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ORIGINAL RESEARCH

# Biao and Ben Acupoints Regulate Mitochondrial Function Through P2X Receptor to Improve Myocardial Ischemia

ShaoKun Li, BM; Saijun Wang, BM; Xiuling Feng, BM; Yanna Chang, MM; Dongling Yan, BM


## ABSTRACT

**Context** • Ischemic heart disease is a common disease in older surgical patients. The current treatments for myocardial ischemia mainly restore blood flow to an extent, but reperfusion inevitably causes reperfusion injury. Single-point acupuncture and moxibustion can strengthen the body's resistance and eliminate pathogenic factors, but medical practitioners haven't considered matching acupoints in treatments for myocardial ischemia.

**Objective** • The study intended to examine the effects of electroacupuncture using the Biao and Ben acupoints on the structure and function of myocardial mitochondria, the changes in the expression of related proteins, and the intraoperative circulation of rats with myocardial ischemia and to provide a theoretical basis for the clinical use of the Biao-Ben acupoints.

**Design** • The research team performed an animal study.

**Setting** • The study took place in Lanzhou Maternal and Child Health Hospital.

**Animals** • The animals were 84 male Sprague-Dawley (SD) rats, weighing 160-220 g.

**Intervention** • The research team divided the rats into seven groups, with 12 rats in each group. The study evaluated two types of interventions: (1) zinc chloride (ZnCl2) and (2) electroacupuncture. The team used two versions of each type of intervention, for four groups in total: (1) a low dose of ZnCl2, the low-dose ZnCl2 group; (2) a high dose of ZnCl2, the high-dose ZnCl2 group; (3) electroacupuncture using a single acupoint, the Neiguan point, for the Neiguan group; and (4) electroacupuncture using three acupoints, the Neiguan point and the Biao and Ben points, for the Biao-Ben group. The study included three control groups—the control group, a positive control group; the sham group, a ZnCl2 control group; and the model group, a negative control group. The team collectively called five of the groups the operation group, which included all four intervention groups and the model group, in which the team induced ischemic heart disease.

**Outcome Measures** • The research team measured: (1) the relative changes in the mitochondrial ultrastructure of the rat cardiomyocytes for each group using a laser confocal, fluorescent indicator assay to detect the concentration of calcium(2+) [Ca2+] in the cytoplasm of cardiomyocytes; (2) the content of adenosine triphosphate (ATP) in myocardial tissue using ATP-detection technology; (3) mitochondrial activity using the fluorescent probe method; and (4) the protein levels of P2X purinoceptor 7 (P2X7) and mitochondria-related oxidative stress factors on the myocardial cell membrane using Western blot technology. The team monitored the physiology of the rats in each group.

**Results** • Compared with the model group, the two ZnCl2 groups and the two electroacupuncture groups showed: (1) a significantly improved mitochondrial structure and function of the ischemic cardiomyocytes, (2) a significant increase in the mitochondrial activity, (3) a significant increase in the permeability of the membrane and thus an increase the concentration of Ca2+ in the cytoplasm, (4) a significant increase in the content of ATP inside and outside the myocardium, (5) at the same time, a significant reduction in the protein levels of the P2X7 receptors on the myocardial cell membrane and the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and manganese superoxide dismutase (MnSOD) in mitochondria, and (6) a significant reduction in the protein levels of nitric oxide (NO) and cytochrome C (CytC).

**Conclusions** • The Biao and Ben electroacupuncture can improve the structure and function of mitochondria in the myocardial cells of rats with myocardial ischemia, reduce the expression levels of the P2X7 receptor, NO, and CytC proteins, increase the expression levels of PGC-1α and MnSOD, and improve the intraoperative circulation, thus having a positive effect on myocardial ischemia. (*Altern Ther Health Med.* 2023;29(4):43-51).


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Ischemic heart disease is a common basic disease in older surgical patients. The reduction or interruption of coronary-artery blood flow causes a myocardial ischemia injury, resulting in an insufficient oxygen supply to the myocardium to maintain the normal function of cardiomyocytes.

Mitochondria are important organelles that provide energy for cells and are the power plant of cardiomyocytes, providing more than 95% of the energy supply for cardiomyocytes.[1] Structural abnormalities and dysfunction

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of mitochondria are one of the main characteristics of myocardial ischemia injury.[2]

Stimulation by ischemia and hypoxia can damage the mitochondrial function and makes the maintenance of the cardiomyocytes' normal function difficult. After myocardial ischemia, the number and morphology of mitochondria,[3] oxidative phosphorylation capacity,[4] permeability to calcium(2+) [Ca2+], mitochondrial enzyme activity,[1] and energy generation capacity can change.

When damage occurs to mitochondria, reactive oxygen species (ROS) increase and can further damage already damaged mitochondria and the surrounding mitochondria. Researchers haven't yet determined the pathogenesis of myocardial ischemia; however, two studies found that ROS production[5] and apoptosis[6] are involved in its development. These processes may be related to the destruction of the structure and function of myocardial mitochondria.

