Effect of Electroacupuncture at Wushu Acupoints of the Cardiopulmonary Meridian on the Autophagy in Rats with Acute Myocardial Ischemia

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1. Introduction

Acute myocardial ischemia (AMI) is a relatively common disease with severe myocardial damage. Changes in coronary flow cause AMI, resulting in an imbalance in oxygen demand between the blood and myocardium. Recently, it has become one of the key reasons for the increase of morbidity and mortality globally. Furthermore, the incidence rate of women is higher than that of men [1–3]. Studies at home and abroad have verified that acupuncture has a good therapeutic effect on myocardial ischemia [4, 5]. Acupuncture at Neiguan (PC 6), Shenmen (HT 7), and Lieque (LU 7) played a part in regulating the core mass, neurotransmitters, autonomic nervous activity, cardiac bioactive substances, ECG, and myocardial infarction size of AMI patients [6–11]. Additionally, in previous animal experiments, relevant researchers compared a single acupoint in the heart meridian with a single acupoint in the lung meridian to examine the difference in effects on AMI [12].

Wushu acupoints are the five acupoint distributions below the human elbow and knee joint. They are all located on the same meridian and divided into five categories: Jing, Ying, Shu, Jing, and He. It has been shown that electroacupuncture (EA) at Shenmen point of heart meridian can improve acute myocardial ischemia (AMI) early. However, it is still unclear if all the Wushu acupoints of the heart meridian can improve AMI. Hence, this study emphasizes Wushu acupoints of heart meridian, compares them with Wushu acupoints of lung meridian, and studies the therapeutic effect of EA at Wushu acupoints on AMI and its possible mechanism. It also discusses the specificity of the heart meridian to heart disease. The AMI model is established by ligation of the left anterior descending coronary artery. The detection methods like the physiological recorder, TTC staining, ELISA, and so forth were used to determine the ECG, myocardial infarct size, serum myocardial enzymes, and myocardial tissue-related protein expression in rats. The heart rate (HR) and ST segment along with creatine kinase (CK), creatine kinase isoenzymes (CK-MB), lactate dehydrogenase (LDH), and myocardial infarctions increased after the induction with AMI. Furthermore, the expressions of PINK1 and Parkin protein also showed an increase. However, EA at Wushu acupoints in the heart meridian can reverse the above changes, whereas EA at the lung meridian exhibits limited effect. It is depicted that the heart meridian has a relatively specific relationship with the heart in a diseased state.
the Wushu acupoints of the lung meridian are as follows: Jing-Shaoshang, Ying-Yuji, Shu-Taiyuan, Jing-Jingqu, and He-Chize. The clinical application of Wushu acupoints is widespread, but each has its own emphasis. At present, there are no reports available on animal research of Wushu acupoints. Therefore, in this experiment, Wushu acupoints were used as the treatment point. A comparison between heart meridian and lung meridian was used to observe the effects of acupuncture at both heart and lung acupoints on the heart rate (HR), myocardial infarct size, the expression of myocardial enzymes, and autophagy proteins in AMI. Finally, we also discussed the specificity of the heart meridian to the heart.

The experimental process and group details are shown in Figure 1.

2. Materials and Methods

2.1. Animals and Groups. 108 specific-pathogen-free Sprague Dawley (SD) rats (190–230g) were provided by Pizhou Dongfang Aquaculture Co., Ltd. [animal license number SCXK (SU) 2017–0003]. A total of 120 SD male rats were prepared in advance. During the experiment, 12 rats died due to improper operation, excessive anesthesia, and euthanasia. Hence, 108 SD rats were involved in the experiment. SD rats were placed in the animal feeding room of Anhui University of Chinese Medicine for one week in a natural light environment and were fed food and water daily. The temperature in the cage was set at 19–21 degrees Celsius, and the relative humidity was maintained at 40–60%. To effectively carry out this experiment, we included nine rats in the sham group. The rest were included into the model group according to a random number table.

