


# Effects of post-treatment electroacupuncture on ventricular monophasic action potential and cardiac function in a rat model of ischemia/reperfusion injury

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## Abstract

**Background:** To determine the effects of post-treatment electroacupuncture (EA) on the electrophysiological properties of ventricular muscle in rats with ischemia/reperfusion (IR) injury.

**Methods:** Male Sprague–Dawley (SD) rats were randomly assigned into sham-operated (SH), IR and IR + EA groups ( $n = 8$  each). The IR model was generated by ligation of the left anterior descending (LAD) coronary artery for 30 min. After establishing the IR model, EA was administered at PC6 for 30 min while opening the coronary artery and allowing reperfusion for 30 min. Heart rate (HR), mean arterial pressure and monophasic action potential (MAP) of cardiac muscle in the outer membrane of the antetheca of the left ventricle before coronary artery ligation ( $T_0$ ), after coronary artery ligation for 30 min ( $T_1$ ) and after reperfusion for 30 min ( $T_2$ ) were recorded. At the same time, ventricular electrophysiological parameters including ventricular effective refractory period (ERP), conduction velocity (CV) and ventricular fibrillation threshold (VFT) were measured. Then, the cardiac function and the levels of creatine kinase-muscle/brain (CK-MB) and cardiac troponin I (cTnI) were monitored. Based on these data, monophasic action potential amplitude (MAPA), the maximum depolarization velocity ( $V_{\max}$ ) and the MAP durations at 50% and 90% repolarization (MAPD<sub>50</sub> and MAPD<sub>90</sub>) were calculated to determine the incidence of arrhythmia during reperfusion.

**Results:** Compared with the SH group, the IR group showed an obviously decreased HR as well as reduced mean arterial pressure,  $V_{\max}$ , CV, ERP and MAPA. All indices of cardiac function except left ventricular end-diastolic pressure (LVEDP) decreased (i.e. ventricular systolic pressure (LVSP), left ventricular ejection fraction (LVEF), fractional shortening (FS) and rate of the ventricular pressure rise/drop ( $\pm dp/dt$ )). Furthermore, the MAPD<sub>50</sub> and MAPD<sub>90</sub> were prolonged, and the levels of CK-MB and cTnI increased ( $p < 0.05$ ). In comparison to the IR group, HR and the mean arterial pressure were increased. All indices of cardiac function except LVEDP increased (LVSP, LVEF, FS and  $\pm dp/dt$ ).  $V_{\max}$ , CV, ERP and MAPA were also increased in the IR + EA group. However, MAPD<sub>50</sub> and MAPD<sub>90</sub> were distinctly shortened, and the levels of CK-MB and cTnI decreased ( $p < 0.05$ ). There were no statistically significant differences in VFT between the three groups ( $p > 0.05$ ).

**Conclusion:** EA post-treatment can relieve prolongation of repolarization and slowed depolarization of ventricular muscle during IR, thus decreasing the rate of incidence of reperfusion arrhythmia.

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acupuncture point, cardiac arrhythmia, electroacupuncture, ischemia/reperfusion, monophasic action potential

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## Introduction

Reperfusion arrhythmia (RA) refers to arrhythmia occurring after a blocked or spasmodic coronary artery regains blood flow. RA generally occurs within 30 min of reperfusion and can cause serious hemodynamic disruption, thus increasing disability and morbidity among patients.<sup>1</sup> During ischemia/reperfusion (IR), electrophysiological disorders are likely to occur, such as prolonged duration of myocardial repolarization and dysfunction of ion channels.<sup>2,3</sup> In arrhythmia studies, the monophasic action potential (MAP), as an index of vector changes in the heart during arrhythmia, can intuitively and stably describe the repolarization phase of myocardial cells. Moreover, it is an important means for observing the electrical activities of myocardial cells.<sup>4,5</sup> The action potential duration recorded by the synchronous recording technique for MAP is closely related to arrhythmia.<sup>6</sup>

Acupuncture is a form of traditional Chinese medicine that has been used for more than 1000 years to relieve pain and treat various diseases including arrhythmia.<sup>7,8</sup> Electroacupuncture (EA) is an effective acupuncture method that has been successfully used to treat many arrhythmia-related diseases such as ventricular premature beats, paroxysmal supraventricular tachycardia and atrial fibrillation.<sup>9–11</sup> Previous studies have demonstrated that acupuncture at PC6 (*Neiguan*) can protect against IR injury and arrhythmia in rats;<sup>12–14</sup> however, the related electrophysiological mechanisms have not been thoroughly elucidated.

The aim of this study was to investigate the effects of post-treatment EA on arrhythmia and the electrophysiological properties of the heart in a rat model of IR injury, to provide translationally relevant data for clinical development.

## Methods

### *Animals and grouping*

All experimental protocols were approved by the Experimental Animal Care and Institutional Animal Ethical Committee of Guizhou Medical University (Guizhou, China). All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 86-23, revised 1985). Twenty-four clean-grade healthy male Sprague–Dawley (SD) rats, weighing 280–320 g, were provided by the Experimental Animal Center of Guizhou Medical University, China. Rats were fed a standard diet and were housed at 25°C and 60% humidity under a 12-h light–dark cycle. All rats were allowed access to food and water ad libitum for 1 week before experiments. In accordance with a random number table method, they were divided into three groups ( $n=8$  each): (1) the

sham-operated group (SH group), in which the left anterior descending (LAD) coronary arteries were threaded but not ligated during the operation; (2) the IR group, in which the LAD coronary arteries were ligated for 30 min and reperused for 30 min; and (3) the IR + EA group, in which the LAD coronary arteries were ligated for 30 min and reperused for 30 min and, at the same time, EA was administered at PC6 for 30 min.