**Mechanisms of Myocardial Ischemia**

The P2X purinoceptor 7 (P2X7) is a key regulator of mitochondrial energy metabolism, and its expression can affect the metabolism of mitochondria and the adaptability of immune cells. P2X7 is an ATP-gated cation channel receptor, which the presence of a large amount of ATP activates. It can participate in the regulation of Ca2+, sodium(1+) [Na+], and potassium(1+) [K+][7]; promote the synthesis and release of inflammatory factors such as nitric oxide (NO) and cytochrome C (CytC), so as to participate in the occurrence and development of inflammation[7]; and also regulate cardiovascular function and pathological change processes. [6]

Some studies have shown that after ATP activates P2X7, Ca2+ enters the cell through P2X7-related channels, which can promote mitochondrial dysfunction [7,8] Toyama et al found that inhibition of P2X7 can alleviate neuronal hypoxia or reoxygenation injury by inhibiting mitochondrial fission and promoting mitochondrial fusion.[9] Chu et al found that using P2X7 antagonists in a rat model of focal, cerebral, ischemia-reperfusion (I/R) injury could effectively protect the rats from cerebral ischemia from reperfusion injury and reduce the area of the cerebral infarction.[10]

Some studies have found that inhibition of P2X7 can enhance the activity of "5' adenosine monophosphate (AMP)-activated protein kinase" (AMPK)[11] and that AMPK can directly or indirectly regulate "peroxisome proliferator-activated receptor gamma coactivator 1-alpha" (PGC-1α) to promote the synthesis of mitochondria.[12,13] " PGC-1α plays a key role in regulating the signaling pathways for mitochondrial biosynthesis and oxidative phosphorylation.[14]

The activation of PGC-1α can improve the function of mitochondria,[15] protect the mitochondrial structure, improve the body's antioxidant capacity, inhibit the mitochondrial dysfunction and oxidative damage that chronic cerebral ischemia induces, and improve the vascular cognitive impairment of rats that chronic cerebral impairment causes.[16]

Wu et al found that significantly inhibiting P2X7 can effectively stabilize the blood pressure and heart rate of mice in a type 2 diabetes model.[17]

**Electroacupuncture**

The current treatments for myocardial ischemia mainly restore blood flow to an extent, but reperfusion inevitably causes reperfusion injury, so it's particularly important to find a new treatment.[18] Western medicine is effective, but it's easy to cause hemodynamic changes and reduce muscle strength during surgeries.

Lin et al found that an electroacupuncture intervention can increase the activities of adenosine-triphosphate-(ATP) ase in cardiomyocytes, thus decreasing myocardial ischemia related to a reperfusion injury.[19] Luo et al found that such an intervention can regulate the nuclear factor-κB (NF- κB) signaling pathway and alleviate inflammatory reactions.[20] However, the mechanism of electroacupuncture in treating myocardial ischemia isn't fully understood.

Single-point acupuncture and moxibustion can strengthen the body's resistance and eliminate pathogenic factors, but medical practitioners haven't considered matching acupoints in treatments for myocardial ischemia.

**Biao-Ben Acupoints**

Chen et al found that using the Biao-Ben acupoints can increase the activity of the mitochondrial respiratory enzymes of ischemic cardiomyocytes; improve the potential of the myocardial mitochondrial membrane; enhance aerobic respiratory productivity; and improve cardiac ATP metabolism, the cardiac energy supply.[6] These activities can reduce the damage to the myocardial cell structure that ischemia causes, significantly reduce the myocardial infarction area, and have a certain protective effect on myocardial tissue.[6]

Using the Biao-Ben acupoints can also regulate the "nuclear factor kappa-light-chain-enhancer of activated B cells" (NF-κB) signaling pathway, inhibit release of tumor necrosis factor alpha (TNF-α), and increase the production of interleukin 10 (IL-10), thereby reducing the inflammatory response.[20] In addition, Lin et al found that an electroacupuncture intervention could reduce the content of IL-8 in the serum of rats with myocardial ischemia, increase the activities of the complex enzymes of the mitochondrial respiratory chain and the ATPase in cardiomyocytes, and downregulate the expression of the FXR gene, thus decreasing myocardial ischemia from reperfusion injury.[19]

**Current Study**

Researchers need to explore whether application at multiple points is better than application at a single point for myocardial ischemia.

The current study intended to examine the effects of electroacupuncture using the Biao and Ben acupoints on the structure and function of myocardial mitochondria, the changes in the expression of related proteins, and the

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intraoperative circulation of rats with myocardial ischemia and to provide a theoretical basis for the clinical use of the Biao-Ben acupoints.

## METHODS

### Animals

The research team performed an animal study. The study took place in Lanzhou Maternal and Child Health Hospital. The animals were 84 male Sprague-Dawley (SD) rats, weighing 160-220 g. The team purchased them from Beijing Weitong Lihua Experimental Animal Technology (Beijing, China).

The research team housed the rats five per cage in an sun protection factor (SPF)-level barrier, with fluorescent-lamp lighting, 12-hour alternating light and dark, a room temperature at 20-25°C, and humidity at 55%-65%. The rats were free to eat and drink water, and the study started after adaptive feeding for one week.

### Procedures

**Main reagents and consumables.** The research team purchased a mitochondrial red fluorescent probe, the C1049B Mito-Tracker Red CMXRos (Beyotime, Shanghai, China) and an ATP quantitative test box, the BC0305 (Solarbio, Beijing, China).