Further, the rats were divided into five acupoint groups in heart meridian (Jing-Shaohong, Ying-Shaofu, Shu-Shenmen, Jing-Lingdao, and He-Shaohai groups) and five acupoint groups in lung meridian (Jing-Shaoshang, Ying-Yuji, Shu-Taiyuan, Jing-Jingqu, and He-Chize group). A total of nine rats were included in each group. The treatment of animals during the experiment strictly abides by the ethical principle of welfare of experimental animals in the Research and Experiment Center of the Anhui University of Chinese Medicine.

2.2. Main Reagents and Instruments. The following reagents were used in this study: enzyme-linked immunoassay kit for creatine kinase (CK), creatine kinase isoenzyme (CK-MB), and lactate dehydrogenase (LDH) (Shanghai Jianglai Biotechnology Co., Ltd.); 2,3,5-triphenyl tetrazolium chloride (TTC) staining solution (Meilun Biotechnology Co., Ltd.); BCA protein quantitation kit (Thermo Fisher Scientific); PINK1, Parkin antibody (Abcam); β-actin (Beijing Zhongshan Jinqiao Company Biotechnology Co., Ltd.); goat anti-rabbit HRP-labeled secondary antibody (ZSGB-BIO); luminescent liquid (MILLIPORE); and PBS buffer (Shanghai Kearton Biotechnology Co., Ltd.).

The following instruments were used in this study: PowerLab 16 physiological recorder (AD Instruments, Australia); R500 universal small animal anesthesia machine (Shenzhen Ruiwode Life Technology Co., Ltd.); microplate reader (Shenzhen Redu Life Science Co., Ltd.); high-speed desktop refrigerated centrifuge (Anhui Jiawen Instruments Co., Ltd.); Hwato brand acupuncture needle (Beijing Luoya Shanchuan Medical Instrument Co., Ltd.); electronic acupuncture therapeutic apparatus (Suzhou Medical Appliance Factory Co., Ltd.); 37-degree incubators; electrophoresis instrument (BIO-RAD Co., Ltd.); and electrokinetic instrument (Dalian Jingmai Technology Co., Ltd.).

2.3. Animal Model. The SD rats were placed in a general anesthesia induction box for small animals to be induced with isoflurane at 5% concentration and then fixed on the rat board in the supine position. The anesthesia state was maintained using isoflurane at 2% to 3%. The skin was prepared at the heart of the chest for incision. Deep and shallow muscles of the chest were separated bluntly. Hemostatic forceps were used to open the 4th and 5th intercostal space and extrude the heart. The left anterior descending branch of the coronary artery was ligated using suture needle No.6–0 with line. The whitened myocardial tissue at the ligated part was visible to the naked eye, and then the heart was reset. Residual air trapped in the thoracic cavity was extruded. The thoracic cavity was sutured, and penicillin ointment was applied postoperatively to prevent infection. ECG was synchronously recorded, and the successful replication of the model was analyzed using ST-segment hunchback elevation in limb lead II and a high T wave. The threading was performed only at the sham group’s left anterior descending coronary artery, and no ligation was executed. During the experiment, abnormal electrocardiograms and failure of modeling were excluded. ECG before and after modeling is revealed in Figure 2.

2.4. The Treatment. The sham and model groups were not treated with EA during the experiment. In the other treatment groups, the Wushu acupoints in the heart meridian and lung meridian were selected based on the acupoint positioning of rats in experimental Acupuncture Science. The disposable sterile acupuncture needles (specification parameter: Φ0.35 × 25 mm, Hwato brand) were inserted into the acupoints on the right side of the rat and subcutaneous part of the right hip. One end was connected to the positive electrode, whereas the other end was connected to the negative electrode. The current intensity and the current frequencies were 1 mV and 2 Hz/15 Hz, respectively. The treatment was conducted once a day, 30 min each time, for three successive days.