### *Establishment of a myocardial IR model*

Under anesthesia via an intraperitoneal injection of pentobarbital (40 mg/kg; Sigma Aldrich; Merck KGaA, Darmstadt, Germany), the SD rats were fixed on a thermostatic surgery table for small animals and connected to the corresponding electrodes in order to record the electrocardiogram (ECG) in lead II. Rats with cardiac dysfunction were excluded. Ventilation was provided via tracheotomy using the following parameters: tidal volume 8 mL/kg, breath frequency 45–60 times/min and inspiration-to-expiration ratio 1:2. An endotracheal tube was inserted into the trachea, fixed and then connected to the animal ventilator for mechanical ventilation. The ECG and mean arterial pressure of the rats were monitored in real time, and hemodynamic changes observed. A longitudinal incision was made at the apex cordis at the left margin of the sternum where the pulse was the strongest for blunt dissection of subcutaneous tissue, and the pericardium was cut to expose the heart. On the right side of the pulmonary conus, the left atrial appendage was lifted with a pair of tweezers to allow a thread to pass through a position 2–3 mm below the lower margin of the left atrial appendage. The LAD coronary artery was ligated with no. 6 silk thread. After ligation, lead II of the ECG was observed. After establishing the acute myocardial ischemia model, ischemia was continued for 30 min, and then the suture was cut to reperuse the ischemic heart for 30 min. The ischemia model was verified by an elevated ST segment or higher magnitude of the QRS complex, plus wider waves and T wave fusion, and a >20 mm Hg decrease in arterial pressure, with cyanosis of the ventricular walls at the ligated region. The reperfusion model was verified as the disappearance of cyanosis, and a >50% decrease in ST segment elevation at 30 min after reperfusion. All subjects that met the above criteria were included in the experimental results and those not conforming to these standards were excluded.

### *Detection of cardiac function*

The left ventricular ejection fraction (LVEF) and fractional shortening (FS) were determined using diagnostic

ultrasonic equipment, and then a micro-catheter with a pressure transducer was inserted into the left ventricle via the right carotid arteries. A multichannel physiological recorder was used to measure and record the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), rate of the ventricular pressure rise (+dp/dt) and rate of the ventricular pressure drop (-dp/dt).

## EA

According to the textbook “Experimental Acupuncture,” PC6 is located in the ulnar and radial seam, about 3 mm from the wrist joint.<sup>15,16</sup> A disposable sterile acupuncture needle (0.30 mm × 25 mm) was used for direct acupuncture to a depth of 2–3 mm at PC6 bilaterally in the upper limbs of the rats. Afterwards, an EA instrument (Huatuo SDZ-II electronic needle therapy apparatus; Suzhou Medical Supply Factory Co., Ltd, Suzhou, China) was connected and adjusted to deliver dilatational waves at a frequency of 1–2 Hz, a current intensity of 2 mA and load voltage of 1 mV. EA was performed for 30 min (reaching the intervention standard that both upper limbs trembled slightly).

## Observation indices

After successful ligation, the compound electrode for measuring the MAP of ventricular muscle was slowly inserted into the antetheca of the left ventricle. By placing electrodes in the outer membrane on the heart’s surface and the reference electrode on the root of the aorta, the waveforms of the MAP and ECG of each rat were recorded. Soon afterwards, the electrode lead was connected to the input line of signals of a BL-420F biofunctional experimental system (Taimeng Software Co., Ltd, Chengdu City, Sichuan Province, China). Based on these data, heart rate (HR), mean arterial pressure, monophasic action potential amplitude (MAPA) in the outer membrane of the antetheca of the left ventricle, conduction velocity (CV), maximum depolarization velocity ( $V_{max}$ ) and MAP durations at 50% and 90% repolarization (MAPD<sub>50</sub> and MAPD<sub>90</sub>, respectively) were recorded before coronary artery ligation ( $T_0$ ), after coronary artery ligation for 30 min ( $T_1$ ) and after reperfusion for 30 min ( $T_2$ ). By referring to the Curtis–Walker grading standard,<sup>17</sup> the incidence of arrhythmia during reperfusion was recorded.

## Measurement of ventricular ERP and VFT

Pairs of platinum stimulating electrodes were deposited on the left ventricular free walls including the ischemic area (under the ligation site, close to the left ventricular apex) and a remote non-ischemic area (near the left ventricular base). All effective refractory periods (ERPs) were measured by the S1–S2 stimulating method containing eight successive stimuli (S1–S1=150 ms) followed by an additional stimulus (S2). The time interval of S1–S2 began at 120 ms and was

gradually shortened to loss capture at 10 ms each time and then at 1 ms. ERP was defined as the longest S1–S2 time interval that failed to capture the ventricle. The paired platinum stimulating electrodes were placed in the right ventricle to perform a burst stimulation (4 ms pulse widths at 50 Hz). Each stimulus lasted for 5 s and every two successive stimuli were separated by 2 s. The stimulating voltage started at 4 V and was increased by 1 V, and the ventricular fibrillation threshold (VFT) was defined as the lowest voltage that induced sustained ventricular fibrillation ( $\geq 3$  s).

