PROTECTIVE EFFECTS OF ELECTROACUPUNCTURE AND SALVIAE MILTIORRHIZAE ON MYOCARDIAL ISCHEMIA/REPERFUSION IN RABBITS

Xiang-Rui Wang, M.D., Ph.D, Professor of Anesthesiology
Han Lin, M.D., Ph.D Candidate
Zhen-Hong Wang, M.D., Ph.D Candidate

RenJi Hospital, Shanghai Second Medical University
Shanghai 200127, P.R. China

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ABSTRACT:
The aim of present study was to observe the protective effects of electroacupuncture (EA) and Salviae Miltiorrhizae (SM) on myocardial ischemia/reperfusion in rabbits. Acute ischemia/reperfusion of myocardium was set up by ligating left anterior descending branch of coronary artery in 24 rabbits which were divided randomly into control, EA, SM, and EA+SM group. Changes of plasma myocardial zymogram were found after ischemia in these groups. EA and SM were observed to decrease plasma IL-8 and epinephrine concentration and to increase $^{99m}$Tc-MIBI intake ratio of myocardial mitochondrial, but enhancing or antagonistic effect between EA and SM was negligible. There was positive correlation between concentrations of plasma epinephrine and IL-8. The results indicated that EA and SM could reduce myocardial ischemia/reperfusion injury and protect myocardial mitochondrial by reducing concentrations of plasma epinephrine and IL-8. EA and SM could reduce the release of endogenic epinephrine, which was one of the mechanisms of lowering plasma IL-8.

KEY WORDS:  Myocardial ischemia/reperfusion; Electroacupuncture (EA); Salviae Miltiorrhizae (SM); Mitochondria; Interleukin-8 (IL-8); epinephrine; $^{99m}$Tc-MIBI

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Correspondence: Xiang-Rui Wang, M.D., Ph.D., Department of Anesthesiology of Renji Hospital, 1630 Dongfang Road Shanghai, Tel: 86-21-58752345-3198, Fax: 86-21-50903239, Email: xiangruiwang@vip.sina.com
INTRODUCTION

Myocardial ischemia/reperfusion injury is a severe adversity, causing death and injury to those in critical care or after cardiac surgery and the aged who is out of condition in cardiovascular function, thus at present it’s still a hot topic in clinical research. Clinical trials in our research showed that EA could help patients to recover in a rapider way with less complications after cardiac surgery, but the mechanism remains unknown\(^1\,2\). We adopted the animal model of acute myocardial ischemia/reperfusion injury in this study to explore the protective effects of combination of EA and SM and the underlying mechanism.

MATERIALS AND METHODS

1. Animals and Anesthesia

24 Chinese rabbits aged 6 months (weight 2.39±0.11kg, Shanghai Pudong Chenhang Experiment Animal Field) were used in the experiment. Anesthesia was induced with intramuscular ketamine (50mg/kg) and diazepam (1mg/kg). Mechanical ventilation (Dräger ventilator, Germany) was instituted via tracheostomy and maintained with tidal volume 20ml/kg and respiratory frequency 30/min. Anesthesia was maintained with fentanyl (50ug/kg per hour) and pancuronium bromide (0.2mg/kg).

2. Surgical Procedure of AMI Model

Right internal jugular vein was carefully isolated and cannulated with venous catheter to right atrium for blood sample and transfusion. A femoral arterial line was placed for blood pressure monitoring. Subcutaneous electrodes were set to monitor changes of HR and ST segment. A median sternotomy was performed, and the pericardium opened. The left anterior descending branch (LAD) of coronary artery was ligated with a nylon thread at the position 1cm close to intersection point with circling branch. Topical myocardium turned to dim-red with ST segment elevated in ECG remarkably, which indicated that the model of acute myocardial ischemia (AMI) was established. After half an hour of occlusion, the thread was drawn out to allow reperfusion for 2 hours.

3. Animal Groups and Treatments

Rabbits were divided randomly into control group, EA group, SM group and EA+SM group, 6 in each group. LAD was ligated for 30 min and then reperfused for 2 h in control group without any intervention. In EA group, EA stimulation was applied after the induction of anesthesia at the points of Neiguan (PC 6) and LieQue (LU 7) with G6805-2 electrostimulator at the frequency of 5Hz (Fig.1). After half an hour, surgical procedure was started and the stimulation was maintained during operation. Rabbits in SM group were injected with SM (1.5 g/ml, made by Hangzhou Upright pharmaceutical Co., Ltd) at the dose of 1.5g/kg before the operation, 1.0g/kg immediately before ligation and reperfusion respectively. In EA+SM group, rabbits were electro-acupunctured by stimulator and injected with SM as the EA group and SM group.
Fig. 1 Schematic diagram showing the EA points and procedure in rabbit with electrical stimulator (G6805-2)

4. Plasma Myocardial Zymogram, IL-8 and Epinephrine Determination

Blood (3 ml) was sampled from right atrium through internal jugular vein cannula and placed into plain glass tubes. This was performed immediately before ligating LAD branch of coronary artery, immediately before reperfusion and 2 hrs after reperfusion. Plasma was prepared by centrifugation of blood at 2,000 g for 15 min at room temperature and then removed and stored at -70°C for later analysis.

