Effects of electroacupuncture stimulation of the Wushu acupoints along the heart channel on brain-derive neurotrophic factor overexpression and angiogenesis in rats with acute myocardial ischemia

Hao-Sheng Wu\textsuperscript{1,3}, Hang Su\textsuperscript{1,3}, Wen-Hui Wang\textsuperscript{1,3}, Chao Zhu\textsuperscript{2,3}\textsuperscript{*}, Sheng-Bing Wu\textsuperscript{1,3}, Mei-Qi Zhou\textsuperscript{1,3}\textsuperscript{*}.

\textsuperscript{1}College of Chinese Medicine, Anhui University of Chinese Medicine, Hefei 230012, China. \textsuperscript{2}Anhui Province Key Laboratory of Meridian Viscera Correlationship, Anhui University of Chinese Medicine, Hefei 230012, China. \textsuperscript{3}College of Acupuncture and Massage, Anhui University of Chinese Medicine, Hefei 230012, China.

\textsuperscript{*}These authors contributed equally to this work and are co-first authors for this paper.

\textsuperscript{Correspondence to:} Chao Zhu, Sheng-Bing Wu, Mei-Qi Zhou. Anhui Province Key Laboratory of Meridian Viscera Correlationship, No. 103 Meishan Road, Shushan District, Hefei 230012, China. E-mail: zc472485026@ahctcm.edu.cn; wsb922@126.com; meiqizhou@163.com.

Abstract

Background: To explore the effect of electroacupuncture Wushu acupoints on angiogenesis and expressions of brain-derived neurotrophic factor (BDNF), as well as to explore the possible mechanism of electroacupuncture on acute myocardial ischemia (AMI). Methods: We randomly divided 42 Sprague Dawley rats into sham AMI group, AMI group, and the heart channel groups comprising Shaochong (HT9) acupoint, Shaofu (HT8) acupoint, Shenmen (HT7) acupoint, Lingdao (HT4) acupoint, and Shaozhi (HT3) acupoint groups, with 6 rats in each group. The AMI model was fabricated via hypodesmus of the left anterior descending coronary artery. After modeling, the corresponding acupoints of each group underwent electroacupuncture treatment 30 min/time, once/day for 3 consecutive days. The cardiogram obtained before and after the intervention was compared, and the pathologic changes of the myocardial tissue were observed via hematoxylin & eosin staining. The amount of serum endothelin-1, prostacycline-2, thromboxane-2, and BDNF indicators were assayed using ELISA, the number of CD31-positive cells in myocardial tissue was calculated using immunohistochemistry, and the expression levels of BDNF and TrKB was measured using western blot. Results: Compared with the sham AMI group, the ST segment on the electrocardiogram was significantly elevated (P < 0.05); the myocardial angiogenesis marker CD31 was remarkably increased (P < 0.05); serum levels of endothelin-1, prostacycline-2 and thromboxane-2 decreased (P < 0.05), whereas serum levels of thromboxane-2 levels were increased (P < 0.05); and those of serum BDNF was decreased (P < 0.05); the protein overexpression contents of BDNF and TrKB in the myocardial tissues of HT8 acupoint and Shenmen acupoints groups were reduced (P < 0.05) in the AMI group. Compared with the AMI group, in the heart channel groups, the ST segment of electrocardiogram was dramatically reduced (P < 0.05); CD31 was dramatically elevated (P < 0.05); the serum levels of endothelin-1, prostacycline-2, and thromboxane-2/2-thromboxane-2 were increased (P < 0.05), thromboxane-2 levels were reduced (P < 0.05); the serum BDNF level was increased (P < 0.05); the protein overexpression levels of BDNF and TrKB in myocardial tissue of HT8 group and Shenmen group was increased (P < 0.05). Conclusion: electroacupuncture of Wushu acupoints along the heart channel may enhance myocardial ischemia by promoting overexpression of serum BDNF and activating the BDNF/TrKB pathway to promote myocardial angiogenesis.