## Enzyme-linked immunosorbent assay

After the relevant indicators were recorded, fresh blood was harvested from the right carotid arteries of the rats and centrifuged at 500g for 10 min at 4°C to extract the serum. Cardiac troponin I (cTnI) content was determined using an enzyme-linked immunosorbent assay (ELISA) kit (E-H149, Nanjing Jiancheng Biological Product, Nanjing, China), according to the detailed instructions provided by the manufacturer. Briefly, after conducting the reaction with related reagents, the absorbance of each well was quantified by a microplate reader. At the same time, various standard cTnI reagents were used to build a standard curve under the same reaction conditions. Then, the content of cTnI in each well was calculated according to the standard curve. Creatine kinase-muscle/brain (CK-MB) activity in rat serum was determined using a commercial CK-MB detection kit (15649, Sigma Aldrich, USA) according to the provided instructions. The detection methods were all based on colorimetry. After the blood collection was finished, all animals were anesthetized with 2% isoflurane and killed by cervical dislocation.

## Statistical analysis

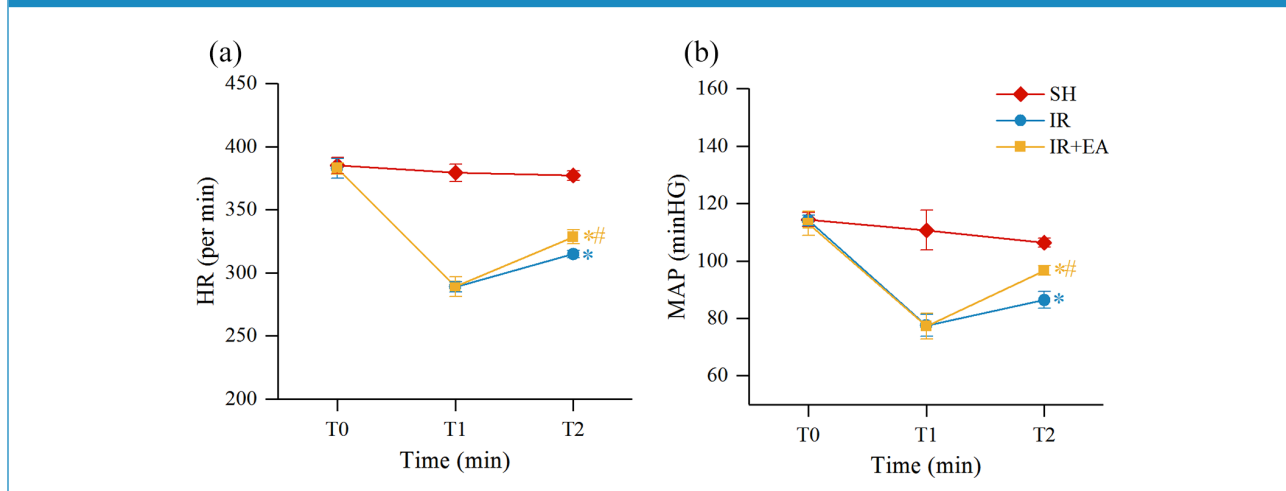
Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 20.0 statistical analysis software (SPSS Inc., Chicago, IL, USA). Arrhythmia scores were expressed as median (lower quantile and upper quantile), while the other data were expressed as mean value  $\pm$  standard deviation after testing for normality. Statistical analysis was carried out using repeated measurement analysis of variance when evaluating data obtained from different time points within a single group. Comparison between groups was carried out using one-way analysis of variance. The  $\chi^2$  test was used for enumeration data, and the values of  $p < 0.05$  were considered statistically significant.

## Results

### Influence of post-treatment EA on the hemodynamics of rats

Compared to the SH group, HR and mean arterial pressure in the IR + EA group were reduced at  $T_1$ – $T_2$  ( $p < 0.05$ ). In comparison with the IR group, HR and mean arterial pressure in

**Figure 1.** Effects of post-treatment electroacupuncture (EA) on: (a) heart rate (HR); and (b) mean arterial pressure of rats with ischemia/reperfusion (IR) injury and sham-operated (SH) controls.  $T_0$ : before coronary artery ligation.  $T_1$ : 30 min after ischemia.  $T_2$ : 30 min after reperfusion. Data are expressed as mean value  $\pm$  standard deviation ( $n=8$ /group). \* $p < 0.05$  compared to SH group. # $p < 0.05$  compared to IR group.



the IR + EA group increased at  $T_2$  ( $p < 0.05$ ). In comparison with that at  $T_0$ , the IR and IR + EA groups showed decreased HRs and reduced mean arterial pressure at  $T_1$ – $T_2$  ( $p < 0.05$ ). HRs in the IR and IR + EA groups were accelerated at  $T_2$ , compared with those at  $T_1$  ( $p < 0.05$ ; Figure 1).

#### Effects of post-treatment EA on MAPA and $V_{max}$ of rats

In comparison with the SH group, MAPA and  $V_{max}$  in the IR and IR + EA groups at  $T_1$ – $T_2$  were reduced ( $p < 0.05$ ), while those in IR + EA group were increased at  $T_2$  compared with the IR group ( $p < 0.05$ ). MAPA and  $V_{max}$  of the IR and IR + EA groups were decreased at  $T_1$ – $T_2$  ( $p < 0.05$ ) compared with those at  $T_0$ , while they were increased in the IR + EA group at  $T_2$  ( $p < 0.05$ ) compared with those at  $T_1$  (Figure 2(a) and (b)).

#### Influence of post-treatment EA on MAPD<sub>50</sub>

Compared to the SH group, MAPD<sub>50</sub> in the IR and IR + EA groups was increased at  $T_1$ – $T_2$  ( $p < 0.05$ ). In comparison with the IR group, MAPD<sub>50</sub> in the IR + EA group was reduced at  $T_2$  ( $p < 0.05$ ). In comparison with that at  $T_0$ , the IR and IR + EA groups showed increased MAPD<sub>50</sub> at  $T_1$ – $T_2$  ( $p < 0.05$ ). Compared with values at  $T_1$ , MAPD<sub>50</sub> was decreased at  $T_2$  in the IR and IR + EA groups ( $p < 0.05$ ; Figure 2(c)).

