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Effects of Zhuang Medicine Longzuan Tongbi Decoction on Left Ventricular Remodeling in Rats with Myocardial Infarction

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Abstract: *Objective:* To investigate the effects of Longzuan Tongbi Decoction on ischemic myocardial angiogenesis in rats with myocardial infarction (MI), and to analyze its regulatory effect on the expression of angiogenesis-related factors. *Methods:* A total of 120 healthy, clean-grade female Sprague-Dawley (SD) rats were randomly divided into six groups (20 rats per group): sham surgery (normal control) group, model group, Shexiang Baoxin Pill (positive control) group, as well as low-, medium-, and high-dose groups of Longzuan Tongbi Decoction. The MI model was established by left coronary artery ligation; the sham surgery group underwent threading without ligation. Postoperatively, each treatment group received daily oral administration of the corresponding medicine (the low-, medium-, and high-dose groups of Longzuan Tongbi Decoction received 1 mL, 2 mL, and 4 mL of the extract, respectively; the positive control group received Shexiang Baoxin Pill), while the sham surgery and model groups received an equal volume of saline. Treatment continued for 6 weeks. At the end of the experiment, the ratio of myocardial infarct size to left ventricular area was measured, and the number and density of microvessels in the MI border zone were assessed. *Results:* Compared with the model group, the ratio of myocardial infarct size to left ventricular area was significantly reduced in each dose group of Longzuan Tongbi Decoction ($p < 0.05$), and the efficacy was comparable to that of the Shexiang Baoxin Pill group. Regarding angiogenesis promotion, the number and density of microvessels in the infarct border zone were significantly higher in each dose group of Longzuan Tongbi Decoction than in the model group ($p < 0.05$); among these, the pro-angiogenic effect of the medium- and high-dose groups was significantly superior to that of the Shexiang Baoxin Pill group ($p < 0.05$), while no significant difference was observed between the low-dose group and the positive control group ($p > 0.05$). *Conclusion:* Longzuan Tongbi Decoction can effectively reduce the extent of myocardial infarction and exert a cardioprotective effect by promoting local angiogenesis in ischemic myocardium, and this effect exhibits a dose-dependent relationship within a certain range.

Keywords: Zhuang Medicine Longzuan Tongbi decoction; Myocardial infarction; Angiogenesis

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1. Introduction

Coronary heart disease and myocardial infarction are among the cardiovascular diseases with the highest mortality and disability rates worldwide. According to statistics, approximately 3.5 million people die from cardiovascular diseases in China each year, with myocardial infarction accounting for over 70% of these cases. The condition not only seriously endangers people's life and health, but also imposes a heavy medical and economic burden on society and families [1]. Following myocardial infarction, restoring blood supply to the ischemic myocardium is the core to improving patient prognosis. Clinically, around 30% of patients with coronary heart disease cannot achieve complete revascularization due to diffuse multi-vessel disease; therefore, promoting the formation of collateral circulation in ischemic areas through pharmaceutical intervention has become a critical scientific challenge in the field of cardiovascular medicine [2]. This study focuses on Zhuang medicine with distinctive ethnic medical characteristics. Longzuan Tongbi Decoction is a compound prescription derived from the folk experience of the Zhuang nationality and formulated based on the Zhuang medical theory of "removing toxin and unblocking vessels". Previous studies have suggested its potential in cardiovascular protection. Through animal experiments, this study aims to systematically investigate the effects of this formula on ischemic myocardium angiogenesis in rats with myocardial infarction. Specifically, by measuring the ratio of myocardial infarct size to left ventricular area and quantitatively analyzing key morphological indicators such as myocardial microvascular count (MVC) and microvascular density (MVD) in the MI border zone, the study preliminarily explores its potential mechanisms in promoting angiogenesis and improving myocardial perfusion. It is expected that this study can provide a potential new pharmaceutical intervention strategy to address the aforementioned clinical challenges, and offer experimental evidence for the modern development and clinical application of traditional ethnic medicine.

2. Experimental materials

2.1. Animals

A total of 120 clean-grade, healthy female SD rats weighing (230 ± 20) g were purchased from the Animal Center of Zhengzhou University (Animal Certificate No.: 410156). The rearing environment complied with the national standard of Laboratory Animal Environment and Facilities (GB 14925-2010). All rats were given free access to food and water. The formal experiment was initiated after one week of adaptive feeding.

