Original Article

Effects of electroacupuncture with dexmedetomidine on myocardial ischemia/reperfusion injury in rats

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Background: To investigate the protective effect of electroacupuncture combined with dexmedetomidine (EA + Dex) on oxidative stress injury in myocardial ischemia/reperfusion (I/R) rats.

Methods: A total of 50 male Sprague-Dawley (SD) rats were randomly divided into 5 groups: sham operation (sham group); I/R group; dexmedetomidine group (Dex group); electroacupuncture group (EA group); and EA + Dex group. The myocardial I/R model was established. The EA group received EA at the Neiguan acupoint [pericardium 6 (PC6)] every day for 1 week before modeling. Rats in the EA + Dex group received EA at PC6 every day for 1 week before modeling, and intraperitoneal injection of Dex was performed 15 minutes before modeling. Dex was injected intraperitoneally in the Dex group 15 minutes before modeling. The rats were sacrificed 1 hour after reperfusion, and myocardial tissue was obtained to measure the myocardial infarction area. The myocardial tissue pathologic changes were shown by hematoxylin and eosin (HE) staining, and the superoxide dismutase (SOD), malondialdehyde (MDA), adenosine triphosphate (ATP), and reactive oxygen species (ROS) content in serum was determined.

Results: Compared with the sham group, the myocardial infarction area was significantly increased ($P<0.01$), SOD and ATP content was significantly decreased ($P<0.01$), and MDA and ROS content was significantly increased ($P<0.01$) in the I/R group; this change was significantly reduced in the Dex, EA, and EA + Dex groups ($P<0.01$). The indicators in the EA + Dex group were better than those in the EA and Dex groups ($P<0.05$). There was no significant change in the above indices in the Dex group compared with the EA group ($P>0.05$).

Conclusions: EA + Dex pretreatment improved the damage of myocardial I/R by increasing SOD and ATP content and reducing the generation of MDA and ROS in an oxygen-free radical system.

Keywords: Electroacupuncture (EA); myocardial ischemia/reperfusion (myocardial I/R); dexmedetomidine (Dex); oxidative stress

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Introduction

Myocardial ischemia/reperfusion (I/R) injury is a complex pathophysiologic process that occurs during hypoxia/reoxygenation treatment. Free oxygen radicals are widely recognized as the mechanism underlying the injury. Myocardial I/R injury can activate the free radical chain reaction to produce more free oxygen radicals, such as reactive oxygen species (ROS), superoxide dismutase

(SOD), and malondialdehyde (MDA). The free oxygen radicals further react with cellular components to cause cell structure damage and energy metabolism disturbances (1). Studies in traditional Chinese medicine (TCM) have shown that needling the Neiguan acupoint [pericardium 6 (PC6)] with electroacupuncture (EA) can increase the mitochondrial SOD and adenosine triphosphate (ATP) content of cardiomyocytes in rats with a myocardial I/R injury. Such pretreatment protects the mitochondrial structure and function, improves cardiac function and cardiomyocyte activity, and decreases the infarct area. Needling the relevant acupoints by EA has an excellent protective effect on cardiomyocytes (2-5). Sinomenine and sodium aescinate can protect the myocardium of I/R rats by inhibiting inflammation (6,7). According to studies in Western medicine, dexmedetomidine (Dex) pretreatment reduces the myocardial I/R injury area in rats and inhibits myocardial apoptosis due to the I/R injury by inhibiting endoplasmic reticulum stress, the inflammatory response, and mitochondrial oxidative stress (8-11). Both EA and Dex have been shown to be protective for cardiomyocytes; however, whether the myocardial protective effect can be enhanced by EA + Dex has rarely been reported. An in-depth investigation is needed to determine the mechanism of action. Herein we discuss the protective effects of EA + Dex against myocardial I/R injury in a rat model of myocardial I/R injury and tentatively propose the underlying mechanism. We present the following article in accordance with the ARRIVE reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-969/rc).