#### Impact of post-treatment EA on MAPD<sub>90</sub>

Compared with the SH group, MAPD<sub>90</sub> in the IR and IR + EA groups at  $T_1$ – $T_2$  was shown to be largely increased ( $p < 0.05$ ). In comparison with the IR group, MAPD<sub>90</sub> in the IR + EA group at  $T_2$  was significantly reduced ( $p < 0.05$ ). Compared with that at  $T_0$ , MAPD<sub>90</sub> in the IR and

IR + EA groups at  $T_1$ – $T_2$  showed an obvious increase ( $p < 0.05$ ), while MAPD<sub>90</sub> was obviously decreased in the IR + EA group at  $T_2$  compared with that at  $T_1$  (Figure 2(d)).

#### Effects of post-treatment EA on RA of rats in each group

We found that the incidence of arrhythmia and the arrhythmia scores were the highest in the IR group (mainly ventricular premature beats, ventricular tachycardia, and ventricular fibrillation) and that there appeared to be a beneficial effect of EA with respect to this phenomenon. The incidence of arrhythmia was not high, so we compared the three groups. In comparison with the SH group, the incidence rates and grades of arrhythmia in the IR and IR + EA groups were shown to be higher, while the IR + EA group showed a decreased incidence rate and grades of arrhythmia ( $p < 0.05$ ) compared with the IR group (Table 1).

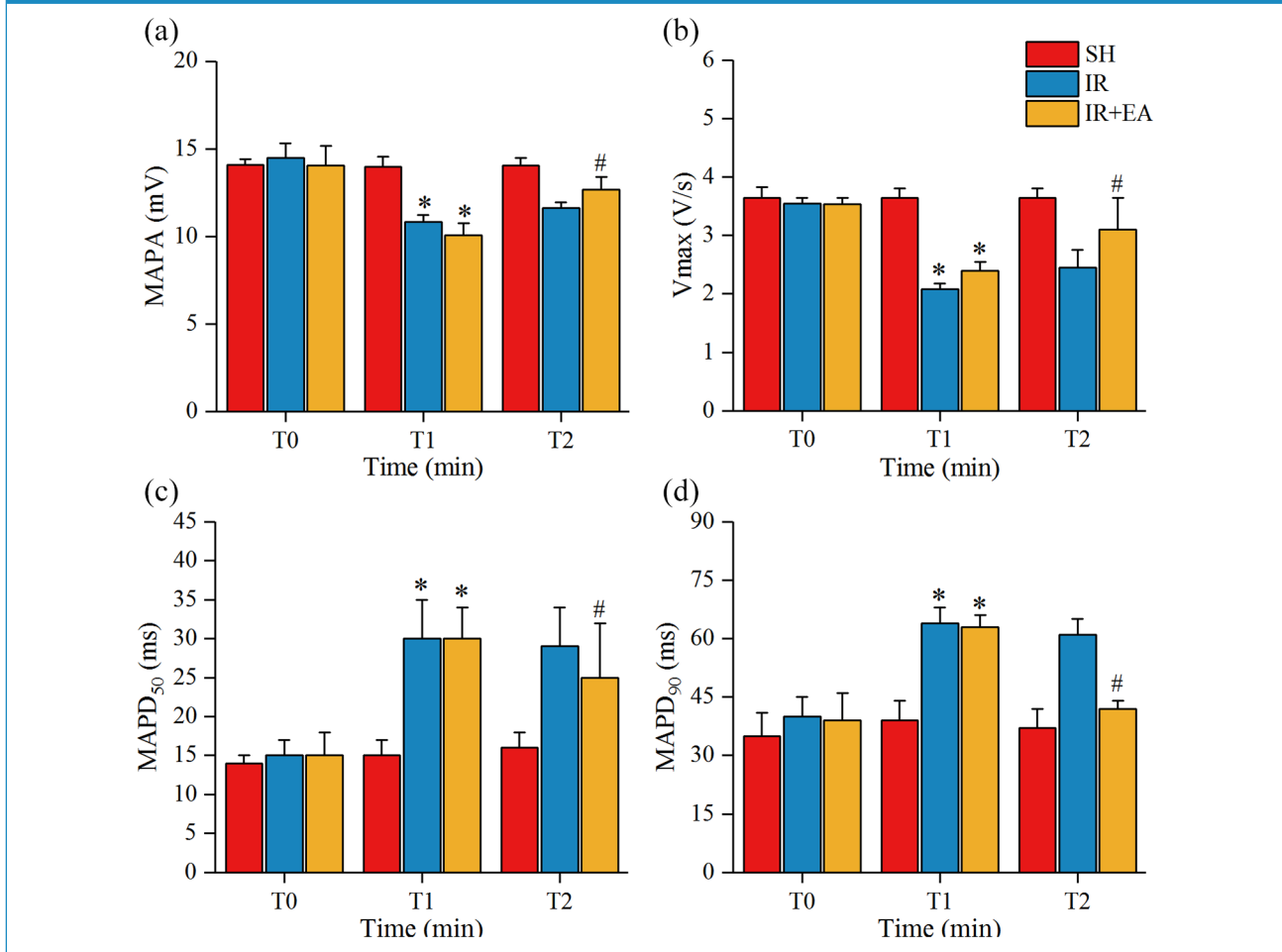
#### Effects of post-treatment EA on CV, ERP and $V_{max}$ of rats

Compared to the SH group, ERP and CV in the IR and IR + EA group were reduced ( $p < 0.05$ ). In comparison with the IR group, ERP and CV in the IR + EA group were increased ( $p < 0.05$ ; Figure 3(a) and (b)). There were no statistically significant differences in VFT between the three groups ( $p > 0.05$ ; Figure 3(c)).

