

Zhuangtongyin Ameliorates Cardiomyocyte Pyroptosis in Rats with Coronary Heart Disease by Regulating Caspase-1

Shumin Zhang¹, Hanqing Tang^{1*}, Xianfeng He², Xuqing Zhang¹, Weizhen Wei³, Wenchong Wang¹, Jinying Wang¹

¹College of Basic Medicine, Youjiang Medical University for Nationalities, Baise, China

²School of Clinical Medicine, Youjiang Medical University for Nationalities, Baise, China

³School of Nursing, Youjiang Medical University for Nationalities, Baise, China

Email: *Phdtanghanqing@163.com

How to cite this paper: Zhang, S.M., Tang, H.Q., He, X.F., Zhang, X.Q., Wei, W.Z., Wang, W.C. and Wang, J.Y. (2024) Zhuangtongyin Ameliorates Cardiomyocyte Pyroptosis in Rats with Coronary Heart Disease by Regulating Caspase-1. *Journal of Biosciences and Medicines*, 12, 94-108.

<https://doi.org/10.4236/jbm.2024.1211008>

Received: September 29, 2024

Accepted: November 2, 2024

Published: November 5, 2024

Copyright © 2024 by author(s) and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Background: Recent studies have demonstrated that pyroptosis has an important impact on the pathological process of many cardiovascular diseases, and inhibiting this process can significantly reduce pathological damage. At the same time, some studies have proved that Zhuangtongyin (ZTY) has a certain protective effect on the myocardium of rats with coronary heart disease. Therefore, the aim of this study is to observe and explore the effects of ZTY on cardiomyocytes and its mechanism of action in rats with coronary heart disease based on the Caspase-1 mediated cellular pyroptosis pathway. **Methods:** Fifty SD rats were randomly divided into blank group, sham-operation group, model group, ZTY group and cysteinyl aspartate specific protease (Caspase-1) inhibitor group. The blank group did not receive any treatment, and the other groups established a rat model of coronary artery disease by ligating the left anterior descending branch of the coronary artery, but the sham-operation group was only threaded without ligation. After that, the sham-operated and model groups were gavaged with 0.1 ml/kg distilled water, the ZTY group was gavaged with 13.6 g/kg ZTY decoction based on the previous study, and the Caspase-1 inhibitor group was injected intraperitoneally with 3 mg/kg Ac-YVAD-cmk solvent once a day for four consecutive weeks. Transmission electron microscopy was used to observe the ultrastructure of cardiomyocytes, HE staining was used to observe the morphology of cardiac tissue, and enzyme-linked immunosorbent assay (Elisa) was used to detect serum interleukin-1 β (IL-1 β), interleukin-18 (IL-18) and C-reactive protein (CRP) in the rats. In addition, fluorescent probe was used to detect serum Reactive oxygen species (ROS). Real-time fluorescence quantitative PCR was used to detect the expression of mRNAs of

ASC, Nlrp3, Caspase-1 and gasdermin-D (GSDMD), and the protein expression of Caspase-1 and GSDMD was detected by immunohistochemistry. **Results:** There was no significant difference between the results of the blank and sham-operated groups. Compared with the blank group, the transmission electron microscopy results showed swollen and ruptured cardiomyocyte membranes in the model group, with pore formation, severe mitochondrial swelling, membrane lysis, and cristae breakage. The staining results showed myofibril breakage, severe intercellular oedema and vacuolation in the model group. The inflammatory factors IL-1 β , IL-18, CRP and ROS were significantly elevated ($P < 0.05$). Besides, the cardiac tissues of the ASC, Nlrp3, Caspase-1, GSDMD mRNA and Caspase-1 and GSDMD protein expression were significantly elevated ($P < 0.05$). Compared with the model group, the staining and electron microscopy results showed that the myocardial pathological damage was reduced in the ZTY and Caspase-1 inhibitor group. The inflammatory factors IL-1 β , IL-18, CRP, ROS and the expression of ASC, Nlrp3, Caspase-1, and GSDMD mRNA were significantly elevated ($P < 0.05$). The protein expression of Caspase-1 and GSDMD was also reduced ($P < 0.05$). **Conclusion:** ZTY can reduce myocardial injury by lowering the expression of ROS and other inflammatory factors, inhibiting Caspase-1 mediated cellular pyroptosis, thus reducing myocardial inflammation and protecting cardiomyocytes.