2.5. Method for Recording Rat Heart Rate and ST-Segment. The PowerLab 16 lead physiological recorder was used for analysis. The ECGs of rats before and after modeling were noted, and the failure in modeling was omitted. All groups were measured for 30 min again, after the final treatment in each treatment group. We recorded the limb II leads in all rats. The three-needle electrodes of the physiological
recorder were divided into three parts and put into the subcutaneous layer of the right upper limb, left lower limb, and right lower limb in rats. The heart rate and ST-segment were recorded after the signal was stabilized.

2.6. Determination of Serum CK, CK-MB, and LDH. After the EA treatment, all rats were anesthetized with isoflurane, and 3 mL of blood was collected from the abdominal aorta into a 5 mL blood collection tube. The blood samples were centrifuged in a 4°C centrifuge at 3500 rpm for 15 min after standing for 1 h. The supernatant was collected and placed in a −80°C refrigerator for subsequent use; CK, CK-MB, and LDH contents were measured according to the manufacturer’s instruction after the ELISA kits were balanced for 30 min at room temperature.

2.7. Measurement of Myocardial Infarction Area by TTC Staining. The myocardial tissue of each group was taken out after the collection of blood from the abdominal aorta. The blood on the tissue surface was washed with 4°C normal saline and stored in the −20°C refrigerator. The myocardial tissue was removed after 20 min and sliced with a blade, one at every 2 mm, and six slices were cut in total. The sections were immersed in TTC staining solution and incubated in the dark at 37°C for 30 min. Further, the sections were turned over once every 10 min to ensure that the myocardial tissues were fully in contact with the staining solution. A camera photographed the sections after incubation, and the infarction size was analyzed by ImageJ software.

2.8. Detection of the Expression Levels of Mitochondrial Autophagy Proteins PINK1 and Parkin in the Myocardial Tissue of Rats Using the Western Blotting Method. The myocardial tissues were weighed, cut into pieces, and added with the lysis solution in a proper proportion. They were then homogenized, lysed, and centrifuged at 12000 g for 15 min at 4°C. The supernatant was collected to quantify the protein, and a PAGE gel was used to separate the protein sample by electrophoresis at 120 V for 60 min. The protein was then transferred to the nitrocellulose (NC membrane) by a semidry transfer membrane at 25 V for 30 min. The antibody was diluted in PINK1 (1 : 1000) and Parkin (1 : 500)
3. Results

3.1. EA at Wushu Acupoints of Heart Meridian Inhibited the Level of HR and ST-Segment. Compared with the sham group, the HR and ST segments in the model group were significantly higher ($P < 0.01$). Compared with the model group, the HR and ST-segment decreased considerably in all Wushu acupoints in heart meridian groups (Shaochong, Shaofu, Shenmen, Lingdao, and Shaohai groups) ($P < 0.01$). Among the five lung acupoints, only the Jingqu group showed a significant decrease in HR ($P < 0.01$) and ST-segment ($P < 0.05$), and the differences among the other groups were not statistically significant.

Comparing the acupoints with the same cross section between heart meridian and lung meridian, the HR reducing effects of the Jing-acupoint Shaochong group and the He-acupoint Shaohai group in the heart meridian were better than those of the Jing-acupoint Shaoshang group and He-acupoint Chize group in the lung meridian ($P < 0.05$). The HR of AMI rats in the Shaofu group at Ying-acupoint in the heart meridian was lower than that in the Yuji group at Ying-acupoint in the lung meridian ($P < 0.05$). Furthermore, the ST-segment reduction was also better than that in the Yuji group ($P < 0.01$). The reducing degree of AMI HR and ST-segment in the Shenmen group of heart meridian was superior to that in the Taiyuan group of lung meridian ($P < 0.01$) (Figures 3(a) and 3(b)).

3.2. EA at Wushu Acupoints of Heart Meridian Reduced the Content of Serum CK, CK-MB, and LDH. Compared with the sham group, the model group’s serum CK, CK-MB, and LDH contents increased significantly ($P < 0.01$). Compared with the model group, the contents of serum CK, CK-MB, and LDH in all five acupoints in heart meridian groups (Shaochong, Shaofu, Shenmen, Lingdao, and Shaohai) reduced considerably ($P < 0.01$). Among the five acupoints in lung meridian, only the serum CK content in the Yuji group was greatly reduced ($P < 0.01$), while the serum CK and CK-MB contents in the Chize group were reduced considerably ($P < 0.01$), and the serum LDH content in the Jingqu group was also significantly reduced ($P < 0.01$). The differences among the other groups were not statistically significant (Figures 4(a)–4(c)).