Methods

General materials

Healthy adult male Sprague-Dawley (SD) rats, each weighing approximately 200 g, were purchased from Beijing Huafukang Biotechnology Co., Ltd. [laboratory animal license No. SCXK (Beijing) 2019-000; Beijing, China]. Animal experiments and raising animals were carried out in Yishengyuan Gene Science and Technology. Animal experiments was approved by institutional ethics board of Yishengyuan Gene Science and Technology (No. YSY-DWLL-2021085), in compliance with Chinese guidelines for the care and use of animals. A protocol was prepared before the study without registration. The reagents and instruments used for the study were as follows: inhaled sevoflurane, manufactured by Shanghai Hengrui Pharmaceutical Co., Ltd. (batch No. 20042731; Shanghai, China); small animal anesthesia machine (ZS-MV-IV series; Beijing Zhongshidichuang Science and Technology Development Co., Ltd., Beijing, China); Hwato SDZ-II Electronic Acupuncture Stimulator Machine (Suzhou, China); centrifuge machine (Eppendorf-5430; Hamburg, Germany); and a microplate reader (DNM-9602; Perlong Medical, Beijing, China).

Study methods

Building the rat model of myocardial I/R injury

The rats were weighed and anesthetized using the small animal anesthesia machine under 3% sevoflurane. After the corneal reflex had disappeared, the rats were immobilized on the operating table in a supine position. The skin of the chest was prepared, followed by conventional disinfection and spreading of the sterile hole towel. The skin was incised from the fourth intercostal space on the left, and blunt dissection of muscles was performed to open the chest cavity. The pericardium was cut open, and the heart was delivered. A thread was passed through the left auricle and the pulmonary conus for ligation 1–2 mm from the starting point of the left anterior descending artery and its branches. A pediatric tympanostomy (PE) tube was placed where the thread was passed. Finally, the thread was tied in a loose knot to complete the modeling. The myocardial I/R injury was induced by subjecting the heart to ischemia for 30 minutes, followed by reperfusion for 60 minutes. Ischemia induction was considered successful if there was a significant ST-segment elevation on electrocardiogram (ECG) and a darkened color appeared in the myocardium below the ligation line. The heart was reperfused for 60 minutes after 30 minutes of ligation. The reperfusion was considered successful upon observing local inflammatory edema, exudation, and congestion with ST-segment depression by >1/2 (3,12). The rats were sacrificed for tissue harvest 1 hour after the reperfusion procedure.

Animal grouping and treatments

A total of 50 male SD rats were randomly divided into 5 groups, with 10 rats in each group. In the sham operation group, after exposure, the heart was only threaded without ligating the left coronary artery. In the I/R group, the left coronary artery was ligated for 30 minutes, followed by reperfusion for 60 minutes to build the I/R injury model. In the EA group, the PC6 acupoint was needled by EA

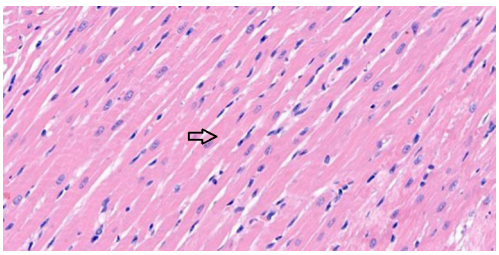

Figure 1 Sham group (HE, 400×). The arrow indicates that the cardiomyocytes are neatly arranged, morphologically intact, rich in cytoplasm, full in nuclei, and clear in the nucleoli. No abnormalities were found in the myocardial interstitium. HE, hematoxylin and eosin.

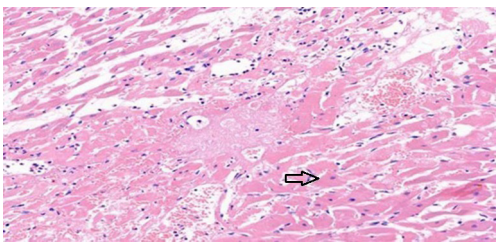

Figure 2 I/R group (HE, 400×). The arrow points to the observation of myocardial fiber rupture, injury and swelling, nuclear chromatin concentration, and unclear nucleoli. Locally, a large amount of inflammatory cell infiltration was noted. I/R, ischemia/reperfusion; HE, hematoxylin and eosin.