#### Effects of post-treatment EA on cardiac function of rats in each group

As expected, the IR rats exhibited higher values of LVEDP as well as lower values of LVSP,  $-dp/dt$  and  $+dp/dt$  when

**Figure 2.** Effects of post-treatment electroacupuncture (EA) on: (a) monophasic action potential amplitude (MAPA); (b) maximum depolarization velocity ( $V_{max}$ ); (c) monophasic action potential durations at 50% ( $MAPD_{50}$ ); and (d) monophasic action potential durations at 90% ( $MAPD_{90}$ ) of rats with ischemia/reperfusion (IR) injury and sham-operated (SH) controls.  $T_0$ : before coronary artery ligation.  $T_1$ : 30 min after ischemia.  $T_2$ : 30 min after reperfusion. Data are expressed as mean value  $\pm$  standard deviation ( $n=8$ /group). \* $p < 0.05$  compared to SH group. # $p < 0.05$  compared to IR group.



**Table 1.** Effects of post-treatment electroacupuncture on arrhythmia scores and incidence of arrhythmia.

Group	<i>n</i>	Incidence of arrhythmia (%)	Arrhythmia scores (M [Q])
SH	8	12	0 [0, 1]
IR	8	88 <sup>a</sup>	2.5 [2, 3] <sup>a</sup>
IR + EA	8	50 <sup>a,b</sup>	1 [1, 1.75] <sup>a,b</sup>

EA: electroacupuncture; IR: ischemia/reperfusion; M: median; Q: interquartile range; SH: sham-operated.

Arrhythmia incidence and arrhythmia score were compared between groups.

<sup>a</sup> $p < 0.05$  compared to SH group.

<sup>b</sup> $p < 0.05$  compared to IR group.

compared with those in the SH group (Figure 4(a) and (b)). Noticeably, in comparison with the IR group, the IR + EA

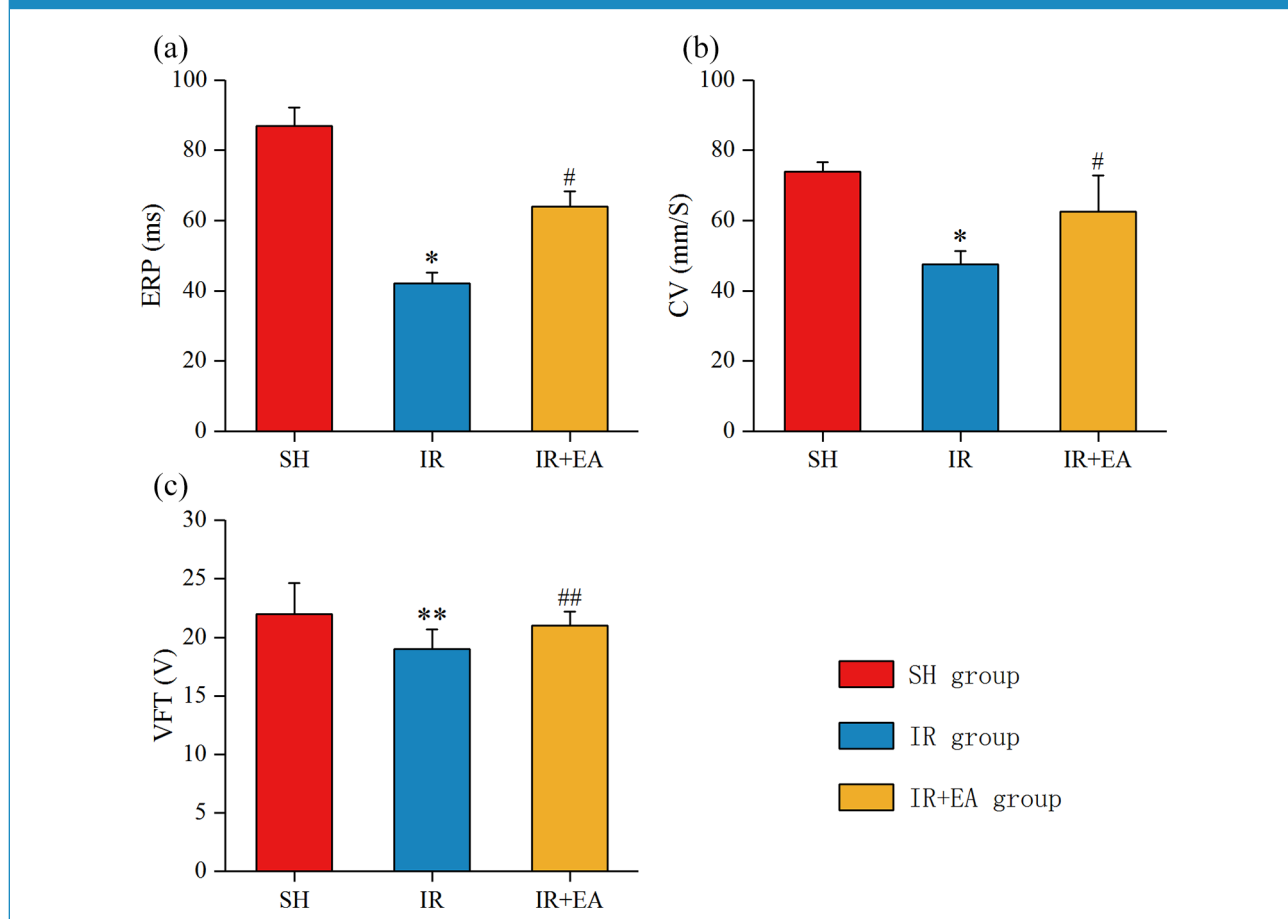
group showed substantially lower values of LVEDP and significantly higher values of LVSP,  $-dp/dt$  and  $+dp/dt$  (Figure 4(c) and (d)). As shown in Figure 5, when compared with these parameters in the sham group, the IR model rats had substantially lower values of LVEF and FS. Interestingly, the IR + EA group had increased values of LVEF and FS when compared to the untreated IR group (Figure 5(a) and (b)).