Keywords

Zhuangtongyin, Coronary Heart Disease, Caspase-1, Pyroptosis, Inflammation

1. Introduction

Coronary atherosclerotic heart disease, referred to as coronary heart disease (CHD), is caused by atherosclerotic changes in the coronary arteries, resulting in narrowing or occlusion of the lumen of the coronary arteries. It ultimately leads to myocardial ischaemia, hypoxia and necrosis if without control [1] [2]. In China, with the improvement of people's living standards and changes in living habits, the incidence of coronary heart disease population is a younger trend. In the meantime, the incidence of coronary heart disease increases year by year with the accelerated aging process of society [3]. The medical burden of cardiovascular diseases in China is increasing and has become a major public health problem, so the prevention and treatment of cardiovascular disease should not be delayed [4]. Recent studies have demonstrated that pyroptosis has an important impact on the pathological process of many cardiovascular diseases [5] [6], and inhibiting pyroptosis can significantly reduce pathological damages [7] [8].

Compared with drugs for the treatment of coronary heart disease, Chinese medicine has the advantages of fewer toxic side effects and multi-targeted actions, which can be effective through more pathways [9] [10]. Zhuang medicine is the main component of traditional Chinese medicine, and ZTY is a commonly used

empirical formula in Guangxi Bourau folklore, which is composed of three flavours of Guangxi characteristic Zhuang medicinal herbs, such as euonymus fortunei, polygala fallax, and pseudo-ginseng. The ZTY has the effect of activating blood circulation and removing blood stasis [11]. It has been proved that ZTY has certain protective effects on the myocardium of rats with coronary heart disease, myocardial ischemia and blood stasis [12], and it can delay the progression of the disease to a certain extent, but there is not enough clarity and depth as to whether ZTY is antiapoptosis and its mechanism of action to protect the cardiomyocytes. Therefore, in this study, based on the Caspase-1 mediated cellular pyroptosis pathway, we investigated the molecular mechanism of ZTY on the expression of cellular pyroptosis pathway factors and explored the molecular mechanism of action on cardiomyocyte pyroptosis [13]-[15]. At the same time, it can provide research ideas for further development of Guangxi characteristic Zhuang medicine in treating coronary heart disease and more research information to deepen and understand the specific mechanism of ZTY in treating cardiovascular diseases [16].

2. Materials

2.1. Instruments

Tissue dehydrator (Thermo Company, USA), KW-100 small animal artificial respirator (Nanjing Calvin Biotechnology Co., Ltd.); digital electrocardiograph (Guangzhou 3ray Electronics Co., Ltd.); ME204E electronic balance (Mettler Toledo Instruments Co., Ltd.); High-speed low-temperature tissue grinder (Wuhan Servicebio Technology Co., Ltd.); RM2245 Slicer (Shanghai Leica Microsystems Co., Ltd.); AD340 enzyme labeller (Beckman Coulter, USA); Transmission electron microscope (HITACHI).

2.2. Drugs and Reagents

Euonymus fortunei, Polygala fallax, pseudo-ginseng (Jiexi Yibai Pharmacy Co., Ltd., batch number: 0000653), Caspase-1 inhibitor Ac-YVAD-cmk (Med Chem Express, batch number: HY-16690), Gluta Fixative ((Beijing Solarbio Science & Technology Co., Ltd., Batch number: P1126), IL-18, IL-1 β , CRP ELISA Test Kit (Elabscience Biotechnology Co., Ltd, Batch number: E-EL-R0567C, E-EL-R0012C and E-EL-R0506C); Hematoxylin Eosin Staining Kit (Beijing Solarbio Science Technology Co., Ltd., batch number: G1120), Reactive Oxygen Demonstration Kit (O13, red fluorescent, RXSH Bio-Tech Ltd., lot number: RXSH0845), Universal immunohistochemistry test kit (Proteintech Group, Inc, lot number: PK10006).

2.3. Animals

Fifty SD rats were purchased from Guangdong Vital River Laboratory Animal Technology Co., Ltd. Animal Qualification Certificate No.: SCXK (Guangdong) 2022-0063. The rats were kept in the SPF Grade Animal Experiment Centre of Youjiang Medical University for Nationalities, and this experiment was approved by the Ethics Committee of Youjiang Medical University for Nationalities.