On comparing the Wushu acupoints with the same cross section of heart meridian and lung meridian, the reductions in the serum CK, CK-MB, and LDH contents in the heart meridian of the Jing-acupoint Shaochong group and the Shu-acupoint Shenmen group were superior to those in the lung meridian of Jing-acupoint Shaoshang group and the Shu-acupoint Taiyuan group ($P < 0.05/0.01$). The LDH content decreased greatly in that heart meridian. Ying-acupoint Shaofu group was superior to the lung meridian of Ying-acupoint Yuji group ($P < 0.05$). The reduction of serum CK content in the heart meridian of Jing-acupoints Lingdao group was greater than that in the lung meridian of Jing-acupoints Jingqu group ($P < 0.05$), and the reduction of serum LDH content in the heart meridian of He-acupoint Shaohai group was significantly superior to that in the lung meridian of He-acupoint Chize group ($P < 0.01$) (Figures 4(a)–4(c)).

3.3. EA at Wushu Acupoints of the Heart Meridian Reduced the Myocardial Infarction Size. The ischemic area of myocardial tissue was grayish-white, and the nonischemic area was red or purplish-red, as shown in Figure 5(a).

Compared with the sham group, the myocardial infarction area in the model group was amplified significantly ($P < 0.01$). In comparison with the model group, the myocardial infarction area of rats in the Shaochong group reduced ($P < 0.05$), while those in Shaofu, Shenmen, Lingdao, and Shaohai groups decreased significantly ($P < 0.01$). There was no major difference in the myocardial...
Figure 3: EA at Wushu acupoints of heart meridian inhibited the level of HR and ST-segment. The blue color in the histogram depicts the five different acupoints of the heart meridian. The red color in the histogram shows the five different acupoints of the lung meridian, such as (a) the HR histogram and (b) the ST histogram. Compared with the sham group, HR \((P < 0.01)\) and ST \((P < 0.01)\) were considerably increased in the model group. Compared with the model group, HR \((P < 0.01)\) and ST \((P < 0.01)\) decreased after EA at Shaochong, Shaofu, Shenmen, Lingdao, and Shaohai points of the heart meridian. Compared with the model group, HR \((P < 0.01)\) and ST \((P < 0.05)\) decreased after EA at the Jingqu points of the lung meridian. HR \((P < 0.05)\) of Shaochong group was lower than that of Shaoshang group, HR \((P < 0.05)\) and ST \((P < 0.01)\) of Shaofu group were lower than those of Yuji group, HR \((P < 0.01)\) and ST \((P < 0.05)\) of Shenmen group were lower than those of Taiyuan group, and HR \((P < 0.05)\) of Shaohai group was lower than that of Chize group. The number of rats in each group was nine, \(*P < 0.01, \# P < 0.05, \#\# P < 0.01, \#\#\# P < 0.05, \#\#\#\# P < 0.01, \& P < 0.01, \&\& P < 0.05.\)

Figure 4: Continued.
infarction among the acupoints groups of the lung meridian. Comparing the acupoints with the same cross section of heart meridian and lung meridian, the Shenmen group via the heart meridian was superior to the Taiyuan group via the lung meridian (P < 0.01). The Shaofu, Lingdao, and Shaohai groups via the heart meridian were better than the Yuji, Jingqu, and Chize groups via the lung meridian (P < 0.05), as shown in Figure 5(b).