2.2. Medicines and reagents

Longzuan Tongbi Decoction (composed of *Radix Toddaliae Asiaticae* (Feilongzhangxue) 30 g, *Radix Kadsurea Coccineae* (Dazuan) 20 g, *Radix Alangii Chinensis* (Bajiaofeng) 15 g, *Radix Zanthoxyli* (Liangmianzhen) 15 g, *Caulis Sinomenii* (Qingfengteng) 15 g, *Rhizoma Smilacis Glabrae* (Tufuling) 15 g, *Caulis Spatholobi* (Jixueteng) 30 g, *Herba Plantaginis* (Cheqiancao) 15 g, *Rhizoma Dioscoreae Hypoglaucae* (Bixie) 15 g and *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) 10 g) was purchased and decocted into extract by the Pharmacy Department of Fusui County Hospital of Traditional Chinese Medicine; 1 mL extract is equivalent to 1 g of crude herb. Shexiang Baoxin Pill (produced by Shanghai Hutchison Pharmaceutical Co., Ltd.; Batch No. A040214; 22.5 mg per pill). DAB Staining Kit (Beijing Zhongshan Company), 1% Pentobarbital Sodium (analytical pure, Sinopharm Chemical Reagents Co., Ltd.), Benzylpenicillin Sodium Injection (North China Pharmaceutical Co., Ltd.).

2.3. Equipment

Small Animal Ventilator (TKR-200C, Jiangxi Teli Anesthesia & Respiratory Equipment Co., Ltd.); Optical

Microscope (CH20BIMF200, OLYMPUS Optical Co., Ltd., Japan); Paraffin Microtome (HM340E, Carl Zeiss AG, Germany); Medical Image Analysis System (BT 2000, Version 3.6 Build 0211, Chengdu Taimeng Technology Co., Ltd.); High-Resolution Color Pathological Image Analysis System (Noesis S.A., France).

3. Experimental methods

3.1. Preparation of animal model

The myocardial infarction model was established according to the Laboratory Animal Science [3]. Rats were anesthetized via intraperitoneal injection of 1% Pentobarbital Sodium, fixed in the supine position, and subjected to tracheal intubation connected to a small-animal ventilator for mechanical ventilation. The chest was opened, and the root of the left coronary artery was ligated. The ligation site was 0.1 cm below the line between the aortic conus and the left atrial appendage on the main trunk of the left coronary artery. Successful model establishment was confirmed by immediate changes in cardiac surface color and significant ST-segment elevation in leads I and aVL of the surface electrocardiogram. After suturing the chest wall and observing that the rats' physiological status had stabilized, artificial respiration was discontinued. Postoperatively, penicillin was administered for 3 days to prevent infection. The surviving rats were randomly divided into the model group, Shexiang Baoxin Pill group, high-dose group of Longzuan Tongbi Decoction, medium-dose group of Longzuan Tongbi Decoction, and low-dose group of Longzuan Tongbi Decoction. Rats in the sham surgery group underwent the same procedure as described above, except that a loose knot was made under the coronary artery without ligation. All rats were euthanized after 6 weeks for subsequent index detection.

3.2. Animal grouping and administration methods

A total of 120 healthy, clean-grade female SD rats weighing 200 ± 20 g were randomly divided into six groups (A, B, C, D, E, and F), with 20 rats in each group. A: normal control group; B: model group; C: Shexiang Baoxin Pill group; D: low-dose group of Longzuan Tongbi Decoction (1 mL); E: medium-dose group of Longzuan Tongbi Decoction (2 mL); F: high-dose Longzuan Tongbi Decoction group (4 mL). (The dosages were converted according to the body surface area of experimental animals. The medication groups were administered via gavage at 8:00 a.m. daily; the control and model groups received an equal volume of saline via gavage, once daily. All rats were reared in separate cages with free access to food and water.

3.3. Collection and processing of myocardial tissue

After anesthetizing the rats, the hearts were quickly removed. The aortic arch, right ventricle, atria, and auricles were excised. Myocardial tissue (including the infarct area, transition area, and intact area) was harvested from the transverse section of the papillary muscles, fixed in 10% neutral buffered formalin, embedded in paraffin, and prepared into 4 μm thick sections. Partial myocardial tissues from the MI border zone were preserved in liquid nitrogen for subsequent use.