**Main instruments.** The research team purchased: (1) an ALC-V8 animal ventilator (Shanghai Alcott, Shanghai, China); (2) a blood pressure meter for rats, the GE VIVID7 cardiac ultrasound diagnostic apparatus (GE, San Francisco, CA, USA); (3) thoracic surgical instruments (Shanghai Medical Instruments, Shanghai, China); (4) the Japan Fukuda FX7200 ECG machine, a JEM1230 transmission electron microscope (JEOL company, Tokyo, Japan), and (5) the Leica EM UC6 ultrathin slicer (Leica, Wetzlar, Germany).

**Groups.** The research team divided the rats into seven groups, with 12 rats in each group, using a random number table.

The study evaluated two types of interventions: (1) zinc chloride (ZnCl2) and (2) electroacupuncture. Zinc chloride is administered by intraperitoneal injection. The research team purchased the zinc chloride from Jiangsu Nhwa Pharmaceutical Co., Ltd. (Xuzhou, Jiangsu, China).

The team used two versions of each type of intervention, for four groups in total: (1) a low dose of ZnCl2 was administered at a dose of 2.5 ug/g, the low-dose ZnCl2 group; (2) a high dose of ZnCl2 was administered at a dose of 10 ug/g the high-dose ZnCl2 group; (3) electroacupuncture using a single acupoint, the Neiguan point, for the Neiguan group; and (4) electroacupuncture using three acupoints, the Neiguan point and the Biao and Ben points, for the Biao-Ben group ZnCl2 was administrated once per day and for 21 days. For the Neiguan and Biao-Ben groups, the rats received the electroacupuncture intervention for 10 minutes per day for 21 days. The team induced ischemic heart disease in all four groups.

The study included three control groups—the control group, a positive control group; the sham group, a ZnCl2 control group; and the model group, a negative control group. The team didn't induce ischemic heart disease in the sham group or the control group but did induce it in the model group. The control group and the model group received no treatments. The sham group received intraperitoneal injection of saline.

The team collectively called five of the groups the operation group, which included all four intervention groups and the model group.

**Induction of ischemic heart disease.** For the induction, the research team used the coronary-artery ligation method to create the rat myocardial ischemia model, following the method that Qi et al used.[21]

For the operation groups, the research team: (1) placed 8-0 gauge sharp needles of the same size only at the position of the anterior descending branch of the left coronary artery between the lower edge of the left atrial appendage and the conus of the pulmonary artery; (2) stitched silk thread without a knot; and (3) injected the rats with 200 000 units per day of penicillin sodium intramuscularly, with the antiinfection operation occurring for five consecutive days. The research team considered elevation of the ST segment at an amplitude of ≥0.1 mV to be successful modeling.

**Specimen collection and processing.** At day 22 for each group, after 21 days of treatment, the research team: (1) anesthetized the rats with 150 mg/kg of sodium pentobarbital using an intraperitoneal injection; (2) opened the rat's chest and exposed the heart; (3) quickly extracted the rat's heart using thoracotomy under aseptic conditions and placed it on an ice bag; (4) cleaned the blood stain on the surface with 0.9% sodium chloride (NaCl) solution; (5) trimmed the excess adipose tissue and rapidly rinsed the heart tissue with phosphate buffered saline (PBS) at 4°C.

**Ultrastructural observation of mitochondria in cardiomyocytes**. The research team: (1) cross-cut the lower one-quarter of the heart and cut it apart into a 1-$mm^3$ small patch; and (2) took that part of the heart-tip tissue and fixed it in 2.5% glutaraldehyde at 4°C for 2-4 h for observation of the mitochondrial structure under the transmission electron microscope. After gradient dehydration and infiltration embedding and sectioning, the team saturated the tissues with uranium acetate and lead citrate for step-by-step staining and then observed them under the microscope and collected pictures.

The team: (1) homogenized another part of the heart-tip tissue and then measured the mitochondrial activity, adenosine triphosphate (ATP) content, intracellular Ca2+ concentration, and cardiac-tissue related proteins. The research team detected the activity of mitochondria using the mitochondrial, red, fluorescent-probe technology.

**Detection of mitochondrial activity.** The team used cardiomyocytes homogenized in an ice bath according to the manufacturer's instructions for the MitoTracker Red (Beyotime) to observe and photograph them with a laser confocal microscope to collect images.

**Detection of intracellular Ca2+ concentration.** The research team measured the intracellular Ca2+ concentration of cardiomyocytes according to Martewicz et al's method, as described in the literature.[23]

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**Detection of ATP content.** After centrifugation, the research team used the supernatant to determine the ATP content in the myocardial tissue, according to the manufacturer's steps in the kit's instructions.

**Detection of cardiac-tissue-related proteins.** The research team measured the P2X7 in the myocardium and the protein expression levels of PGC-1α, manganese superoxide dismutase (MnSOD), NO, and CytC in the mitochondria using Western blot. The downregulation of MnSOD can alleviate the mitochondrial peroxidation damage of airway epithelial cells.[24]

The team took an appropriate amount of fresh heart tissue and added 200 μL of single detergent lysate, fully homogenized it, lysed it on ice for 30 min, centrifuged it at 12 000 rpm for 5 min at 4°C, and collected the supernatant.

The team determined the total protein concentration using the bicinchoninic acid (BCA) method. The team loaded 40 ug of total protein for electrophoresis and gel running. After that, the team transferred the antibody, blocked it, incubated it, and then exposed it to light to analyze the gray value. The team used the ratio of the gray value of the target protein and the internal reference protein for relative analysis.