#### Effects of post-treatment EA on CK-MB and cTnl of rats in each group

We investigated the effect of EA on serum levels of myocardial infarction markers (CK-MB and cTnl) in IR rats, measured using ELISA. As shown in Figure 5(c) and (d), in comparison with the SH group, the IR rats had evidently higher serum levels of CK-MB and cTnl. As expected, the IR + EA group had lower serum levels of CK-MB and cTnl when compared to the IR group.



**Figure 3.** Effects of post-treatment electroacupuncture (EA) on: (a) effective refractory period (ERP); (b) conduction velocity (CV); and (c) ventricular fibrillation threshold (VFT) of rats with ischemia/reperfusion (IR) injury and sham-operated (SH) controls. ERP, CV and VFT were measured and compared between the three different groups ( $n=8/\text{group}$ ). Data are presented as mean value  $\pm$  standard deviation. \* $p < 0.05$  compared to SH group. # $p < 0.05$  compared to IR group. \*\* $p > 0.05$  compared to SH group. ## $p > 0.05$  compared to IR group.



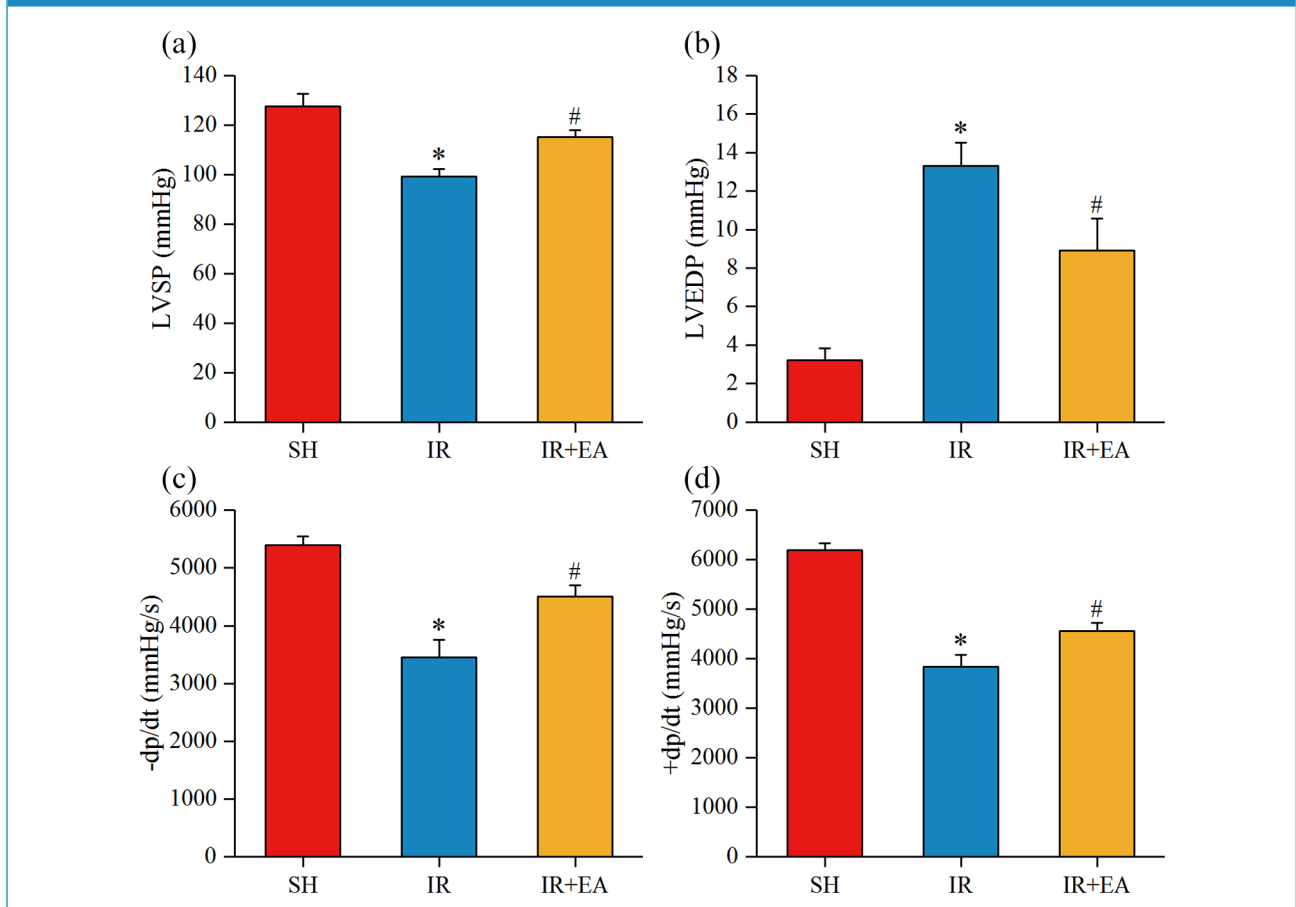
## Discussion

IR arrhythmia is a common clinical complication and a significant cause of death in patients;<sup>18,19</sup> however, its underlying mechanisms have yet to be fully elucidated. At present, it is considered that the electrophysiological mechanisms of IR arrhythmia include post-depolarization triggered activities, abnormal automaticity, abnormal intercellular communication and abnormal ion channels. The biochemical basis of the condition is thought to be associated with calcium overload and excessive production of oxygen free radicals.<sup>20</sup> There are many available methods to treat and prevent RA. Compared to antiarrhythmic drugs commonly used in clinical practice, acupuncture is an effective therapy with the advantages of being safe and cost-effective, and having minimal side effects.<sup>9,16</sup> A large number of studies suggest that acupuncture at PC6 can significantly reduce arrhythmia but, to our knowledge, there has been no in-depth analysis of its electrophysiological mechanisms.