3.4. Observation indicators and detection methods

3.4.1. Measurement of myocardial infarct size

The fixed heart was embedded in paraffin and sectioned, followed by hematoxylin and eosin (HE) staining. Myocardial necrosis was observed under a light microscope. The ratio of myocardial infarct size to left

ventricular area was measured using a high-resolution color pathological image analysis system (Noesis S.A., France). Infarct size (%) = Myocardial infarct size / Left ventricular area × 100%.

3.4.2. Determination of microvascular count (MVC) and microvascular density (MVD) in the MI border zone

Tissue sections were stained via the immunohistochemical SP method. After staining, vascular endothelial cells appeared yellow and brown.

(1) Criteria for microvessel identification

Vessels were not identified based on the presence of red blood cells or vascular lumens. Any single brownish-yellow endothelial cell or cluster of endothelial cells was counted as one vessel. Vessels with lumens larger than the size of eight red blood cells or those surrounded by thick muscular layers were not counted.

(2) Procedures

First, locate the MI border zone under 40 × magnification. Then, count the microvessels in this region under 400 × magnification. Randomly select five visual fields for each section, record the MVC of each field, and calculate the mean as the MVC value of the rat. Since MVD was expressed as the number of microvessels per visual field, the MVC value for each rat is equivalent to the MVD value for that rat.

3.5. Statistical methods

All data were analyzed using SPSS 32.0 statistical software. Measurement data were expressed as "mean ± standard deviation ($\overline{x} \pm s$)"; comparisons between groups were performed using the independent samples *t* test. Enumeration data were presented as "number of cases (percentage)"; comparisons between groups were performed using the χ^2 test. Ranked data were analyzed by rank sum test. $p < 0.05$ was considered statistically significant.

4. Results

4.1. Comparison of myocardial infarct size among groups

After 6 consecutive weeks of intervention, the ratio of myocardial infarct size to left ventricular area was compared across groups. Compared with the normal control group, the ratio was significantly elevated in the model group and all treatment groups (Shexiang Baoxin Pill group, and the low-/medium-/high-dose group of Zhuang Medicine Longzuan Tongbi Decoction), with statistically significant differences ($p < 0.05$); compared with the model group, the ratio of infarct size to left ventricular area was significantly reduced in each dose group of Longzuan Tongbi Decoction and Shexiang Baoxin Pill group, with statistically significant differences ($p < 0.05$); compared with the Shexiang Baoxin Pill group, there were no statistically significant differences in this ratio among the low-, medium-, and high-dose groups of Longzuan Tongbi Decoction ($p > 0.05$), indicating that Zhuang Medicine Longzuan Tongbi Decoction exerted a comparable effect on reducing infarct size to Shexiang Baoxin Pill group. Detailed data are shown in **Table 1**.

Table 1. Effect of different dose groups of Zhuang Medicine Longzuan Tongbi Decoction on myocardial infarct size in rats ($\overline{x} \pm s$, $n = 20$)

Group	*n*	Left ventricle area	Myocardial infarct size	Infarct size (%)
Normal control group	20	6.52 ± 1.45	6.18 ± 0.76	85.37 ± 7.82
Model group	20	11.38 ± 1.26	9.65 ± 0.83	86.42 ± 4.53
Shexiang Baoxin pill group	20	8.45 ± 1.21	7.79 ± 0.72	87.26 ± 8.15
Low-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	8.39 ± 1.32	7.68 ± 0.75	87.35 ± 8.21
Medium-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	7.96 ± 1.35	7.37 ± 0.65	87.51 ± 8.34
High-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	7.38 ± 1.29	7.22 ± 0.61	87.63 ± 8.42

Compared with the normal control group, $p < 0.05$; Compared with the model group, $p < 0.05$; Compared with Shexiang Baoxin Pill group, $p > 0.05$