3.4. EA at Some Acupoints Decreased the Protein Expression of PINK1 and Parkin in Myocardial Tissue. Compared with the sham group, the protein expression levels of PINK1 and Parkin in the myocardial tissue of the model group increased (P < 0.01). Compared with the model group, the protein expression levels of PINK1 and Parkin in the Shaofo group and Shenmen group of the Wushu acupoints in the heart meridian decreased (P < 0.01). Further, the expression level of Parkin in the Shaochong group decreased significantly (P < 0.05), and the expression level of PINK1 also showed a reduction (P < 0.01). The Parkin level was reduced in the Taiyuan group (P < 0.05), and the PINK1 level decreased significantly (P < 0.01) among the Wushu acupoints in the lung meridian. Still, no major differences among the other groups were observed (Figures 6(a)–6(c)).

Moreover, there was no statistical significance in the difference between the heart meridian and lung meridian at the same cross-sectional acupoints.

4. Discussion

Wushu acupoints are the points and treatment points of human diseases. At present, there are few experimental studies on Wushu acupoints at home and abroad. Studies on meridians or acupoints mostly focus on the comparison of a single meridian or single acupoint with nonmeridian nonacupoints, because parameters such as meridian (or the size and depth of meridian acupoints) are not described in detail, and it is difficult to define the differences between meridians and nonmeridians. This study surpassed this point by comparing the heart meridian of Shaoyin in hand with the lung meridian of Taiyin in hand, adopting the comparison of "meridians–meridians," taking the Wushu acupoints of the two meridians as the focus, and selecting the acupoints with the same cross section to observe the differences in intervention effects, thus avoiding the problem of meridian-nonmeridian definition. It was possible to more closely study the intervention effects of meridians rather than acupoints in heart meridian on such diseases as myocardial ischemia and prove the specificity of meridian
therapy for diseases. In addition, the heart meridian and lung meridian of human body are short in route and single in function, which can reduce the experimental errors caused by various factors to a certain extent.

Our earlier studies displayed that EA at Shenmen can reduce AMI rats’ heart rate, ST-segment, and myocardial infarction area and relieve myocardial injury. In this study, the HR, ST-segment, and myocardial infarction area were amplified after myocardial injury in rats, which showed that the heart function was damaged. After EA at Shenmen, all the above indexes decreased, proving that myocardial injury increased following the results of previous studies. Additionally, this study revealed that the acupoints of Jing, Ying, Shu, Jing, and He in the heart meridian exhibited regulatory effects on HR, ST-segment, serum CK, CK-MB, LDH content, and myocardial infarct size of AMI rats. There were individual acupoints in Wushu acupoints of lung meridian that could exert the effects, but the number of effective acupoints was smaller than that in the heart meridian. As per the results of this study, the impact of acupoints in the heart meridian on the enhancement of different indicators in rats with AMI was better than that in the lung meridian. This can provide some evidence for the specificity of heart meridian acupoints in treating heart diseases to some extent.

Serum CK, CK-MB, and LDH are the sensitive indicators for early diagnosis of AMI and are the representative serum biomarkers of myocardial ischemia and necrosis [13–15]. CK is widely present in the cytoplasm of the myocardial cells. Once the myocardial injury occurs, CK is released into the bloodstream, which sharply increases the content of CK in the blood [16], revealing high specificity. CK-MB, one of CK, also exists in the myocardium, and an increase in its content

![TTC staining picture of myocardial tissue in each group.](image1)

![The infarction size of the heart of AMI rats.](image2)
reflects the scope of ischemic necrosis [17, 18]. LDH is a key myocardial functional enzyme [19]. After myocardial damage, LDH will leak from the inside to the outside of the cells, and its quantity and quality will directly affect the body's energy metabolism. The finding of the LDH content has an important reference value for the clinical diagnosis of myocardial damage [20]. In this study, after the AMI rat model was established, the CK, CK-MB, and LDH contents increased in the model group, showing that myocardial ischemia and necrosis had occurred. The above indicators were reduced to different degrees after EA at the Wushu acupoints of the heart meridian, signifying that EA at the Wushu acupoints of the heart meridian could relieve the AMI state, regulate body energy metabolism, and improve the damaged myocardium. Moreover, only limited Wushu acupoints in the lung meridian affected the serum index, and the result was worse than that of the acupoints in the heart meridian. Besides, all the Wushu acupoints in the heart meridian could improve the serum CK, CK-MB, and LDH contents, so it could be deduced that the acupoints in the heart meridian exhibited specificity for the treatment of myocardial ischemia.