Keywords: electroacupuncture; Wushu acupoints; heart channel; brain derived neurotrophic factor; angiogenesis; acute myocardial ischemia
**Background**

Acute myocardial ischemia (AMI) is a pathological condition where myocardial function is impaired, and necrosis occurs due to obstruction of coronary blood supply. Myocardial ischemia and hypoxia is the principal disease leading to increased mortality and disability rates [1, 2]. Based on the Guidelines for Cardiovascular Risk Assessment and Management in China released in 2019, more than 1 million people died from cardiovascular disease [3]. Although advances in surgical intervention and drug therapy in the past have improved the survival rate of patients with myocardial ischemia and infarction, these patients continue to develop cardiac remodeling and heart failure, which is an economic burden on the patient’s family and society [4]. Therefore, there is an urgent need to investigate the pathogenesis of myocardial ischemia and explore appropriate interventions for its prevention.

Previous studies revealed that the heart channel in traditional Chinese medicine theory may have a specific link to the heart in the disease condition, and electroacupuncture (EA) at the Wushu acupoints on the heart vein can improve the heart function of AMI rats [5]. In recent years, numerous clinical trials and experimental studies have confirmed that acupuncture can effectively treat angioarciopathy via regulation of oxidative stress, inhibition of the cardiac fibrosis process, cardiovascular autonomic regulation, and other pathways [5-17]. Therefore, investigating the mechanism of Wushu acupoints on the improvement of myocardial ischemia is helpful in interpreting the traditional medicine of the great Chinese nation and constantly firmly safeguards the historical consciousness and cultural confidence of the excellent traditional Chinese culture.

Angiogenesis is a process wherein endothelial cells of mature blood vessels proliferate and migrate, generating new blood vessels in a budding or nonbudding form [18, 19]. With the concept of therapeutic angiogenesis, several studies continue to prove that acupuncture can promote angiogenesis, thereby effectively increasing the blood supply to ischemic tissues [20-23]. Brain-derived neurotrophic factor (BDNF) molecules and their TrkB receptors are found in coronary endothelial cells, and blood BDNF concentrations increase following myocardial infarction [24-26]. It has been reported that overexpression of BDNF in cardiac tissue increases capillary density [27, 28]. Therefore, acupuncture may have a role in improving myocardial ischemia and increasing angiogenesis by enhancing the expression of the BDNF/TrkB pathway. This study focused on angiogenesis by comparing and analyzing the effect of Wushu acupoints on angiogenesis, this study compared and analyzed the effect of Wushu acupoint of the heart channel. The possible mechanism of improving myocardial ischemia via EA at Wushu acupoints was investigated by observing the effects on heart-related indexes, myocardial angiogenesis, levels of vascular balance factor, and expression of BDNF/TrkB protein, which are the basis for the Wushu acupoints on the heart channel to promote angiogenesis and improve myocardial ischemia through BDNF/TrkB signaling pathway.

**Materials and methods**

**Animals**

Fifty Sprague Dawley male rats of SPF grade, three-month-old, weighing 200 ± 20 g purchased from Pizhou Dongfang Aquaculture Co., Ltd. (Pizhou China, Certificate No. SCXK (SU) 2017–0003) were included in the study. All rats were placed in an automatic ventilation cage and allowed free access to standard animal feed and drinking water in an orderly cage with a 12 h light-dark cycle. All rats’ experimental processes were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and performed with the approval of the Institution Ethics Committee of Anhui University of Chinese Traditional Medicine (Certificate No. AHUCM-Rats-2021014).

**Animal grouping and models establishment**

Following adaptive feeding for one week prior to the experiment, of the 50 rats, 6 were randomly chosen to be sham-operated and were known as the sham AMI group. In the sham AMI group, we made punctures only at the back of the chest without ligation of the left anterior descending coronary artery. The remaining 44 rats were all modeled by using 6-0 surgical sutures to ligature the left anterior descending branch of the coronary artery, and 8 rats died during the modeling process. Following model replication, electrocardiogram (ECG) lead II recordings of ST-segment elevation greater than 0.1 mV and high T-wave were considered as the success criteria for the model [29] (Figure 1). The remaining surviving and successfully modeled 36 rats were stochastically split into six groups with six rats in each group: AMI, Shaochong (HT9), Shaofu (HT8), Shenmen (HT7), Lingdao (HT4) and Shaoai (HT3) groups.