bilaterally 7 days before the modeling for the I/R group. The acupoint was located according to the Rat Experimental Atlas (13). The EA procedure was implemented under the following parameters: needling depth, approximately 5 mm; electric current,1 mA; and frequency, 2 Hz (3). Each electrical simulation continued until a mild limb tremor was observed. The EA was delivered once daily for 30 minutes per treatment for 7 consecutive days. The I/R injury was induced as planned 7 days later. An equal volume of normal saline was injected intraperitoneally 15 minutes before inducing the I/R injury. In the Dex group, Dex (5 μg/kg) was injected intraperitoneally 15 minutes before inducing the I/R injury (7). In the EA + Dex group, PC6 was stimulated bilaterally by EA for 7 consecutive days. Dex (5 μg/kg) was injected intraperitoneally 15 minutes before inducing the I/R injury, followed by the same modeling procedure as in the I/R group. The animals were kept in the specific-pathogen-free standard animal room, and the feeding conditions were as follows: the temperature of the animal room was 20–25 ℃, the relative humidity was 50%±10%, and the water was freely accessible.

Detection indicators

Infarct area calculation

The modeling was established over 1 hour after all specified procedures were completed. The whole heart was fixed in formaldehyde and subjected to 2,3,5-triphenyletetrazolium chloride (TTC) staining. The normal tissues stained blue, while the infarct tissues were white and not stained. The infarct area-to-total myocardial ratio was calculated using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA).

Determination of serum SOD, MDA, ATP, and ROS content

We obtained and centrifuged 100 g of myocardial tissues. The centrifuge tube was placed in a boiling water bath for 10 minutes, then removed, shaken well, and centrifuged at 1,301 g for 10 minutes. The supernatant was collected for detection of SOD, MDA, ATP, and ROS. All of the above indicators were determined according to the instructions provided with the kits. The kits were manufactured by the Nanjing Jiancheng Biological Engineering Research Institute (Nanjing, China).

Statistical analysis

Statistical analyses were performed using SPSS 18.0 software (IBM Corp., Chicago, IL, USA). Measurement data are expressed as the mean ± standard deviation ($\bar{x}$±s). Tests for normality and homogeneity of variance were performed. The means of the groups were compared by one-way analysis of variance (ANOVA). Dunnet's test was used for multiple comparison tests. A P value <0.05 was considered statistically significant.

Results

Results of myocardial hematoxylin and eosin (HE) staining

In the sham operation group, the cardiomyocytes were neatly aligned, with intact morphology, abundant cytoplasm, plump nuclei, and clear nucleoli. No abnormalities were found in the myocardial interstitium (*Figure 1*). In the I/R group, fractured, damaged, and swollen myocardial fibers, nuclear chromatin concentration, and unclear nucleoli were observed. Massive inflammatory cell infiltration was noted locally (*Figure 2*). In the EA and Dex groups, myocardial

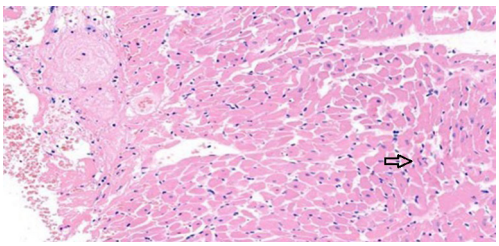

Figure 3 EA group (HE, 400×). The myocardial fiber rupture and swelling shown by the arrow improved compared to the I/R group. Mild inflammatory cell infiltration was observed locally. EA, electroacupuncture; HE, hematoxylin and eosin; I/R, ischemia/reperfusion.

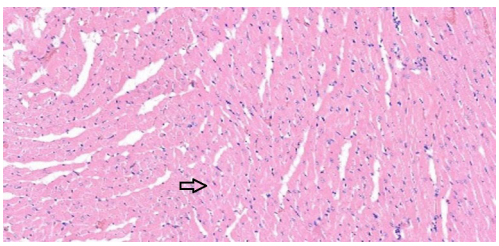

Figure 4 Dex group (HE, 400×). The rupture and swelling of the myocardial fibers pointed to by the arrow are less than I/R. Mild inflammatory cell infiltration was observed locally. Dex, dexmedetomidine; HE, hematoxylin and eosin; I/R, ischemia/reperfusion.