4.2. Comparison of microvascular count (MVC) and microvascular density (MVD) in the MI border zone across groups

New microvessels were observed in the MI border zone of all groups. Comparison of MVC and MVD revealed that, compared with the normal control group, both MVC and MVD were significantly elevated in the model group and all treatment groups, with statistically significant differences ($p < 0.05$), suggesting a compensatory angiogenic response following myocardial infarction; compared with the model group, MVC and MVD were significantly higher in Shexiang Baoxin Pill group and all dose groups of Zhuang Medicine Longzuan Tongbi Decoction, with statistically significant differences ($p < 0.05$), indicating that drug intervention can enhance the angiogenis in ischemic myocardium; compared with Shexiang Baoxin Pill group, MVC and MVD were significantly elevated in the high- and medium-dose groups of Zhuang Medicine Longzuan Tongbi Decoction ($p < 0.05$), whereas there was no statistically significant difference in MVC and MVD between the low-dose group of Zhuang Medicine Longzuan Tongbi Decoction and Baoxin Tongbi Pill group ($p > 0.05$); suggesting that the angiogenic effect of the Zhuang Medicine Longzuan Tongbi Decoction is dose-dependent, with superior efficacy at high and medium doses. Detailed data are shown in **Table 2**.

Table 2. Comparison of MVC and MVD in the MI border zone across groups ($\overline{x} \pm s$, $n = 20$）

Group	N	MVC, MVD
Normal control group	20	44.17 ± 2.53
Model group	20	59.32 ± 10.87
Shexiang Baoxin pill group	20	78.26 ± 9.35
Low-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	79.05 ± 7.58
Medium-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	85.42 ± 4.96
High-dose group of Zhuang medicine Longzuan Tongbi Decoction	20	86.13 ± 5.97

Note: Compared with the normal control group, (1) $p < 0.05$; compared with the model group, (2) $p < 0.05$; compared with the Shexiang Baoxin Pill group, (3) $p > 0.05$

5. Discussion

Coronary heart disease falls into the category of "Amen" in Zhuang medicine. It refers to a condition characterized by sudden precordial pain (which may radiate to the shoulder and back), dyspnea, and inability to lie flat, resulting from blockage of the Longlu (blood circulation vessel in Zhuang medicine). From the perspective of Zhuang medicine, the onset of "Amen" is attributed to the invasion of pathogenic factors such as wind, dampness, filth, and miasma, or to constitutional weakness and stagnation of qi and blood, resulting in obstruction of the Longlu and Huolu (sensory-conduction vessel of Zhuang medicine) [4].

Longzuan Tongbi Decoction is an empirical formula developed by our research team based on the Zhuang medicine therapeutic principle "removing toxins and unblocking vessels," and drawing upon traditional Zhuang folk herbal remedies for coronary heart disease. It consists of ten medicinal herbs, including *Radix Toddaliae Asiaticae* (Feilongzhangxue), *Radix Kadsurea Coccineae* (Dazuan), *Radix Alangii Chinensis* (Bajiaofeng), *Radix Zanthoxyli* (Liangmianzhen), *Caulis Sinomenii* (Qingfengteng), *Rhizoma Smilacis Glabrae* (Tufuling), *Caulis Spatholobi* (Jixueteng), *Herba Plantaginis* (Cheqiancao), *Rhizoma Dioscoreae Hypoglaucae* (Bixie) and *Pseudobulbus Cremastrae seu Pleiones* (Shancigu). According to Zhuang medicine theories, the onset of coronary heart disease is closely associated with stasis and stagnation of the Longlu and Huolu. Its core pathogenesis is "the mutual entanglement of toxins and stasis, and obstruction of the Qi pathway," leading to a disruption in the synchronized circulation of the three primordial Qi of heaven, human, and earth. This formula acts on the network of connections where the Longlu and Huolu intersect at the body's surface to unblock vessels, eliminate pathogenic factors, and restore the synchronized circulation of the three Qi, thereby exerting its therapeutic effects.