**Main reagents and apparatus**

The following main experiment reagents were purchased for this study: Hematoxylin & eosin dye solution (ebiogo, Anhui, China, lot number: 09232110, 09122109; rat BDNF, endothelin-1 (ET-1), prostacyclin-2 (PGI2), thromboxane A2 (TXA2) ELISA Kits (Genmed Technology Co., LTD., Wuhan, China, lot number: JYM0666RA, JYM0591RA, JYM0805RA, JYM0893RA); CD31 antibody (Santa, Texas U.S.A., lot number: sc-376764); vascular endothelial growth factor-A (VEGF-A) antibody (Bios, Boston, U.S.A., lot number: sc-13133R); GAPDH (Zsibo, Wuhan China, lot number: TA-08); BDNF antibody (Boster, Wuhan China, lot number: BA0565); TrkB antibody (Abcam, Cambridge, U.K., lot number: ab6178341); goat anti-mouse IgG (Zsibo, Wuhan, China, lot number: ZB-2305); and goat anti-rabbit IgG (Zsibo, Wuhan, China, lot number: ZB-2301).

The following instruments were used for the study: Enzyme-label analyzer (Leidu Company, Shenzhen, China, RT-6000), electroacupuncture therapy instrument (Medical Supplies Factory Co., Ltd., Suzhou, China, SDZ-II), Powerlab 16-guide physiological recorder (AD instruments, Sydney, Australia, ML870), general anesthesia machine for small animals (Reward Life Technology Co., Ltd., Shenzhen, China, R500), high-speed desktop refrigerated centrifuge (Thermo Fisher, Massachusetts, U.S.A., ST16R), electrophoresis apparatus (Bio-Rad Company, California, U.S.A., mini-protein 3 cell) dehydrator, and encapsulation machine (Junjie Electronics Co., Ltd., Wuhan, China, JJ-12J; JB-P5), and pathological micrometre (Leica Instrument Co., Ltd., Witzlitz, Germany, RM2016).

**Electroacupuncture treatment**

The acupuncture intervention positioning map of the rats was in accordance with the Name and location of common acupoints in laboratory animals and a previous study [30]. The HT9 acupoint is located at the radial fingernail root corner of the little finger. The HT8 acupoint is located between the 4th and 5th metacarpal bones of the palm. The HT7 acupoint is located on the edge of the ulna of the

**Highlights**

Wushu acupoint (It is five acupoints arranged from the end of the limbs to the elbow and knee on the same meridian, used to treat the corresponding visceral disease) is an acupoint selection method in traditional Chinese medicine. Although their distribution varies, they are located on the same channel and can possess a similar role. Wushu acupoints on the heart channel were found to likely significantly improve the symptoms of acute myocardial ischemia, which may be related to angiogenesis.

**Medical history of objective**

The concept of Wushu acupoints in traditional Chinese medicine first appeared in the book Inner Canon of Yellow Emperor: Spiritual Pivot - Nine Needles and Twelve Original (unknown author, 221 B.C.E.–220 C.E.). In ancient times, the Wushu acupoint along the heart channel can treat heart-related diseases and emotional diseases, such as angina, forgetfulness, insomnia, etc.
transverse wrist of the forelimb. The HT4 acupoint is located at the radial edge of the wrist flexor tendon on the ulnar side of the forearm, about 3 mm away from the HT7 acupoint. The HT3 acupoint is located in the medial depression of the internal epicondyle of the humerus. HT9 acupoint, HT8 acupoint, HT7 acupoint, HT4 acupoint, and HT3 acupoint are the Wushu acupoints of the heart channel. The acupuncture angle was straight, and the depth of acupuncture was 2 mm. The EA intervention parameters were as follows: the waveform is a continuous wave; the current intensity is 1 mA; the current frequency is 2 Hz; the electroacupuncture duration is 30 min, once a day. The EA intervention therapy was operated 1 day after AMI for three consecutive days in all acupuncture intervention groups.