Autophagy is an extremely conservative lysosomal degradation pathway of the eukaryotic cells, which can degrade damaged organelles, intracellular invasive microorganisms, long-lived proteins, and so forth [21]. It is a self-protecting behavior during the process of growth and development of the body. Reports have shown that autophagy is closely related to cardiovascular disease [22]. Mitochondrial autophagy is a targeted phenomenon that can specifically recognize and degrade damaged mitochondria. It plays a key role in maintaining the stability of the intracellular environment and its functional state [23, 24]. Activating the mitochondria appropriately during AMI can protect cardiomyocytes and maintain mitochondrial homeostasis. Still, excessive and long-term upregulation of autophagy will disorganize the mitochondria, induce cardiomyocyte apoptosis, and aggravate the myocardial injury [25, 26]. Therefore, inhibition of autophagy may be a key point to improve myocardial injury.

The PINK1/Parkin pathway is one of the main mitochondrial-mediated autophagy pathways, in which PINK1 is an extremely conservative serine/threonine-protein kinase that is present abundantly in the myocardial tissue. Under
physiological conditions, PINK1 will be transported from the mitochondrial outer membrane to the mitochondrial inner membrane and then cleaved and decomposed by presenilins-associated rhomboid-like (PARL) protein to maintain a low level [27]. However, mitochondria are damaged after injury to the body, and PINK1 cannot be transferred smoothly, cleaved, and degraded. Thus, they gather in a large amount in the mitochondrial outer membrane, leading to spatial structure changes and Parkin protein activation. The Parkin protein located in the cytoplasm is transferred to the mitochondria after activation, which in turn causes P62 and LC3 to bind to the mitochondrial matrix and induce mitochondrial autophagy [28–30]. In this study, the protein levels of PINK1 and Parkin in AMI rats were significantly increased as compared with the sham group, indicating that PINK1 and Parkin were activated and autophagy was exerted, confirming that PINK1/Parkin pathway was involved in the pathophysiological process of AMI. After EA, the protein levels of PINK1 and Parkin in the Shaochong, Shaofu, and Shenmen groups of heart meridian and the Taiyuan group of lung meridian were significantly decreased, indicating that autophagy was inhibited and myocardial cell necrosis was reduced.

In conclusion, according to the number of effective acupoints used in the treatment of myocardial ischemia at the Wushu acupoints of the heart meridian and the superior effect exerted by it when compared with the same cross section of the lung meridian, it can be inferred that the treatment of myocardial ischemia at the Wushu acupoints in the heart meridian is much more specific than that in the lung meridian. The protective effect of EA at Wushu acupoints on AMI may be attained by inhibiting the PINK1/Parkin autophagy pathway.

**Abbreviations**

- **AMI**: Acute myocardial ischemia
- **EA**: Electroacupuncture
- **Wushu acupoints of heart meridian**: Jing-Shaofu, Ying-Shaofu, Shu-Shenmen, Jing-Lingdao, and He-Shaohai
- **Wushu acupoints of lung meridian**: Jing-Shaoshang, Ying-Yuji, Shu-Taiyuan, Jing-Jingqu, and He-Chize
- **HR**: Heart rate
- **SD**: Creatine kinase
- **CK-MB**: Creatine kinase isoenzymes
- **LDH**: Lactate dehydrogenase
- **ECG**: Electrocardiograph
- **TTC**: Triphenyl tetrazolium chloride
- **PINK1**: PTEN induced putative kinase protein 1
- **ST**: ST-segment

**Data Availability**

The analyzed data sets generated during the present study are available from the corresponding author upon reasonable request.

**Conflicts of Interest**

All authors declare no conflicts of interest.