Cardiac zymogram, including creatine phosphokinase (CPK), aspartate transaminase (AST) and lactic dehydrogenase (LDH) were determined by detecting all the plasma samples. Plasma taken 2 hours after reperfusion was measured for the concentration of IL-8 with CLIA (IMMULITE enzyme-labeling apparatus) and epinephrine with HPLC (Electrochemical Detector Model 1640, BIO-RAD).

5. Measurement of $^{99m}$Tc-MIBI in Myocardial Mitochondria

The $^{99m}$Tc-MIBI dose in test kit was measured with the radioactivity meter (RM905, Chinese Academy of Metrology, Beijing) and $^{99m}$Tc-MIBI 185MBq (5mCi) was injected intravenously before perfusion. Myocardial tissue about 1g was collected 2 hours after reperfusion at the ischemia/perfusion region. Myocardial mitochondria was prepared.
according to the measure as recommended by Regitz\textsuperscript{[3]} and 1ml NaOH (0.1mol/L) was titrated to the sediment for dissolving mitochondria. Concentration of mitochondrial protein was measured with an ELISA. $\gamma$-calculation in 1ml mitochondrial solution was performed with SN-682 radioimmunoassay $\gamma$-calculator and expressed as CPM/mg mitochondrial protein.

6. Statistical Analysis

All the data were expressed at mean $\pm$ SD. Repeated measurements analysis of variance (ANOVA) was used for comparisons between time points, factorial ANOVA for multiple comparisons and mutual action, and correlation analysis for relationship of two variances. Results of these tests were considered statistically significant when $P$ was less than 0.05.

RESULTS

1. The changes of myocardial zymogram

Plasma CPK concentration in all groups showed higher before reperfusion and 2 h after reperfusion than that before ligation, and LDH concentration changed just as CPK did ($P<0.05$, see figure 2 and 4). AST concentration showed higher 2 h after reperfusion than that before ligation ($P<0.05$, shown in figure 3). LDH concentration was higher before perfusion and 2h before perfusion than before ligation.

![Graph showing changes in plasma CPK concentration](image)

**Fig2. Changes of plasma CPK concentration**

* $P<0.05$ vs Before ligation
**PROTECTIVE EFFECTS OF ELECTROACUPUNCTURE**

**Fig 3. Changes of plasma AST concentration**

* $P < 0.05$ vs Before ligation

**Fig 4. Changes of plasma LDH concentration**

* $P < 0.05$ vs Before ligation
2. Comparison of plasma IL-8, epinephrine concentration and $^{99m}$Tc-MIBI intake ratio of myocardial mitochondrials

It was found that IL-8 concentration was lower in EA group, SM group and EA+SM group than that in control group respectively ($P < 0.05$ or $P < 0.01$), but there’s no mutual effect between EA and SM according to the factorial ANOVA. Epinephrine concentrations and $^{99m}$Tc-MIBI intake ratio in the three groups were lower than that in control group ($P < 0.05$ or $P < 0.01$), but the mutual effects between EA and SM were not found.

Table 1. Plasma IL-8, epinephrine and $^{99m}$Tc-MIBI intake ratio (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>IL-8 (ng/L)</th>
<th>epinephrine (ng/L)</th>
<th>$^{99m}$Tc-MIBI intake ratio (cpm/mg pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.3±8.9</td>
<td>2015.4±75.1</td>
<td>8542±1774</td>
</tr>
<tr>
<td>EA</td>
<td>21.9±6.0 *</td>
<td>1926.5±58.9 *</td>
<td>20210±8631 *</td>
</tr>
<tr>
<td>SM</td>
<td>18.3±3.3 **</td>
<td>1921.3±93.8 *</td>
<td>20412±3115 *</td>
</tr>
<tr>
<td>EA+SM</td>
<td>13.6±3.5 **</td>
<td>1812.8±88.6 **</td>
<td>30609±10464 **</td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs Control group  
** $P < 0.01$ vs Control group

3. Relation between plasma concentration of IL-8 and epinephrine

A linear correlation analysis was carried out to explore the relationship between plasma concentration of IL-8 and epinephrine (correlation coefficient $r = 0.454$, $P < 0.05$).