3. Methods

3.1. Preparation of ZTY Water Extract

In accordance with the requirements of Chinese medicine taking dose and experimental zoology as a reference standard, according to the adult (about 60 kg) ZTY taking dose should be: euonymus fortunei 30 g, polygala fallax 20 g, pseudo-ginseng 15 g as a reference dose [17]. After soaking the above three medicines in distilled water overnight, heat them to boiling and then decoct them with mild fire for 1 h, filtering and removing the dregs. Add distilled water to boiling and then decoct them with mild fire for 1 h. Combine the decoctions, divide them into packages and store them at 4 °C for spare use [18].

3.2. Grouping, Modelling and Drug Administration

The rats were randomly divided into blank group, sham-operation group, model group, ZTY group and Caspase-1 inhibitor group. Except for the blank group, the rats in each group were anaesthetized by intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg), connected to a small-animal ventilator for mechanically assisted ventilation (respiratory rate: 80 breaths/min, tidal volume: 30 - 40 mL/kg, respiratory time ratio: 1:1), and electrocardiogram monitoring. After the left side of the chest was opened, the heart was exposed, and the rat model of coronary artery disease was established by ligating the left anterior descending branch of the coronary artery [19]. The myocardium below the ligature became greyish in colour (ischemia occurred), and myocardial tissue around the anterior wall of the left ventricle and the apical region of the heart was weakened in movement, and the presence of a 0.2 mm elevation of the S-T segment or the presence of a higher and wider QRS wave on the ECG was considered as the success of constructing the model [20], as shown in **Figure 1**. After suturing the incision, the rats were kept in cages and injected with 160,000 units of penicillin sodium intramuscularly into the thighs (once a day for 3 d) to prevent infection. In the sham-operation group, only the thorax was opened but not ligated, and the rest of the operation was the same as above [21]. Three days after the operation, the blank group was left untreated, the rats in the sham-operation group and the model group were gavaged with an equal volume of pure water, and the others were gavaged with the corresponding drugs and injected once a day for 4 consecutive weeks [22].

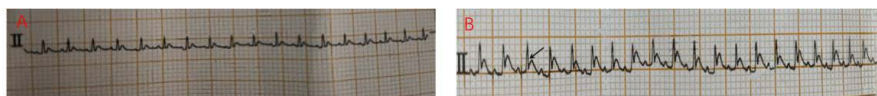


Figure 1. (A) Electrocardiogram before coronary ligation; (B) Electrocardiogram after coronary ligation (arrow indicates ST segment elevation).

3.3. Transmission Electron Microscopy Observation of Myocardial Tissue Ultrastructure

Sample myocardial tissues in the ischemic area with 2 mm × 2 mm size, as thin as

possible. The blade should be sharp to avoid bruising the tissue. Tissues are put into the electron microscope fixative at room temperature avoiding light for 2 h after removal. Then they are transferred to 4°C preservation and transported with ice packs in time for inspection [23].

3.4. Morphological Observation of Myocardial Tissue

The myocardial tissues in the ischemic area were taken in appropriate quantities, washed with saline, fixed in 4% paraformaldehyde solution for 24 h, routinely dehydrated with ethanol gradient, transparent with xylene, embedded in paraffin, and sliced (thickness of about 5 µm). After that, dewax and hydrate to water, stain nucleus with Hematoxylin Solution for 5 - 20 min and rinse in running tap water. Then differentiate with Differentiation Solution for 3 min, and wash with tap water twice for 2 min each. Re-dyeing with Eosin Y Aqueous Solution for 10 seconds to 2 min and dehydrate in alcohol (75%, 85%, 95%, 100% alcohol (I)), each for 2 - 3 s, and rinse in 100% alcohol (II) for 1 min. Finally, transparent by xylene and seal with resinene. The morphological characteristics of myocardial tissues of the rats in each group were observed using microscope and photographed [24].

3.5. Detection of IL-1 β , IL-18 and CRP Levels in Myocardial Tissue

The levels of IL-1 β , IL-18 and CRP in rat myocardial tissues were detected by ELISA method using an enzyme marker. The operation was performed in strict accordance with the instructions of the corresponding reagent kits.

3.6. Fluorescent Probe for Detection of Reactive Oxygen Species

First, add 100 µl of fresh serum sample and 10 - 20 µl of O13 probe to a 96-well plate blowing with a pipette to mix well. Be careful to choose a suitable 96-well plate, and the fluorescence detection requires black plate. Secondly, incubate at 37°C for 15 - 30 minutes, protected from light. Finally, place the plate in a fluorescence photometer or an enzyme labeller to measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 610 nm. Differences in the degree of reactive oxygen species can be compared by using the fluorescence intensity values of the serum samples from the blank group in the experiment as a benchmark, and the fluorescence intensity values of the serum from the model group to be tested as a percentage of the control serum.