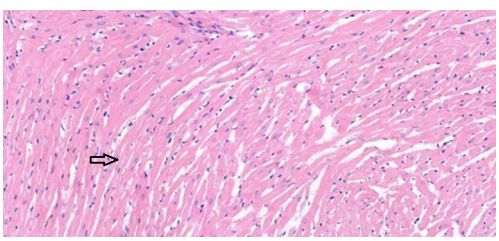

Figure 5 EA + Dex group (HE, 400×). The arrow shows the myocardial fibers neatly arranged, with some mild swelling. A small number of myocardial fibers are broken. EA + Dex, electroacupuncture combined with dexmedetomidine; HE, hematoxylin and eosin.

fiber fracture and swelling were less significant than the I/R group. Mild inflammatory cell infiltration was observed locally (*Figures 3*,*4*). In the EA + Dex group, the myocardial fibers were neatly aligned, and some were mildly swollen. A few myocardial fibers were fractured (*Figure 5*). The arrows in each figure show the observation site of pathological changes in myocardial tissue in each group.

Table 1 Infarct areas calculated for each group

Group	Case	Infarct area: total tissue area ratio
Sham group	10	0
I/R group	10	2.32±0.04*
Dex group	10	1.52±0.02**
EA group	10	1.55±0.05**
EA + Dex group	10	0.18±0.02**$^{\Delta}$

Data are present as $\bar{x}$±s. *P<0.01 for the comparison between the I/R and sham groups; **P<0.01 for the comparison of the EA, Dex, and EA + Dex groups against the I/R group; $^{\Delta}$P<0.05 for the comparison of the EA + Dex and EA groups against the Dex group; P>0.05 for the comparison between the EA and Dex groups. I/R, ischemia/reperfusion; Dex, dexmedetomidine; EA, electroacupuncture; EA + Dex, electroacupuncture combined with dexmedetomidine; $\bar{x}$±s, mean ± standard deviation.

Compared with the sham operation group, the infarct area of the I/R group was significantly increased (P<0.01). The infarct area of the Dex, EA, and EA + Dex groups were significantly decreased compared with the I/R group (P<0.01). In addition, the infarct area of the EA + Dex group was considerably smaller than the EA and Dex groups (P<0.05). There was no significant difference in the infarct area between the Dex and EA groups (P>0.05; *Table 1*).

Changes in the serum SOD, MDA, ATP, and ROS content

Compared with the sham group, the SOD and ATP content of the I/R group decreased significantly (P<0.01), while the MDA and ROS content increased considerably (P<0.01). Compared with the I/R group, the SOD and ATP content of the Dex, EA, and EA + Dex groups increased significantly (P<0.01), while the content of the MDA and ROS decreased dramatically (P<0.01). The EA + Dex group outperformed the Dex and EA groups in all indicators (P<0.05). There were no significant differences between the Dex and EA groups in the above indicators (P>0.05; *Table 2*).

Discussion

Myocardial I/R injury is a complex pathophysiologic process that occurs during hypoxia/reoxygenation treatment. Free oxygen radicals are considered a primary cause of I/R injury (1). Under normal situations, the intracellular

Table 2 Serum SOD, MDA, ATP, and ROS content

Group	Case	SOD activity (U/mL)	MDA content (mmol/L)	ATP [μmol/(g·prot)]	ROS
Sham group	10	4.37±0.12	3.99±0.13	78.93±50.18	70.20±9.84
I/R group	10	2.52±0.19*	5.73±0.03*	14.60±13.09*	103.26±10.83*
Dex group	10	3.49±0.05**	4.69±0.04**	27.35±1.78**	94.66±1.12**
EA group	10	3.55±0.01**	4.87±0.01**	26.39±1.67**	94.57±1.90**
EA + Dex group	10	3.87±0.04**$^{\Delta}$	4.39±0.01**$^{\Delta}$	31.21±2.17**$^{\Delta}$	97.20±0.47**$^{\Delta}$