Detection indexes and methods

**Hematoxylin and eosin staining.** After anesthesia with isoflurane, the myocardial tissue of rats was fixed in 4% paraformaldehyde orderly, dehydrated in an ethanol gradient, paraffin-embedded, and cut into 4 µm sections. The sections were baked (66 °C for 30 min), cleared with xylene (thrice for 10 min each), and immersed in 100%, 90%, 80%, 70%, and 50% ethanol gradients for 5 min each. The sections were washed with water, stained with hematoxylin (1 min), washed with water, and the sections were blued with 1% ammonia (1 min). This is followed by a differentiation step with 1% hydrochloric acid ethanol for 5 s. The sections are stained with eosin for 40 s to 1 min, followed by dehydration with an ethanol gradient (75%, 85%, 100%, and 100% ethanol for 2 min each) and clearing with xylene (2 min), which makes the tissue transparent. Finally, the sections were sealed with neutral gum and observed under a microscope.

**ELISA.** On the fourth day post-surgery, the rats were anesthetized with 2%–3% isoflurane, and then a collection tube was used to collect 3 mL of blood. After standing at room temperature for 30 min, the blood samples were centrifuged at 3,000 rpm for 30 min, and the supernatant was collected and placed in a −80 °C refrigerator for subsequent use. The ELISA kit was equilibrated at room temperature for 20 min in advance, and the levels of BDNF, ET-1, PG-1, and TXA-2 were independently detected under the guidance of the kit's instructions.

**Capillary density measurement.** The capillary density of the myocardium was measured via CD31 immunohistochemical staining of the left heart tissue. A single endothelial cell or a population of endothelial cells stained with brown were counted as a blood vessel, and vessels in the smooth muscle layer were excluded. Counting method: The highest capillary area in the section was selected at low magnification, and then 3 different fields were selected at 400 × magnification. The ImagePro Plus 6.0 image analysis system was used to calculate the capillary number per unit area (capillary/mm²).

**Western blot.** Myocardial tissue below the ligation site of rats in each group was quickly removed and weighed, the surface blood was cleaned with 4 °C normal saline, and water was filtered with absorbent paper. The myocardial tissue was cut into pieces and added to lysis solution at a ratio of 300–500 µL per 40 mg tissue. Then, the lytic tissue was omogenized and centrifuged at 12,000 rpm (15 min at 4 °C). The supernatant was withdrawn by pipette and prepared to perform the following protein quantification. An sodium dodecyl sulfate–polyacrylamide gel electrophoresis was prepared. The required protein was added to an appropriate amount of buffer solution and boiled for 10 min to denature the protein, followed by centrifugation to collect the supernatant. The prepared sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel was placed in the electrophoresis tank and filled with the appropriate buffer before loading the samples. The protein was transferred onto a polyvinylidene fluoride membrane and blocked with 5% skimmed milk for 1 h (room temperature). This polyvinylidene fluoride membrane was incubated by using the primary antibodies overnight (BDNF 1:1,000, TrkB 1:1,000, GAPDH 1:2,000, 4 °C). After incubation, the membrane was washed thrice for 5 min each with tris-buffered saline with Tween 20. The membrane was incubated with a secondary antibody conjugated to horseradish peroxidase (1:10,000) at 37 °C for 1 h, followed by washing thrice for 5 min each with tris-buffered saline with Tween 20. Protein expression was detected using enhanced chemiluminescence, and quantification of protein bands was performed using Image J software.

**Statistical methods**

The SPSS 25.0 statistical and GraphPad Prism 8.0 were used for statistical analysising and drawing the graphs. The data were expressed as mean ± standard deviation (mean ± SD). One-way analysis of variance and Sidak's multiple comparisons test were used to compare and analyze the intergroup differences. Pearson correlation coefficient was used to test whether a linear relationship exists between two variables. P < 0.05 was considered statistically significant.
**Results**

Wushu acupoints in the heart channel reduce myocardial ischemia

Compared with the sham AMI group, the ST segment of ECG in the AMI group was significantly increased ($P < 0.05$). Compared with the AMI group, the ST segment of ECG in the Wushu acupoint groups in the Heart channel was significantly decreased ($P < 0.05$) (Figure 2).

