propranolol, a general antagonist of β-ARs, was administered intraperitoneally in the EAP group at a dose of 10 mg/kg 15 min before EA pretreatment once a day on 3 consecutive days. In the EAN group, the same EA pretreatment was applied at bilateral nonacupoints, which are about 0.5 cm below the Zusanli acupoints (ST36), according to the textbook of experimental acupuncture. On the day of experiment, the chests of all the rats were opened surgically under anesthesia with urethane. After a recovery of 20 min following the operation, the left descending branch of coronary artery in the rat was ligated with silk snare for 30 min, followed by a 15-min reperfusion among the IR, EA, EAP, and EAN groups. The arterial blood pressure was monitored throughout the experiment. The same operation without the ligation of coronary artery was performed on rats of the NC group.

**ECG analysis**

1. **Evaluation.** Electrocardiograms (ECG) were recorded continuously with standard lead II before, during, and after myocardial ischemia and reperfusion in all the animals by use of a computerized PowerLab system (ADInstruments, Australia). The ST segment of ECG elevated over 100 μV from the baseline was recruited as an index of ischemia. The amount of elevated voltage in μV in the different groups was compared statistically.

2. **Arrhythmia scoring system.** The atrial arrhythmias, such as premature atrial contraction (PAC) and the ventricular arrhythmias, including premature ventricular contraction (PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF), were observed for 15 min, beginning from the onset of reperfusion. Their severity was evaluated with a modified arrhythmia scoring system [11] with principles as follows. (1) Ventricular arrhythmias were more severe than atrial ones. (2) VF, VT, frequent PVC, and occasional PVC were in a descending order of severity. (3) The more frequent or longer duration of the incidence of arrhythmia, the greater its severity. In the scoring system, an occurrence of three or more PVCs within 1 min was considered frequent arrhythmia, but occasionally less than three occurred occasionally. The most severe arrhythmia was scored in each individual heart. The details of the scoring system are as follows:

<table>
<thead>
<tr>
<th>Arrhythmia score</th>
<th>Type of arrhythmia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no arrhythmia</td>
</tr>
<tr>
<td>1</td>
<td>atrial arrhythmias or an occasional PVC</td>
</tr>
<tr>
<td>2</td>
<td>frequent PVC</td>
</tr>
<tr>
<td>3</td>
<td>VT (1–2 episodes)</td>
</tr>
<tr>
<td>4</td>
<td>VT (&gt;3 episodes) or VF (1–2 episodes)</td>
</tr>
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**Determination of ischemic risk zone and infarct size.** When the experiment in vivo was finished, the heart was cut off and perfused with Krebs solution in a retrograde manner at the Langendorff perfusion system (Radnoti, USA) as prescribed previously [12]. Evans blue at a concentration of 0.25% was infused into the heart to determine the myocardial risk zone. The heart was then frozen and cut into 2 mm slices. The parts of the right ventricle and connective tissue were removed. The slices were incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC) buffer at pH 7.4 for 15 min at 37°C, then immersed overnight in 10% formalin. The infarct (TTC negative) and risk zone (TTC stained) were measured by a computerized planimetric technique (Minichromax; Biolab) [13]. In the present study the infarct severity was presented as the ratio of infarct size/risk zone.

**Drugs and chemicals.** Propranolol, Evans blue, and 2,3,5-triphenyltetrazolium chloride were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in double-distilled water.

**Statistical analysis.** All data in the present study were presented as mean±SE. A one-way ANOVA and *t*-test were used to analyze the data in different groups. A *P* value of less than 0.05 was considered statistically significant.