**Intraoperative circulation monitoring.** At the end of the study, the research team: (1) clamped the tails of the rats with a rat blood pressure meter to monitor their heart rates and blood pressures and (2) measured and recorded the left ventricular ejection fraction (LVEF), left ventricular short axis shortening rate (LVFS), ventricular septal thickness (IVS), left ventricular posterior wall (LVPW), ratio of peak E to peak A (E/A ratio), left ventricular internal diameter at end systole (LVIDs), and left ventricular internal diameter at end diastole (LVIDd).

**Outcome measures.** The research team measured: (1) the relative changes in the mitochondrial ultrastructure of the rat cardiomyocytes for each group using a laser confocal, fluorescent indicator assay to detect the concentration of Ca2+ in the cytoplasm of cardiomyocytes; (2) the mitochondrial activity using the fluorescent probe method; (3) the content of ATP in myocardial tissue using ATP-detection technology; and (4) the protein levels of P2X7 receptors and mitochondria-related oxidative stress factors on the myocardial cell membrane using Western blot technology. The team also monitored the physiology of the rats in each group.

## Intervention

The research team selected the acupuncture points according to the rat-point position atlas,[22] as formulated by Hua Xingbang and others.

**Neiguan group.** The Neiguan point was located on the inner side of the rat's forelimb between the ulnar and radial sutures, about 3 mm away from the wrist joint, with a straight puncture of 5 mm. At 0.5 cm to the right of Neiguan point, the research team shallow-pricked a needle as an auxiliary electrode to form a pair of electrodes with Neiguan point.

**Biao-Ben group.** The research team needled the rats at the Neiguan point on both sides and at the Zusanli and Guanyuan points on both sides, connected with an electroacupuncture apparatus that had a density-wave, frequency conversion of 2-100 Hz, a density wave, a conversion of 14 times/min, a voltage of 1.6 V, an intensity of 1mA, taking an obvious tremor sensation on the limbs of rats as the degree.

## Outcome Measures

**Intracellular Ca2+ concentration of cardiomyocytes.** We applied the should probe method to measure the calcium ion concentration in the cytoplasm of cardiomyocytes, which is based on the change of fluorescence signal after the fluorescent probe binds to Ca2+, and can achieve real-time monitoring and quantitative measurement of Ca2+ concentration. The results obtained by the fluorescent probe method can reflect the changes of intracellular Ca2+ concentration. For example, during myocardial ischemia-reperfusion, elevated Ca2+ concentrations can cause adverse effects such as intracellular Ca2+ overload and impaired mitochondrial function. Fluorescent probe methods can help researchers monitor and quantify these effects in real time.

**ATP content of myocardial tissue.** The following results may occur during the measurement of ATP content in myocardial tissue. Normal ATP content: Under normal conditions, the ATP content in myocardial tissue is usually high enough to maintain the normal metabolism and function of myocardial cells. In this case, the result of ATP content measurement should match the normal reference value. Reduced ATP content: When cardiomyocytes suffer from ischemia, hypoxia and other injuries, ATP synthesis is inhibited, resulting in reduced ATP content. In this case, the result of ATP content measurement will show a tendency to decrease, reflecting the impaired myocardial energy metabolism; elevated ATP content: Under certain conditions, such as contraction and excitation of cardiac myocytes, the rate of ATP breakdown will be accelerated, leading to an increase in ATP content. In addition, elevated ATP content in myocardial tissue may also be a manifestation of some diseases, such as hyperthyroidism. In such cases, the results of ATP content measurements will show a tendency to increase.

**Changes in muscle tissue related protein levels.** Changes in muscle tissue-related protein levels may result in several possible outcomes, depending on the specific protein and the nature of the change. Some possible results are:Increased protein expression: An increase in the expression of certain muscle tissue-related proteins, such as myosin heavy chain or actin, may result in increased muscle mass, strength, and function. This can occur as a result of exercise training, anabolic steroid use, or other interventions that promote muscle growth; Decreased protein expression: A decrease in the expression of muscle tissue-related proteins can result in muscle atrophy and weakness. This can occur as a result of aging, immobilization, or other conditions that

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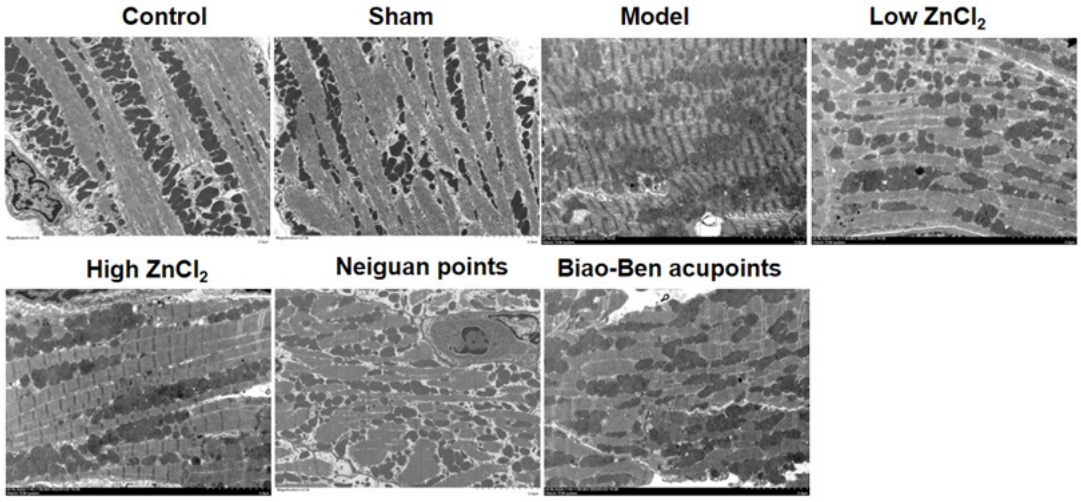
**Figure 1.** Ultrastructure of Myocardial Mitochondria of Rats in Each Group