Wushu acupoints in the heart channel reduce myocardial ischemic injury

In the sham AMI group, the myocardial fibers were neatly arranged, with clear texture, and no ruptured or bleeding myocardial cells. In the AMI group, myocardial fibers were disordered, dissolved, and ruptured, and myocardial cells were ruptured, with swelling and extensive bleeding. In the Wushu acupoint groups, myocardial fiber rupture and the swelling and rupture of myocardial cells were reduced, with improved blood infiltration (Figure 3).

**Figure 2** Comparison of electrocardiogram before and after treatment. (A) Sham AML. (B) AML. (C) Shaochong (HT9). (D) Shaofu (HT8). (E) Shenmen (HT7). (F) Lingdao (HT4). (G) Shaohai (HT3). (H) Comparison of ST segment in ECG. *$P < 0.05$ vs. Sham AMI group, $P < 0.05$ vs. AMI group. AMI, acute myocardial ischemia.

**Figure 3** Comparison of cardiac muscle tissue before and after electroacupuncture treatment. (A) Sham AML. (B) AML. (C) Shaochong (HT9). (D) Shaofu (HT8). (E) Shenmen (HT7). (F) Lingdao (HT4). (G) Shaohai (HT3). AMI, acute myocardial ischemia.
Wushu acupoints in the heart channel enhance the myocardial angiogenesis
No significant neovascularization was observed in the sham AMI group. Compared with the sham AMI group, the AMI group had more VEGF-A expression and new blood vessels. Compared with the AMI group, there were more VEGF-A expressions, and neovascularization was observed in each Wushu acupoint group in the heart channel. Statistical analysis showed that compared with the sham AMI group, the myocardial angiogenesis marker CD31 was remarkably increased in the AMI group ($P < 0.05$). Compared with the AMI group, the myocardial angiogenesis marker CD31 was vastly improved in all Wushu acupoint groups ($P < 0.05$) (Figure 4).

Wushu acupoints in the heart channel improve the vascular balance
Compared with the sham AMI group, serum levels of ET-1, PGI-2, and PGI-2/TXA-2 in the AMI group were decreased ($P < 0.05$), whereas TXA-2 levels were increased ($P < 0.05$). Compared with the AMI

![Figure 4](https://www.tmrjournals.com/tmr)

**Figure 4** Comparison of blood vessel density before and after treatment. (A) Comparison of immunohistochemistry of VEGF-A in each group. (B) Comparison of immunohistochemistry of CD31 in each group. (C) Comparison of angiogenesis density in each group. *$P < 0.05$ vs. AMI group, $^*P < 0.05$ vs. Sham AMI group. AMI, acute myocardial ischemia; VEGF-A, vascular endothelial growth factor-A.
group, serum levels of ET-1, PGI-2, and PGI-2/TXA-2 in the treatment groups were increased ($P < 0.05$), and TXA-2 levels were reduced ($P < 0.05$) (Figure 5).

**Wushu acupoints in the heart vein enhance the serum BDNF levels**

Compared with the sham AMI group, the serum BDNF levels in the AMI group were decreased ($P < 0.05$). Compared with AMI group, the serum BDNF levels in all the Wushu acupoint groups were increased ($P < 0.05$) (Figure 6).

**Wushu acupoints in the heart channel enhance BDNF/TrKB protein expression levels**

Compared with the sham AMI group, the protein expression levels of BDNF and TrKB in myocardial tissues of the AMI group were decreased ($P < 0.05$). Compared with AMI group, the protein expression levels of BDNF and TrKB in the myocardial tissues of the HT8 and HT7 groups were increased ($P < 0.05$) (Figure 7).

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**Figure 5 Comparison of vascular equilibrium before and after treatment.** (A) Comparison of serum ET-1. (B) Comparison of serum TXA-2. (C) Comparison of serum PGI-2. (D) Comparison of serum PGI-2/TXA-2. $P < 0.05$ vs. Sham AMI group. $^*$ $P < 0.05$ vs. AMI group. ET-1, endothelin-1; TXA-2, thromboxane A2; PGI-2, prostacyclin-2; AMI, acute myocardial ischemia.