**Authors’ Contributions**

Meiqi Zhou supervised the study; Meiqi Zhou and Chao Zhu designed the experiments; Chao Zhu and Shengbing Wu carried out the test; Chao Zhu, Xin Wu, Kun Wang, and Shuai Cui analyzed data; Chao Zhu and Meiqi Zhou wrote and modified the manuscript; Chao Zhu and Jie Zhou carried out subsequent partial revision work.

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**References**

[1] M. Sobajima, T. Nozawa, and H. Ihori, "Repeated sauna therapy improves myocardial perfusion in patients with chronically occluded coronary artery-related ischemia," *International Journal of Cardiology*, vol. 167, no. 1, pp. 237–243, 2015.

[2] N. K. Wenger, "Clinical presentation of CAD and myocardial ischemia in women," *Journal of Nuclear Cardiology*, vol. 23, no. 5, pp. 976–985, 2011.

[3] P. Lounsbury, A. S. Elokda, J. M. Bunning, R. Arena, and E. E. Gordon, "The value of detecting asymptomatic signs of myocardial ischemia in patients with coronary artery disease in outpatient cardiac rehabilitation," *Journal of Cardiovascular Nursing*, vol. 32, no. 3, pp. E1–e9, 2017.

[4] H. R. Zhang, J. L. Tao, and H. Bai, "Changes in the serum metabolome of acute myocardial ischemia rat pretreatment with electroacupuncture," *The American Journal of Chinese Medicine*, vol. 47, no. 5, pp. 1025–1041, 2011.

[5] J. Xie, Z. B. Chen, and S. Wu, "[Comparison of protective effect of electroacupuncture on myocardial ischemia injury between different acupoint formulas in rats]," *Zhen Ci Yan Jiu*, vol. 42, no. 2, pp. 131–135, 2015.

[6] P. Li, A. L. S. C. Tjen, and J. C. Longhurst, "Excitatory projections from arcuate nucleus to ventrolateral periaqueductal gray in electroacupuncture inhibition of cardiovascular reflexes," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 290, no. 6, pp. H2535–H2542, 2011.

[7] A. L. S. C. Tjen, P. Li, and J. C. Longhurst, "Midbrain vPAG inhibits rVLM cardiovascular sympathoexcitatory responses during electroacupuncture," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 290, no. 6, pp. H2543–H2553, 2015.

[8] C. Ji, F. Song, and G. Huang, "The protective effects of acupoint gel embedding on rats with myocardial ischemia-reperfusion injury," *Life Sciences*, vol. 211, pp. 51–62, 2008.

[9] S. Lu, Y. Tang, Y. Ding, M. Yu, S. Fu, and B. Zhu, "[Effects of electroacupuncture on the expression of adenosine receptors}
in the heart tissue of myocardial ischemia rats],” Zhongguo Zhen Jiu, vol. 38, no. 2, pp. 173–179, 2013.

[10] S. Wang, L. Ren, and L. Jia, “Effect of acupuncture at Neiguan (PC 6) on cardiac function using echocardiography in myocardial ischemia rats induced by isoproterenol,” Journal of Traditional Chinese Medicine, vol. 35, no. 6, pp. 653–658, 2003.

[11] Q. Zeng, H. He, and X. B. Wang, “Electroacupuncture preconditioning improves myocardial infarction injury via enhancing AMPK-dependent autophagy in rats,” BioMed Research International, vol. 2018, Article ID 1238175, 2018.

[12] Q. Yu, L. Hu, and Z. J. Wu, “[Effects of electroacupuncture stimulation of “Shenmen”(HT 7)-“Taiyuan” (LU 9) on auditory sensory gating P 50 in acute myocardial ischemia rabbits],” Zhen Ci Yan Jiu, vol. 39, no. 6, pp. 472–476, 2015.

[13] F. Foroughinia, J. Salamzadeh, and M. H. Namazi, “Protection from procedural myocardial injury by omega-3 polyunsaturated fatty acids (PUFAs): is related with lower levels of creatine kinase-MB (CK-MB) and troponin I?” Cardiovasc Theory, vol. 31, no. 5, pp. 268–273, 2016.