**Abbreviations:** ZnCl2, zinc chloride.

cause disuse of the muscles;Altered protein isoform expression: Many muscle tissue-related proteins exist in different isoforms, which have different functional properties. Changes in the expression of specific isoforms can affect muscle function in different ways. For example, a shift in myosin heavy chain isoform expression from slow-twitch to fast-twitch fibers may result in increased muscle power but decreased endurance; Abnormal protein aggregation: In some diseases, such as amyotrophic lateral sclerosis (ALS) or inclusion body myositis (IBM), abnormal protein aggregation occurs in muscle tissue. This can lead to muscle weakness and wasting; Dysregulated protein signaling: Many muscle tissue-related proteins are involved in signaling pathways that regulate muscle growth, metabolism, and function. Dysregulation of these pathways, such as activation of the ubiquitin-proteasome system or inhibition of the Akt/mTOR pathway, can result in muscle wasting and weakness.

### Statistical Analysis

The research team entered the data into the computer and analyzed it with Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM, Armonk, NY, USA). The team expressed all data as means ± standard deviations (SDs) and used one-way analysis of variance (ANOVA) for comparisons between groups. The significance level was $P < .05$.

## RESULTS

### Ultrastructural Observation

Figure 1 shows the ultrastructure after induction of myocardial ischemia in the operation groups. For the control group: (1) the mitochondria in the ventricular myocardium of rats were abundant; (2) the cell size was uniform; (3) all parts were complete, without rupture, loss, swelling, or deformation; (4) all parts were arranged in an orderly manner; (5) the membrane's outline was clear and complete; (6) the mitochondrial ridge was clear; (7) the sarcomere was arranged in an orderly manner, and (8) the light and dark bands were clear. For the sham group, the morphology of the mitochondria was basically the same as that of the control group.

For the model group, the mitochondria: (1) in the ventricular myocardium of rats were of different sizes, arranged in disorder; (2) showed hypertrophy, swelling, displacement, and aggregation, accompanied by the loss of mitochondrial membrane; and (3) showed breakage of the mitochondrial spine and disorder of the fiber arrangement.

For the low-dose ZnCl2 group, changes had occurred, but the changes were lighter than those of the model group: (1) the number of some mitochondria significantly decreased, (2) some mitochondria swelled, (3) some mitochondrial ridges were blurred or broken, and (4) the muscle fibers were arranged irregularly.

For the high-dose ZnCl2 group: (1) some mitochondria were slightly swollen but were arranged in order, (2) some mitochondrial ridges were fuzzy, (3) the sarcomeres were arranged in order, and (4) the degree of morphological change was smaller than that in the other intervention groups.

The Neiguan group's results were comparable to those of the low-dose ZnCl2 group, but the mitochondrial morphology in the group was worse.

The Biao-Ben group had fewer mitochondrial morphological changes than the Neiguan point group did, but the effects weren't as good as those of the high-dose ZnCl2 group.

### Mitochondrial Activity

Figure 2 shows the mitochondrial activity after induction of myocardial ischemia in the operation groups. The control group had stronger red fluorescence and higher mitochondrial activity, and the sham group's mitochondrial activity was similar to that of the control group. The model group's mitochondrial activity was weak, and the low-dose ZnCl2 group's mitochondrial activity was slightly better than that of the model group.

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**Figure 2.** mitochondrial activity of each group