**Figure 6 Comparison of blood serum BDNF levels before and after treatment.** $P < 0.05$ vs. Sham AMI group, $^*$ $P < 0.05$ vs. AMI group. BDNF, brain derived neurotrophic factor; AMI, acute myocardial ischemia.
Correlation analysis

The results of correlation analysis indicated that the improvement of myocardial ischemia was closely related to angiogenesis and serum BDNF levels, where positive expression of CD31 and serum BDNF expression levels were negatively correlated with ST-segment altitude (R = −0.5523, P = 0.0005 < 0.05; R = −0.5837, P = 0.0002 < 0.05), respectively. Angiogenesis was closely related with serum BDNF levels and vascular balance, where the serum levels of BDNF, ET-1, and PG-2 were positively correlated with CD31 (R = 0.9491, P < 0.0001; R = 0.9416, P < 0.0001; R = 0.8600, P < 0.0001), respectively. In contrast, TXA-2 expression levels were negatively correlated with CD31 (R = −0.8849, P < 0.0001). Angiogenesis was closely related to the expression levels of BDNF/TrKB protein, where the overexpression levels of BDNF and TrKB proteins were positively correlated with CD31 (R = 0.9311, P < 0.0001; R = 0.8830, P < 0.0001), respectively (Figure 8).

Discussion

Our previous research showed that the EA of HT7 and Tongli (HT5) in rats can inhibit the autophagy level of myocardial cells in rats with myocardial ischemia [5]. Numerous studies have confirmed that acupuncture can promote endothelial cell multiplication, differentiation, and migration, activate endogenous angiogenesis mechanism, and regulate vascular blood flow velocity, and improve blood viscosity and microcirculation [31, 32]. Therefore, it is inferred that therapeutic angiogenesis may be a key mechanism in the treatment of myocardial ischemia via acupuncture. TXA-2 and ET-1 are potent vasoconstrictors affecting the vascular tone, and PG-2 is a potent vasodilator; they act as important modulators to maintain. PG-2/TXA-2 levels are often used to measure the maintenance of vascular tone and act as important indicators of cardiovascular system homeostasis [33, 34]. Myocardial ischemia is generally considered to be a disruption of the balance of oxygen consumption and coronary blood supply to the heart, and PGI-2/TXA-2 reflects this level of vascular balance and remodeling of vascular smooth muscle cells in the human body [35]. These studies have shown that acupuncture is effective in promoting angiogenesis, maintaining vascular balance, and improving myocardial ischemia, which is manifested by promoting CD31 expression in myocardial tissue, alleviating ST segment elevation (P < 0.05), increasing serum levels of ET-1, PGI-2, and PGI-2/TXA-2 (P < 0.05), and decreasing TXA-2 levels (P < 0.05) following acupuncture.

Traditional Chinese medicine believes that the Qi (Qi refers to the basic substance that constitutes the human body and maintains life activities, and is the unity of substance and function) of the five internal organs operates in the Yin channel (The channel that morphologically runs along the inside of the limbs and functionally has some specific connection with internal organs), the Qi of the six hollow organs operates in the Yang channel (The channel that morphologically runs along the outside sides of the limbs and functionally has some specific connection with hollow organs), and the five specific acupoints are the places where the Qi of the channels enter and exit, so the five specific acupoints can not only identify the diseases of the internal organs associated with the channels but also treat them. For the treatment of heart disease, there are five specific acupoints along the heart channel, called Jing (well point, HT9), Ying (ditch point, HT8), Shu (stream point, HT7), Jing (branch point, HT4) and He (river point, HT3) points (Figure 9). These five specific acupoints along the heart channel in human and rats are all mainly distributed under the elbow to the hand. They are collectively known as Wushu acupoints, and their relative specificity for the improvement of myocardial ischemia was reported in this study.