**RESULTS**

The effects of pretreatment with electroacupuncture (EA) on the ST segment of ECG in rats with ischemia and reperfusion

As shown in Fig. 1, before a ligation of the coronary artery, the values of ST segment elevated in the NC, IR, EA, EAP, and EAN groups were 42.8 ± 8.4, 43.4 ± 10.8, 36.0 ± 4.6, 44.9 ± 11.6, and 40.2 ± 6.7 μV respectively, indicating that there was no significantly ischemic elevation of ST segment among all the different groups. The values of the ST segment in the IR group 30 min after ischemia and 10 min after reperfusion were increased, respectively, to 162.5 ± 30.2 and 110.5 ± 23.8 μV, which were both significantly different from 43.5 ± 9.7 and 44.2 ± 10.6 V in the NC group separately. It is interesting that the values of the ST segment were 103.0 ± 16.0 and 50.8 ± 10.3 μV, respectively, in the EA group at the same recording time points as above, showing a significant attenuation in the elevated ST segment in comparison with the matched values of the
ST segment in the IR group. When propranolol was given intraperitoneally at a dose of 10 mg/kg 15 min before the treatment of EA in the EAP group, the matched values of the ST segment recorded 30 min after ischemia and 10 min after reperfusion were $142.4 \pm 24.4$ and $93.2 \pm 19.2 \mu V$, respectively, indicating a significant inhibition on the EA-induced attenuation in the elevation of the ST segment. In the EAN group, the corresponding values of the ST segment 30 min after ischemia and 10 min after reperfusion were $151.24 \pm 18.7$ and $100.07 \pm 14.3 \mu V$, respectively, almost the same as in the IR group, exhibiting that EA at the nonacupoints did not produce significant influence on the IR-induced elevation of the ST segment.

The effects of pretreatment with EA on cardiac arrhythmias in rats with ischemia and reperfusion

Figure 2 showed the changes in arrhythmic scores evaluated by the aforementioned scoring system in different groups. The arrhythmic score was zero in the NC group, suggesting that there was no arrhythmia. In the IR group there were different arrhythmias, including atrial and ventricular arrhythmias induced by myocardial ischemia and reperfusion. The arrhythmic score was $3.167 \pm 1.169$ in this group. With the pretreatment of EA, the arrhythmias produced by ischemia and reperfusion were significantly blunted in the EA group, exhibiting a lower arrhythmic score of $1.100 \pm 0.994$. When the animals were pretreated intraperitoneally with propranolol proceeding the treatment of EA in the EAP group, the arrhythmic score was $2.965 \pm 0.987$, indicating an appearance of severe arrhythmia. The blockage of the EA-induced attenuation of arrhythmias by propranolol suggests an involvement of the cardiac $\beta$-adrenergic receptor in the mediation of the attenuation of arrhythmias induced by EA. In the EAN group, there were also different arrhythmias, including...
propranolol was given before EA, the ratio of infarct size/risk zone was presented by a comparatively lower ratio of risk zone. Dial injury was significantly diminished by EA, which injury in myocardium was well established. The myocardial ischemia and reperfusion via desensitizing cardiac sympathetic activity [8]. Thus the repetitive EA pretreatment may also induce the desensitization of β-ARs via stimulating sympathetic activity to achieve the cardioprotective effect. In the present study, the cardioprotection of EA pretreatment was abolished by the administration of propranolol prior to each time of EA stimulation, which is in agreement with results in the previous studies on the cardioprotection of β-adrenergic preconditioning [6, 21]. Accordingly, propranolol seems to temporarily "protect" atrial and ventricular arrhythmias, during myocardial ischemia and reperfusion. The arrhythmic score was 3.790 ± 0.548 in this group. No statistical difference was found in the arrhythmic score between the IR and EAN groups, suggesting that EA at nonacupoints produces no significant antiarrhythmic effect in rats subjected to myocardial ischemia and reperfusion.

The effects of EA pretreatment on risk zone/infarct size in rats with ischemia and reperfusion

As shown in Fig. 3, the ratio of infarct size/risk zone was 1.2 ± 0.1 and 38.6 ± 2.9 in the NC and IR groups, respectively, indicating that the animal model of ischemic injury in myocardium was well established. The myocardial ischemia injury was significantly diminished by EA, which was presented by a comparatively lower ratio of risk zone/infarct size of 21.5 ± 1.7 in the EA group. However, when propranolol was given before EA, the ratio of infarct size/risk zone was 36.6 ± 3.7, being almost the same as that in the IR group. The results showed that the protective effect of EA on myocardium subjected to ischemia and reperfusion was blocked by the selective antagonist of cardiac β-adrenergic receptor. The ratio of infarct size/risk zone was 34.8 ± 1.6 in the EAN group, which was still significantly higher than the ratio in the NC group. The result showed that no significant effect was produced by the EA at nonacupoints.