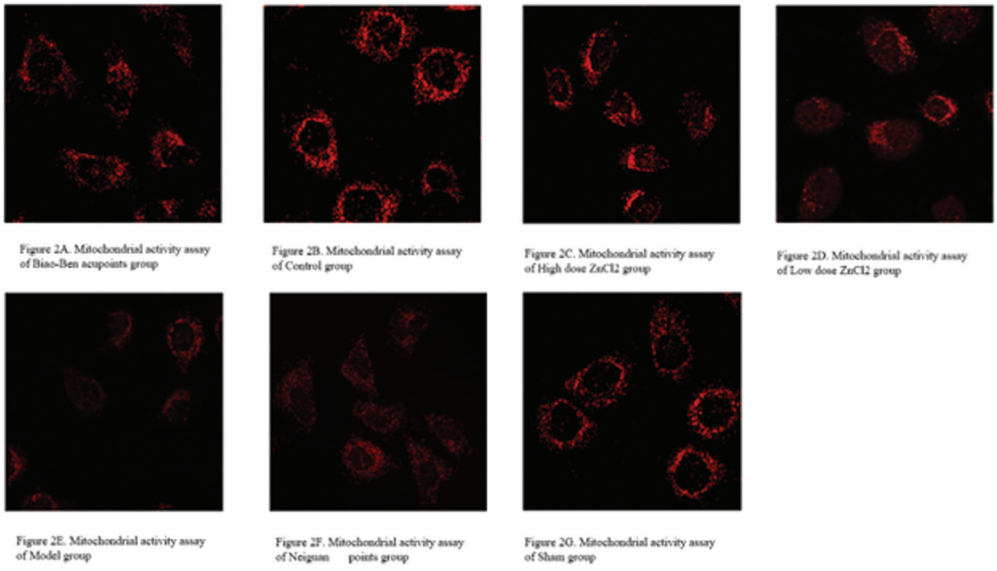
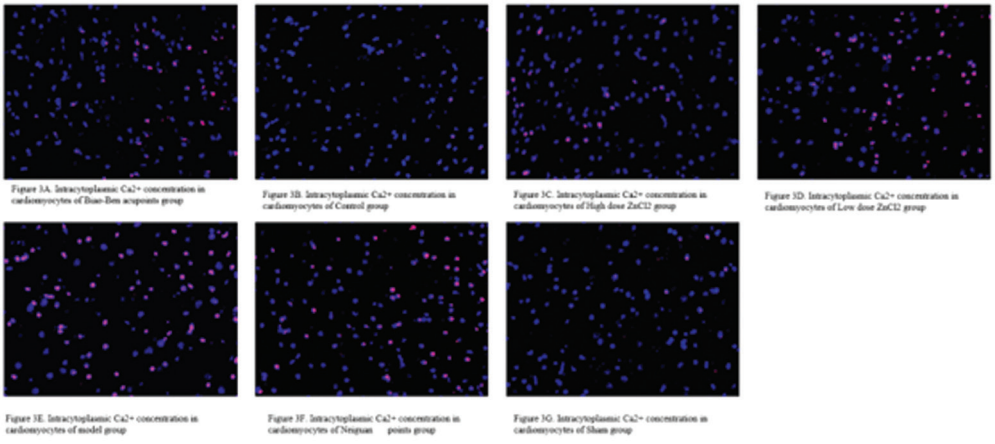



**Figure 3.** Ca2+ concentration of cardiomyocytes in each group




The high dose ZnCl2 group's mitochondrial activity was significantly better than that of the model group. The mitochondrial activity of the Neiguan group was similar to that of the low-dose ZnCl2 group, and the mitochondrial activity of the Biao-Ben group was similar to that of the high-dose ZnCl2 group.

### Ca2+ Concentration

Figure 3 shows the Ca2+ concentration of cardiomyocytes after induction of myocardial ischemia in the operation groups. The concentration in the model group was significantly higher than that of the control and sham groups postintervention (both $P < .05$). The concentration decreased significantly between baseline and postintervention after ZnCl2 administration in a dose-dependent manner. The high-dose ZnCl2 group had a significantly lower concentration of Ca2+ postintervention than the low-dose group did ($P < .05$).

The reduction in Ca2+ between baseline and postintervention in the Neiguan group was similar to that in the low-dose ZnCl2 group. The reduction in Ca2+ between baseline and postintervention in the Biao-Ben group was slightly smaller ($P > .05$) than that of the high-dose ZnCl2 group but higher ($P < .05$) than that of the low-dose ZnCl2 group and the Neiguan group.

### ATP Content

Figure 4 shows the ATP content in tissues and cardiomyocytes after induction of myocardial ischemia in the operation groups. The ATP content in the tissues and cardiomyocytes of the Neiguan group was detected. Compared with that of the control and sham groups, the model group's ATP content was lower($P < .05$). The ATP content increased from baseline after ZnCl2 administration, with the increase in the high-dose ZnCl2 group being greater ($P < .05$).

The Neiguan group's ATP didn't increase between baseline and postintervention, but the Biao-Ben group's increase in ATP content was higher ($P < .05$) than that in the low-dose ZnCl2 group and lower ($P < .05$) than that in the high-dose ZnCl2 group.