BDNF is a protein with nerve-promoting growth activity, which is

![Figure 7](https://www.tmrjournals.com/tmr)
Figure 8 Correlation analysis of myocardial ischemia and angiogenesis. (A, B) The improvement of myocardial ischemia was closely related to angiogenesis and serum BDNF levels. (C-F) Angiogenesis was closely correlated with serum BDNF levels and vascular balance. (G, H) Angiogenesis was closely related to the expression levels of BDNF/TrkB protein. BDNF, brain derived neurotrophic factor; ET-1, endothelin-1; TXA-2, thromboxane A2; PGI-2, prostacyclin-2; AMI, acute myocardial ischemia.
mainly released during the month of the central nervous system, peripheral nervous system, endocrine system, bone, and cartilage tissue. However, in recent years, researchers have found that BDNF is also expressed in cardiomyocytes and plays a key role in myocardial physiological activities [36]. In myocardial infarction experiments, injection of BDNF significantly increased microvascular density in myocardial ischemia and improved blood perfusion and cardiac function [37]. During myocardial ischemia, EA treatment exerts its protective effects by increasing BDNF levels in the peripheral blood, thereby activating angiogenesis and repairing the damaged myocardium [38, 39] (Figure 10). In this finding, compared with the AMI group, the levels of serum BDNF ($P < 0.05$) and the protein expressions of BDNF and TrKB were increased in all groups after EA ($P < 0.05$).

Moreover, through the correlation analysis of the ST segment height with CD31 and serum BDNF levels, we found that the improvement of myocardial ischemia was closely related to serum BDNF levels and angiogenesis. The correlation analysis between CD31 and serum BDNF levels revealed that serum BDNF levels were closely related to angiogenesis. Finally, the correlation analysis of CD31 with serum BDNF, brain derived neurotrophic factor; AMI, acute myocardial ischemia; VEGF, vascular endothelial growth factor.

Figure 9 Location of the Wushu acupoints along the heart channel

Figure 10 Electroacupuncture of Wushu acupoints in the heart channel improves myocardial ischemia by activating the BDNF/TrKB pathway to promote angiogenesis. BDNF, brain derived neurotrophic factor; AMI, acute myocardial ischemia; VEGF, vascular endothelial growth factor.
ET-1, PGI2, TAX-2, and BDNF/TrkB protein expression in myocardial tissue revealed that angiogenesis was closely related to vascular balance and BDNF/TrkB pathway expression in myocardial tissue.

Conclusion
In summary, our results indicate that EA of the Wushu acupoints shows an improved effect on myocardial ischemia by possibly promoting serum BDNF overexpression and activating the BDNF/TrkB pathway to promote myocardial angiogenesis. Our study not only presents a new direction for the mechanism of action of traditional acupuncture Wushu acupoints to improve myocardial ischemia but also provides an experimental basis for the clinical application of Wushu acupoints. This study provides a better understanding of traditional Chinese medicine and enhances our confidence in the field of Oriental medicine, which possesses a long and successful therapeutic history. The study involved several groups of Wushu acupoints in the study, which impacted the use of specific inhibitors for regulating the BDNF/TrkB pathway. Future studies should verify the role of the BDNF/TrkB pathway by selecting the best acupoint group and determine the effect of a specific inhibitor on the regulation of this pathway in improving myocardial ischemia.

References
3. Chinese guidelines for risk assessment and management of cardiovascular disease. Chin J Circ. 2019;34(01):4–28. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=0kbmF0AymBBljy747aq2pCjg3l_g_adhnaVinhnb78kTko69CqCqKqBG_Sm_ah7plumCDoDbkLRV0R1ZV2eooEFlsc71uruSUq8hhftw56AES1u0369mX81mWmPfPsf6HyAQ4CT_B–MRNXGDmWqU7w=m=&niplatform=NZXKTPlanguage=CHS
23. Fu Y, Li J, Wu S, Wang H. Electroacupuncture pretreatment promotes angiogenesis via hypoxia-inducible factor 1α and


30. Name and location of common acupoints in laboratory animals-Part 2: Rats. *Acupuncture Res.* 2019;46(4):351–352. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=0kbnF0AYmB-CwnisYbHq-m9f7ZIUZ1AlweduN8M12g4W-hbYSalIMWrp2nw300Q3zp7N6C98va96bITsITx7IXw7icVjEJSFZ-35EL1rINs5dGtaR5ouX1f2wMVjJ3aJHSoHunGVeyHOptwyXXAO9A==&uniplatform=NZKPT&language=CHS