This study established an *in vivo* acute myocardial IR model. Furthermore, using MAP technology, this research demonstrated the effects of post-treatment EA at PC6 on the electrophysiological properties of ventricular muscle with IR injury in rats. Our data provide an experimental basis for the prevention and treatment of RA after myocardial ischemia using clinical acupuncture.

Acupuncture at traditional acupuncture point locations can treat disease and is also conducive to maintaining the homeostasis of patients and accelerating recovery.<sup>21</sup> PC6 is the most commonly selected traditional acupuncture point location for the treatment of cardiovascular diseases, and stimulation at this site has been shown to achieve bidirectional regulation of arrhythmia.<sup>22</sup> Acupuncture at PC6 can adjust the excitability of cardiac sympathetic nerves, inhibit activity of the sympathetic-adrenal medulla system and reduce the release of catecholamine neurotransmitters. This improves the excitability and conductivity of myocardial cells and protects the heart.<sup>23,24</sup> A previous study indicated

**Figure 4.** Effects of post-treatment electroacupuncture (EA) on: (a) left ventricular systolic pressure (LVSP); (b) left ventricular end-diastolic pressure (LVEDP); (c) rate of the ventricular pressure drop ( $-dp/dt$ ); and (d) rate of the ventricular pressure rise ( $+dp/dt$ ) of rats with ischemia/reperfusion (IR) injury and sham-operated (SH) controls.  $n = 8/\text{group}$ . Data are presented as mean value  $\pm$  standard deviation. \* $p < 0.05$  compared to SH group. # $p < 0.05$  compared to IR group.



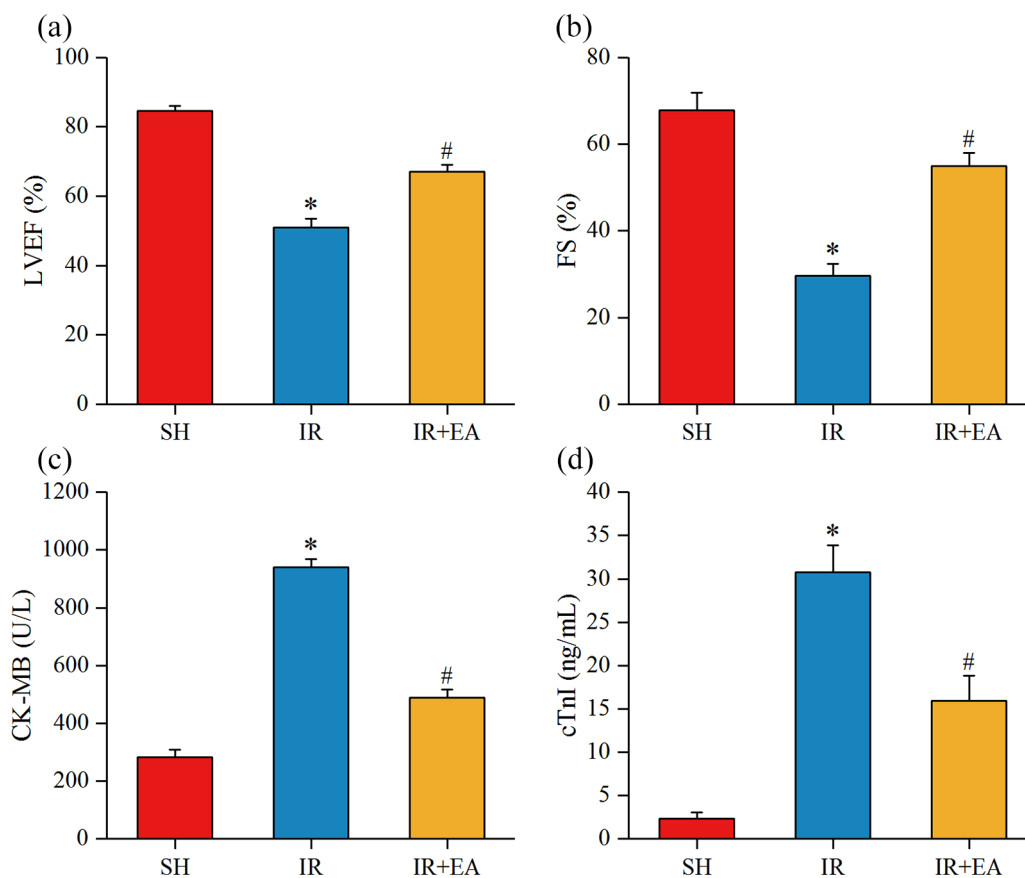
that acupuncture at PC6 can reduce the incidence rate of RA,<sup>25</sup> although its electrophysiological mechanisms have not been clearly elaborated.