DISCUSSION

$^{99m}$Tc-MIBI as a kind of lipophilia univalent positive ion, which can produce γ radial, a technetium complex for non-invasive cardiac perfusion and imaging. Clinically it could be evaluated to be the mitochondrial function of cardiac muscle of patients. The transmembrane potential of cardiac muscle cells is negative, while that of mitochondrial even more. Transmembrane potential of mitochondrial is -150--200mV$^{[41]}$. $^{99m}$Tc-MIBI intake of cardiac muscle cells mainly takes place in mitochondrial and be related to its transmembrane potential. It enters mitochondrial by electrochemical gradient$^{[31]}$. Change of permeability of mitochondrial membrane can lead to the change of membrane potential, therefore $^{99m}$Tc-MIBI could be an excellent functional indicator to the degree of myocardial mitochondrial injury. No matter what kind of mechanism leads to ischemia/reperfusion injury of cardiac muscle, eventually it could be ascribed to the dysfunction of mitochondrial.

It has been reported frequently in many articles about the cardiac toxicity of the endogenous responsive hormone–epinephrine, which leads to the increase of oxygen-derived free radicals and Ca$^{2+}$ over-load of cardiac muscle cells$^{[6]}$. IL-8 plays a significant role in the reperfusion injury of cardiac muscle cells induced by neutrophilic granulocytes. During the several hours at the beginning of reperfusion, IL-8 are largely produced by neutrophilic granulocyte$^{[7]}$. IL-8, as a very powerful chemotactic factor can
leads to the retardation of neutrophilic granulocytes in microcirculation, then injure the cardiac muscles. IL-8 can also activate the CD11b/CD18 the surface of neutrophilic granulocyte and induce myocardial cells to express ICAM-1, which can enhance the combination of neutrophilic granulocyte and myocardial cells. The oxygen-derived free radical released by neutrophilic granulocyte can induce the production of secondary free radical, Ca\(^{2+}\) over-load, then bring death to cardiac cells (necrosis and apoptosis) featured by the change of permeability of mitochondrial and transmembrane potential.

EA was invented according to the analgesia and improvement to physical function of acupuncture. The injury caused by free radical, the release of myocardial zymogram and the production of melonic aldehyde after myocardial ischemia/reperfusion could be reduced by acupuncturing Neiguan (PC 6), an acupoint on human body. It also can inhibit the excitation of peripheral sympathetic-catecholamine system and reduce the release of catecholamine by acupuncturing Neiguan. When myocardial ischemia, phosphorylase is phosphorilated to be activated form, while glycogen synthetase is phosphorilated to be inactivated form, in the way enhancing the glycogen in cardiac muscle to be transformed into the glucose, which provides energy by anaerobic glycolysis. The restoration of glycogen in ischemic zone, therefore can protect myocardial cells. Acupuncturing Neiguan could decrease reperfusion injury of myocardium, probably by increasing glycogen, glycogensynthetase and phosporylase in myocardial cells and improving cardiac contractility.

SM injection is TCM preparation. The effective ingredient is tanshitone. It was found that MDA production of damaged myocardial tissue could be reduced and SOD increased by injection of tanshitone-a DS201. Probably the protective function of tanshitone was accomplished by clearing away oxygen-derived free radical. Other components such as caffeic acid and its polymers, and redoot acid could clear out free radicals. Moreover, Salvia Miltiorrhizae could counteract the Ca\(^{2+}\) over-load.

In the experiment, changes of myocardial zymogram, together with changes of arterial pressure, heart rate and ST segment during surgery procedure proved that myocardial acute ischemia/reperfusion model be reliable. EA and SM decreased plasma IL-8 and epinephrine concentration, and increased the \(^{99m}\)Tc-MIBI intake ratio of myocardial mitochondrial. But enhancing or antagonistic effect between electroacupuncture and SM was negligible. Protection effect of EA and SM was eventually displayed by the increase of intake ratio of \(^{99m}\)Tc-MIBI of mitochondrial in ischemia/reperfusion zone probably by decrease of plasma epinephrine and IL-8 concentration. Plasma IL-8 and epinephrine concentrations were proved be positively correlated through double Pearson correlation analysis. Some overseas laboratories had studied the relation between IL-8 and epinephrine at the molecular and cellular level. Epinephrine could enhance monomacrophage system to produce IL-8, while study on animal model was not done yet.

Our experiment had proved that epinephrine could enhance IL-8 release and EA and SM might inhibit the release of IL-8 by inhibiting the release of endogenous epinephrine.

In summary, EA and SM could decrease myocardial ischemia/reperfusion injury by decreasing plasma epinephrine and IL-8 concentration. IL-8 could be increased by epinephrine and might play an important role in myocardial reperfusion injury, whether other IL-8 inhibitor such as IL-8\(_{AB}\) could have protective effects to myocardial reperfusion injury should be studied.
REFERENCES