Data are present as $\bar{x}$±s. *P<0.01 for the comparison between the I/R and sham groups; **P<0.01 for the comparison of the EA, Dex, and EA + Dex groups against the I/R group; $^{\Delta}$P<0.05 for the comparison of the EA and Dex groups against the EA + Dex group; P>0.05 for the comparison between the EA and Dex groups. SOD, superoxide dismutase; MDA, malondialdehyde; ATP, adenosine triphosphate; ROS, rective oxygen species; I/R, ischemia/reperfusion; Dex, dexmedetomidine; EA, electroacupuncture; EA + Dex, electroacupuncture combined with dexmedetomidine; $\bar{x}$±s, mean ± standard deviation.

antioxidants can clean up the free oxygen radicals to achieve a balance between the production and removal of free oxygen radicals (1). Myocardial I/R results in the production of large amounts of ROS via the xanthine/xanthine oxidase system, activated neutrophils, and damage to the mitochondrial respiratory chain (1,2). As the balance between the production and removal of free oxygen radicals is disrupted, the cardiomyocytes are damaged structurally and functionally (1). The resulting disturbance of myocardial energy metabolism further inhibits the activity of SOD, an oxygen-free radical scavenging enzyme (1,2). Moreover, intracellular calcium overload inhibits oxidative phosphorylation, leading to an ATP synthase disturbance (1,2). The lipid peroxidation reaction increases cell membrane permeability, promoting the reaction between the free oxygen radicals and the unsaturated fatty acids to produce MDA, which further aggravates cell damage (1,2,12). Studies have shown that needling PC6 by EA increases the mitochondrial SOD and ATP content of rat cardiomyocytes with an I/R injury (2). Cardiac function and cardiomyocyte activity is significantly improved following I/R injury, reducing the infarct area and the degree of mitochondrial swelling, thus protecting mitochondrial integrity (2,3,14). Our study showed that stimulating PC6 by EA significantly increased the SOD and ATP content, while reducing MDA and ROS production. The myocardial damage caused by free oxygen radicals was alleviated, which was consistent with the findings of the aforementioned studies.

Dex is a highly selective α_2-adrenergic receptor agonist and has been widely used in the perioperative setting due to its anti-sympathetic and analgesic effects, and the resulting reduction in anesthetic dosage. A growing number of studies have confirmed the vital role of Dex in I/R injury (15). The protective effect of Dex has been evidenced against cerebral I/R injury, alleviating the inflammatory and stress responses (16). Pretreatment with Dex can reduce the area of myocardial I/R injury in rats. Dex can also inhibit apoptosis of rat cardiomyocytes with I/R injury by upregulating Bcl-2 expression and downregulating Bax expression (17). Intestinal I/R injury activates the autophagic apoptosis of cells. Dex inhibits the autophagic apoptosis of cells induced by intestinal I/R injury via the HMGB1/TLR4/NF-κB pathway, thereby exerting a protective effect against intestinal I/R injury (18). We also found that Dex increased SOD and ATP content, reducing MDA and ROS production and having a protective effect against myocardial I/R injury. The above finding agrees with the viewpoint that Dex has a protective effect against I/R injury in cells.

Both EA and Dex have a protective effect against myocardial I/R injury. However, few reports have involved the EA + Dex pretreatment in I/R injury. There is also a scarcity of investigations from the perspective of the potential impact on the free oxygen radical-scavenging ability. Here, we delivered EA + Dex pretreatment in a rat model of myocardial I/R injury. The results showed that the combination treatment group had increased SOD and ATP content, while reducing MDA and ROS production to alleviate myocardial I/R injury. In addition, combination treatment outperformed either monotherapy group in all indicators.

To conclude, EA + Dex reduced MDA and ROS production and alleviated myocardial I/R injury by increasing the SOD and ATP content. Combining TCM and Western medicine may offer a new pathway to prevent and treat I/R injury.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-969/rc

Data Sharing Statement: Available at https://apm.amegroups.com/article/view/10.21037/apm-22-969/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-969/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments was approved by institutional ethics board of Yishengyuan Gene Science and Technology (No. YSY-DWLL-2021085), in compliance with Chinese guidelines for the care and use of animals.

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