DISCUSSION

Acupuncture has been shown to attenuate the cardiac injury induced by myocardial ischemia and reperfusion (MIR) [10, 14]. However, patients with coronary heart disease (CHD) confined to clinics rarely go to an acupuncturist because CHD at an acute stage is usually too urgent to be treated only by acupuncture. Therefore it is interesting to find a proper way to exert the beneficial effect of acupuncture, an economic and simple therapy, in the treatment and prevention of CHD. Pretreatment with acupuncture, i.e., the treatment of patients susceptible to CHD before they are attacked, may be a better way for acupuncture to treat and prevent the disease.

The important findings in the present study are that cardiac injury and arrhythmias induced by ischemia and reperfusion were both significantly attenuated by the repetitive pretreatment with electroacupuncture (EA). One possible underlying mechanism for EA pretreatment to produce cardiac protection and an antiarrhythmic effect may be to influence the activity of the sympathetic nervous system so as to protect the cardiac β-ARs from sympathetic overstimulation during subsequent ischemia and reperfusion.

Sympathetic activity [2, 15], catecholamines [2], and β1-AR [16, 17] are shown to be associated with both myocardial injury of ischemia and reperfusion. The ischemic injury of myocardium was due, at least partially, to the overexcitation of the sympathetic nervous system [1] and hyperactivity of cardiac β-ARs [18]. A receptor-binding study showed that the number of β-ARs was increased during the reperfusion following a one-hour blockage of coronary blood flow [19]. Furthermore, the enhancement of oxygen consumption caused by the increment of myocardial contractility following the excitation of β-ARs can worsen the oxygen deficit of cardiac cells and aggravate the ischemic arrhythmias. During ischemia and reperfusion, a sensitization of β-AR was induced so that the release of catecholamines at only 10% to 15% of normal concentration could elicit cardiac arrhythmias [20].

It was shown that 5-min beta-adrenergic preconditioning with isoproterenol significantly attenuates myocardial dysfunction induced by following a prolonged ischemia and reperfusion via desensitizing cardiac β-ARs [21]. This cardioprotective effect was similar to that of ischemic preconditioning, which was known to increase transiently sympathetic activity and the release of catecholamines [22], leading to a desensitization or adaptation of β-ARs to the sympathetic overstimulation induced by prolonged ischemia and reperfusion. It is interesting that the activity of the sympathetic nervous system was reported to be affected by somatic stimulation [23, 24]. It was also reported that EA stimulation significantly enhanced sympathetic activity [8]. Thus the repetitive EA pretreatment may also induce the desensitization of β-ARs via stimulating sympathetic activity to achieve the cardioprotective effects. In the present study, the cardioprotection of EA pretreatment was abolished by the administration of propranolol to each time of EA stimulation, which is in agreement with results in the previous studies on the cardioprotection of β-adrenergic preconditioning [6, 21].
β-ARs from stimulation by the sympathetic hyperactivity following EA stimulation. Therefore pretreatment with it before EA stimulation may first attenuate the desensitization of the receptors, which should be normally induced by repetitive EA stimulation, and then blunt the cardioprotection of repetitive EA pretreatment.

CONCLUSION
Repetitive EA pretreatment at Neiguan acupoints significantly attenuated the elevated ST segment of ECG, myocardial infarct size and arrhythmias induced by ischemia and reperfusion in rats, in which cardiac β-adrenergic receptors were involved. EA at nonacupoints produced no significant cardioprotection. The results suggest a very likely better way to exert the cardioprotective effect of acupuncture in the prevention and treatment of CHD. Further study needs to explore the subtype(s) of β-adrenergic receptors and the signaling pathway(s) in the mediation of the cardioprotection of EA pretreatment.

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REFERENCES