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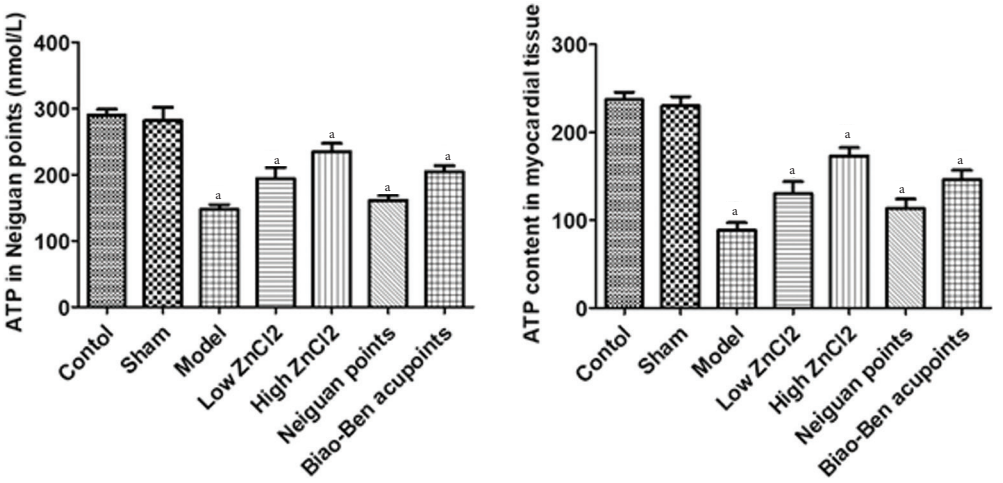
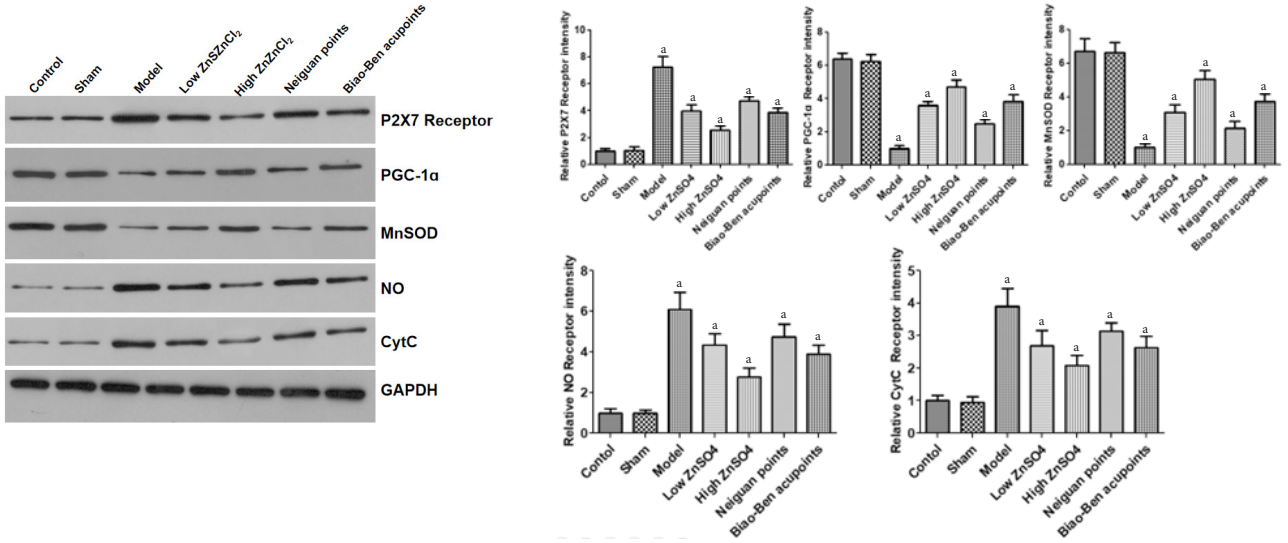
**Figure 4.** ATP content in tissues and cardiomyocytes after induction of myocardial ischemia in the operation groups




[a]indicated that when compared with Control and sham groups $P < .05$

**Figure 5.** The Expression Level of Related Proteins in Myocardial Tissue of Rats in Each Group




[a]indicated that when compared with Control and sham groups $P < .05$

**Abbreviations:** CytC, cytochrome C; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MnSOD, manganese superoxide dismutase; NO, nitric oxide; P2X7, P2X purinoceptor 7; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ZnCl2, zinc chloride.

**Protein Levels**

Figures 5A and 5B show that the expression of P2X7 on the myocardial cell membrane and the NO and CytC in the mitochondria of myocardial cells in the operation groups were significantly higher postintervention than those of the control and sham groups (all $P < .05$).

However, in the two ZnCl2 groups and the two electroacupuncture groups, the protein levels was significantly lower postintervention than in the model group (all $P < .05$).

Compared with the control and sham groups, the expression of MnSOD and PGC-1α in the model group was significantly lower postintervention (all $P < .05$). However, in the two ZnCl2 groups and the two electroacupuncture groups, the protein levels were higher ($P < .05$) postintervention than that of the model group, and the difference was most significant in the high-dose ZnCl2 group (both $P < .05$), followed by the Biao-Ben group (both $P < .05$).

**Circulation Monitoring**

The heart rate of rats in each group was normal, and no significant differences existed between the groups postintervention. Compared with the control and sham groups, the blood pressure of the rats in the operation groups was slightly higher after induction of myocardial ischemia in