In this formula, *Radix Toddaliae Asiaticae* (Feilongzhangxue) serves as the monarch herb [5]. It can dissipate stasis, stop bleeding, remove toxins, and unblock collaterals, directly targeting the core pathogenesis of toxin-stasis obstructing collaterals, which aligns with the key therapeutic principle of "unblocking stasis in Longlu" in Zhuang medicine. *Radix Kadsurea Coccineae* (Dazuan) and *Caulis Spatholobi* (Jixueteng) act as minister herbs. *Radix Kadsurea Coccineae* (Dazuan) promotes Qi movement, activates blood circulation, unblocks collaterals, and relieves pain. It reinforces the stasis-dissipating effect of the monarch herb. Following the theory that free flow of Qi ensures unobstructed blood circulation, it ameliorates Qi stagnation in the Longlu and Huolu. *Caulis Spatholobi* (Jixueteng) tonifies and activates blood, unblocks collaterals, and relaxes tendons [6]. It remedies blood deficiency in the pattern of Qi deficiency and blood stasis, and prevents damage to healthy Qi caused by excessive stasis dissipation. *Radix Alangii Chinensis* (Bajiaofeng), *Radix Zanthoxyli* (Liangmianzhen), *Caulis Sinomenii* (Qingfengteng), *Rhizoma Smilacis Glabrae* (Tufuling) and *Rhizoma Dioscoreae Hypoglaucae* (Bixie) are designated as adjuvant herbs. *Radix Alangii Chinensis* (Bajiaofeng) dispels wind, eliminates dampness, dissipates stasis and unblocks collaterals, targeting the accompanying pattern of damp-toxin combined with stasis. *Radix Zanthoxyli* (Liangmianzhen) activates blood circulation, dissipates stasis, removes toxins and relieves swelling, further potentiating the detoxifying and stasis-resolving effects of the monarch herb. The combined use of *Caulis Sinomenii* (Qingfengteng), *Rhizoma Smilacis Glabrae* (Tufuling) and *Rhizoma Dioscoreae Hypoglaucae* (Bixie) clears heat and drains dampness, dispels wind and relieves impediment. They eliminate damp-toxin and heat-toxin, unblock stagnated collaterals, and relieve internal accumulation of phlegm-dampness commonly seen in patients with coronary heart disease. *Herba Plantaginis* (Cheqiancao) and *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) function as guide herbs. *Herba Plantaginis* (Cheqiancao) clears heat, promotes diuresis, and

guides pathogenic toxins downward, facilitating the excretion of toxins from the lower jiao. *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) clears heat, removes toxins, dissipates abscesses and resolves masses. It enhances the overall detoxifying effect of the formula, moderates the harsh properties of *Radix Alangii Chinensis* (Bajiaofeng) and *Radix Zanthoxyli* (Liangmianzhen), and harmonizes all medicinal ingredients. The whole formula exerts the combined effects of removing toxins and dissipating stasis, promoting Qi movement and unblocking collaterals, and regulating both Qi and blood. It conforms to the traditional Chinese medicine therapeutic strategy of tonifying Qi, activating blood, removing toxins and unblocking collaterals, as well as the Zhuang medicine theory of unblocking the Longlu and Huolu and restoring synchronous movement of the three Qi. With simultaneous treatment of root cause and manifestations and balanced regulation of cold and heat nature, this formula is applicable to coronary heart disease (chest impediment and heart pain) manifested as the pattern of Qi deficiency and blood stasis accompanied by toxin-stasis obstructing collaterals.

Angiogenesis is a critical process in the repair of ischemic myocardium; it promotes the survival, proliferation, and lumen formation of endothelial cells, and the synergistic action of these processes accelerates the formation of a new vascular network in the ischemic region. The results of this study showed that all dose groups of Zhuang Medicine Longzuan Tongbi Decoction significantly reduced the infarct size and increased MVC and MVD in the MI border zone in rats. It suggests that Zhuang Medicine Longzuan Tongbi Decoction exerts a protective effect on ischemic myocardium, promotes local angiogenesis in the ischemic myocardium, and improves myocardial blood supply.

6. Conclusion

In summary, Longzuan Tongbi Decoction effectively reduces myocardial infarct size and promotes angiogenesis in the ischemic myocardium of rats after MI, as evidenced by increased microvascular count and density in the infarct border zone. These cardioprotective effects exhibit a dose-dependent relationship, with the medium and high doses demonstrating superior pro-angiogenic efficacy compared to the positive control Shexiang Baoxin Pill. Our findings suggest that Longzuan Tongbi Decoction, formulated under the Zhuang medical principle of “detoxifying and unblocking vessels”, holds promise as a potential therapeutic agent for improving myocardial perfusion and limiting infarct expansion. Further studies are warranted to elucidate its underlying molecular mechanisms and signaling pathways.

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Disclosure statement

The authors declare no conflict of interest.

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