[14] S. Mythili and N. Malathi, “Diagnostic markers of acute myocardial infarction,” Biomed Rep, vol. 3, no. 6, pp. 743–748, 2014.

[15] I. Kodatsch, J. Finsterer, and C. Stöllberger, “Serum creatine kinase elevation in a medical department,” Acta Medica Austriaca, vol. 28, no. 1, pp. 11–15, 2001.

[16] U. Schlattner, M. Tokarska-Schlattner, and T. Wallimann, “Mitochondrial creatine kinase in human health and disease,” Biochimica et Biophysica Acta, vol. 1762, no. 2, pp. 164–180, 2005.

[17] D. J. Blomberg, W. D. Kimber, and M. D. Burke, “Creatine kinase isoenzymes. Predictive value in the early diagnosis of acute myocardial infarction,” Americas Journal of Medicine, vol. 59, no. 4, pp. 464–469, 2009.

[18] H. M. Suleiman, I. S. Aliyu, S. A. Abubakar et al., “Cardiac Troponin T and creatine kinase MB fraction levels among patients with acute ischemic stroke in Nigeria,” Nigerian Journal of Clinical Practice, vol. 20, no. 12, pp. 1618–1621, 2012.

[19] X. K. Zhang, Q. Hu, Q. H. Chen, and W. X. Wang, “The effect of continuous perfusion of esmolol on cardiovascular risk in elderly patients undergoing noncardiac surgery,” Die Pharmazie, vol. 72, no. 8, pp. 487–489, 2018.

[20] X. Teng, M. J. Emmett, M. A. Lazar, E. Goldberg, and J. D. Rabinowitz, “Lactate dehydrogenase C produces S-2-Hydroxyglutarate in mouse testis,” ACS Chemical Biology, vol. 11, no. 9, pp. 2420–2427, 2016.

[21] S. Bialik, S. K. Dasari, and A. Kimchi, “Autophagy-dependent cell death - where, how and why a cell eats itself to death,” Journal of Cell Science, vol. 13118 pages, 2017.

[22] J. Mialet-Perez and C. Vindis, “Autophagy in health and disease: focus on the cardiovascular system,” Essays in Biochemistry, vol. 61, no. 6, pp. 721–732, 2015.

[23] E. Dombi, H. Mortiboy, and J. Poulton, “Modulating mitophagy in mitochondrial disease,” Current Medicinal Chemistry, vol. 25, no. 40, pp. 5597–5612, 2016.

[24] P. Denny, M. Feuermann, D. P. Hill, R. C. Lovering, H. Plun-Favreau, and P. Roncaglia, “Exploring autophagy with gene ontology,” Autophagy, vol. 14, no. 3, pp. 419–436, 2014.

[25] J. M. Bravo-San Pedro, G. Kroemer, and L. Galluzzi, “Autophagy and mitophagy in cardiovascular disease,” Circulation Research, vol. 120, no. 11, pp. 1812–1824, 2011.

[26] Y. Wang, Q. Wang, and L. Zhang, “Coptisine protects cardiomyocyte against hypoxia/reoxygenation-induced damage via inhibition of autophagy,” Biochemical and Biophysical Research Communications, vol. 490, no. 2, pp. 231–238, 2013.

[27] C. Meissner, H. Lorenz, A. Weiھofen, D. J. Selkoe, and M. K. Lemberg, “The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking,” Journal of Neurochemistry, vol. 117, no. 5, pp. 856–867, 2012.

[28] D. P. Narendra, S. M. Jin, and A. Tanaka, “PINK1 is selectively stabilized on impaired mitochondria to activate Parkin,” PLoS Biology, vol. 8, no. 1, Article ID e1000298, 2017.

[29] S. Sekine and R. J. Youle, “PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol,” BMC Biology, vol. 16, no. 1, p. 2, 2013.

[30] C. T. Chu, “Mechanisms of selective autophagy and mitophagy: implications for neurodegenerative diseases,” Neurobiology of Disease, vol. 122, pp. 23–34, 2012.