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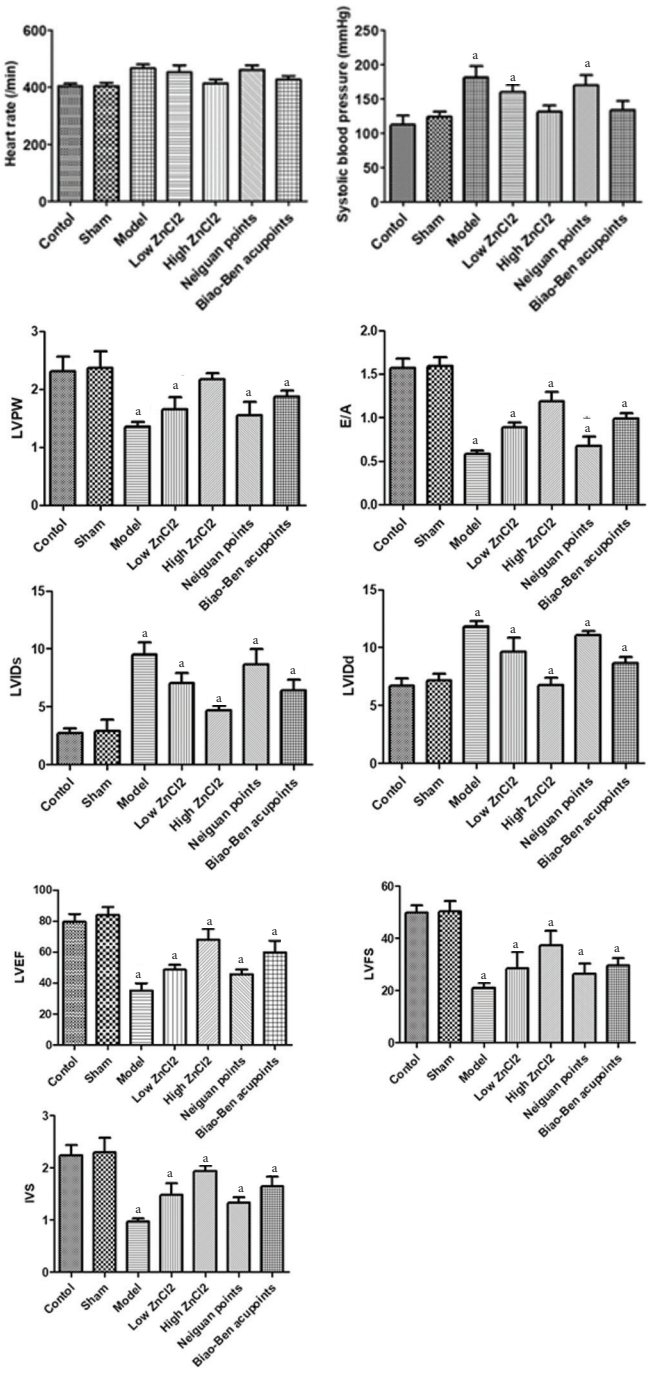
**Figure 6.** Different circulation monitoring parameters of each group




[a]indicated that when compared with Control and sham groups $P < .05$

the operation groups, but all groups had recovered postintervention. Among them, the high-dose ZnCl2 group and the Biao-Ben group improved the most significantly between baseline and postintervention.

Compared with the control and sham groups, the LVEF, LVFS, IVS, LVPW, and E/A ratio of rats in operation groups decreased more after induction of myocardial ischemia in the operation groups, but all groups had recovered postintervention. Among them, the high-dose ZnCl2 group and the Biao-Ben group improved the most significantly between baseline and postintervention (all $P > .05$).

Compared with the control and sham groups, the LVIDs and LVIDd values of rats in the operation groups increased more after induction of myocardial ischemia in the operation groups, but all groups had recovered postintervention. Among them, the high-dose ZnCl2 group and the Biao-Ben group decreased most significantly between baseline and postintervention.

## DISCUSSION

The current study found that the electroacupuncture using the Biao-Ben acupoints was better than that using the Neiguan acupoint, with respect to the changes in the myocardial mitochondrial microstructure and function in rats. This suggests that the Biao-Ben electroacupuncture can delay or repair the morphological changes of mitochondria caused by myocardial ischemia and protect the structure of myocardial mitochondria.

In addition, the current study found that the Biao-Ben electroacupuncture improved the mitochondrial activity the rats, increased the permeability of the mitochondrial membrane, maintained the stability of the mitochondrial dynamics, and thus improved the symptoms of myocardial ischemia in the rats. The expression level of P2X7 receptor protein decreased significantly and the expression level of PGC-1α improved significantly after the Biao-Ben electroacupuncture. This is consistent with the reported results.[1,25]

The current study found that all postoperative cardiac function indexes of the model rats improved after receiving the Biao-Ben acupoints electroacupuncture. This may be due to the reduction of P2X7 expression, which may have inhibited the P2X7 pathway, reducing the expression of a series of pro-inflammatory factors, such as NO and CytC, and reducing the excitability of the nervous system, which ultimately may have improved the intraoperative circulation of the rats with myocardial ischemia model.

The current study showed that Biao-Ben electroacupuncture could significantly reduce the protein expression level of P2X7 receptor, and the decrease in P2X7 receptor promoted the protein expression level of PGC-1α. This could improve the mitochondrial structure and function of myocardial ischemia rats, thus improving the myocardial energy supply.

In addition, the decreased expression of P2X7 inhibited the synthesis and release of various downstream inflammatory

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factors and improved the intraoperative circulation and physiological state the rats.

The present study has some limitations. The study uses 84 male rats, which are divided into several groups, including control, administration, and electroacupuncture intervention groups. The sample size for each group is not specified, making it difficult to determine the statistical power of the study. While the study reports several changes in mitochondrial function and protein levels, the mechanisms underlying these changes are not fully explained. More studies are needed to investigate the signaling pathways and molecular mechanisms involved in the observed effects. Although the study suggests that "Biao and Ben acupoints" electroacupuncture treatment has a positive effect on myocardial ischemia, the clinical relevance of these findings is uncertain. The study was conducted on rats, and it is unclear whether the results can be extrapolated to humans. Further studies are needed to assess the safety and efficacy of this treatment in humans.

## CONCLUSIONS

The Biao-Ben electroacupuncture can improve the structure and function of mitochondria in the myocardial cells of rats with myocardial ischemia, reduce the expression levels of the P2X7 receptor, NO, and CytC proteins, increase the expression levels of PGC-1α and MnSOD, and improve the intraoperative circulation, thus having a positive effect on myocardial ischemia.

### AUTHORS' DISCLOSURE STATEMENT

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