Change in the electrophysiological properties of myocardial cells is a direct cause of arrhythmias.<sup>26</sup> MAP refers to the average extracellular potential of a local myocardial cell population. It can accurately reflect the whole process of local myocardial depolarization and repolarization, and so has been widely used in experimental studies of myocardial electrophysiology.<sup>13,27,28</sup> MAPA and  $V_{\max}$  mainly reveal zero-phase action potentials and represent the opening degree of fast  $\text{Na}^+$  channels and the velocity of  $\text{Na}^+$  inflow during myocardial depolarization.<sup>14</sup>  $\text{MAPD}_{50}$  shows a phase-2 action potential. The main reasons for forming this phase are  $\text{Ca}^{2+}$  inflow and  $\text{K}^+$  outflow. These involve a fast-activated component ( $I_{\text{kr}}$ ) and a slowly activated component ( $I_{\text{ks}}$ ) of the inward rectifying potassium current, as well as a L-type calcium current ( $I_{\text{Ca-L}}$ ).<sup>29</sup>  $\text{MAPD}_{90}$  mainly reflects phase-3 repolarization, which is related to  $\text{K}^+$  outflow, mainly involving a fast-activated component ( $I_{\text{kr}}$ ) and

a slow-activated component ( $I_{\text{ks}}$ ) of the inward rectifying potassium current. When these flows are inhibited,  $\text{K}^+$  outflow slows down and the duration of repolarization of the action potential is prolonged.<sup>30,31</sup>

The results of this study demonstrate that IR can reduce MAPA and  $V_{\max}$ , indicating that IR inhibits the degree of opening of fast  $\text{Na}^+$  channels and velocity of  $\text{Na}^+$  inflow in the depolarization process. Post-treatment EA can raise  $V_{\max}$  and MAPA, suggesting that it influences myocardial depolarization, possibly through a mechanism related to  $\text{Na}^+$  inflow. This study found that  $\text{MAPD}_{50}$  and  $\text{MAPD}_{90}$  were prolonged after IR, while they were shortened in the IR + EA group at  $T_2$ . This suggested that ion channels, including myocardial delayed rectifying potassium and calcium channels, show dysfunction during IR. However, post-treatment EA appears to relieve these impaired ion channel functions, indicating that EA inhibits the change in the electrophysiology of cardiac muscle during IR, thus protecting cardiac muscle. Compared with the SH group, HRs in the IR and IR + EA groups decreased at  $T_1$ , while that in the IR + EA

**Figure 5.** Effects of post-treatment electroacupuncture (EA) on: (a) left ventricular ejection fraction (LVEF); (b) fractional shortening (FS); (c) creatine kinase-muscle/brain (CK-MB); and (d) cardiac troponin I (cTnI) of rats with ischemia/reperfusion (IR) injury and sham-operated (SH) controls. EF and FS were measured and compared between the three different groups ( $n = 8/\text{group}$ ). Data are presented as mean value  $\pm$  standard deviation. Levels of CK-MB and cTnI in the serum were examined by ELISA and compared between the three different groups. \* $p < 0.05$  compared to SH group. # $p < 0.05$  compared to IR group.



group significantly increased at  $T_2$ . In addition, in comparison with the SH group, the incidence rates of arrhythmia in the IR and IR + EA groups increased at  $T_1$ . In comparison with the IR group, the incidence rate of arrhythmia in the IR + EA group was reduced, indicating that EA intervention can mitigate the reduction in HR caused by myocardial ischemia and decrease the incidence rate of RA. The underlying mechanism of these effects is likely to be correlated with decreased calcium overload, inhibition of  $\text{Ca}^{2+}$  inflow and reduction of  $\text{K}^+$  outflow,<sup>32,33</sup> while its specific mechanisms, as related to changes in ion channel current, need to be further investigated.

At the same time, we found that EA application at bilateral PC6 attenuated the decreases in HR, arterial blood pressure and other parameters related to cardiac function. Many previous studies have confirmed that EA application at PC6 could enhance cardiovascular function in cardiovascular diseases.<sup>34</sup> We speculate that these beneficial effects on cardiac function are primarily due to the reduction of

sympathetic outflow through EA application at bilateral PC6. An excessive increase in sympathetic nerve activity can attenuate cardiac function by increasing myocardial oxygen consumption.<sup>35</sup> Vagus nerve stimulation reduces HR and decreases myocardial contractility. Consequently, decreases in HR, arterial blood pressure and other parameters of cardiac function secondary to IR injury-induced stress might be mediated by changes in sympathetic and vagal nerve activity. EA application at bilateral PC6 can inhibit vagal activity, and thus attenuate the decrease in HR and arterial blood pressure.<sup>36</sup> Reduction of sympathetic outflow can reduce myocardial oxygen consumption, thereby contributing to the recovery of cardiac function.<sup>37</sup> However, these possibilities require further investigation. Further studies are necessary to determine whether and how neurotransmitter modulation is involved in the regulation of cardiac function during EA application. In conclusion, post-treatment EA can relieve the prolongation of repolarization and slowed depolarization of ventricular



muscle with IR injury in rats, resulting in reduced incidence of RA.

### Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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The authors received no financial support for the research, authorship and/or publication of this article.

### Ethical approval and consent to participate

The study was approved by the Ethics Committee of Affiliated Hospital of Guizhou Medical University (no. 142).

### Contributors

Y. L. and H.G. conceived and designed the study. J.T., W.R. and S.H. obtained the data. Y.W. analyzed and interpreted the data. J.T. was a major contributor in writing the manuscript. Y.L. and Y.W. reviewed the manuscript. All authors read and approved the final version of the manuscript accepted for publication.

### Availability of data and materials

The data sets used and/or analyzed during this study are available from the corresponding author on reasonable request.

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