3.7. Real-Time Quantitative PCR Method to Detect Myocardial Tissue ASC, Nlrp3, Caspase-1 and GSDMD mRNA Expression

Cardiac tissue was cut, and total tissue RNA was extracted by Trizol, then total tissue mRNA was used as the template to reverse transcribe cDNA, which was firstly incubated at 50°C for 15 min, and then at 85°C for 5 s. After the reaction was finished, it was briefly centrifuged, and then cooled on ice. The PCR reaction system was 20 µL, including 0.4 µL each of upstream and downstream primers, 2 µL of Template, 7.2 µL dd H₂O and 10 µL 2x SYBGREEN PCR Master Mix. The

expression levels of target genes in each sample group relative to those in the blank group were expressed as $2^{-\Delta\Delta Ct}$. $-\Delta\Delta Ct = \text{blank group } \Delta Ct - \text{sample group } \Delta Ct$, $\Delta Ct = \text{target gene} - \text{reference gene}$. The ASC, Nlrp3, Caspase-1 and GSDMD gene sequences were synthesised by Shanghai Bioengineering.

3.8. Immunohistochemical Method to Detect the Expression of Caspase-1 and GSDMD in Myocardium

Paraffin sections were dewaxed to water, microwave heating repair antigen, cold to room temperature. PBS washed 3 times. Add closure buffer closure 30 min and rinse 3 times with PBS. Add appropriate primary antibody diluent in each slice of tissue, 4°C overnight, and rinse 3 times with PBS. Add the secondary antibody (50 – 100 µL of anti-rabbit/mouse HRP-labelled polymer), incubate at room temperature for 30 min and rinse 3 times with PBS; dropwise addition of DAB chromogenic solution 50 - 100 µL. The sections were incubated at room temperature, reaction time controlled under the microscope, washed with distilled water; hematoxylin re-staining, rinsed with distilled water, returned to the blue by PBS, dehydrated, transparent, blocked and observed under the microscope. The results were analysed semi-quantitatively by Images J for the staining intensity of Caspase-1 and GSDMD.

3.9. Statistical Methods

SPSS 24.0 software was used to statistically analyse the data. One-way ANOVA was used to compare multiple groups when normality and chi-square were satisfied. $P < 0.05$ was taken as statistically significant difference.

4. Results

4.1. Survival Status of Rats in Each Group

During the modelling process and drug administration, there were no deaths in the sham-operation group, 4 rats died in the model group, 3 rats died in the ZTY group, and 1 rat died in the Caspase-1 inhibitor group. Finally, the blank group (10 rats), the sham-operation group (10 rats), the model group (6 rats), the ZTY group (7 rats), and the caspase-1 inhibitor group (9 rats).

4.2. Ultrastructural Results of Rat Cardiomyocytes in Each Group

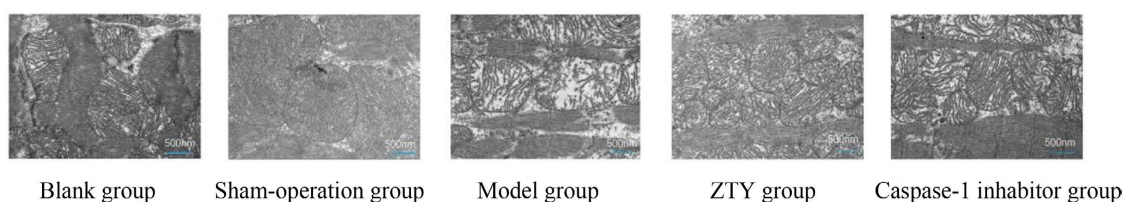


Figure 2. Ultrastructural results of rat cardiomyocytes in each group.

In the blank group and the sham-operation group, the cytomembrane of

cardiomyocytes was continuous and intact without pore formation. In the model group, the cytomembrane of cardiomyocytes was swollen and damaged, with pore formation, and the mitochondria were severely swollen, with membrane lysis and cristae fracture. In the ZTY group and the Caspase-1 inhibitor group, the cytomembrane of cardiomyocytes was more continuous and intact and the mitochondria were better than model group, as shown in **Figure 2**.

4.3. HE Staining Results of Myocardial Tissue Morphology of Rats in Each Group

The myocardial fibres of rats in the blank group and the sham-operation group were clear in structure, regular in arrangement, well delineated, uniform in staining, and the nuclei of the cells were clearly visible. In the model group, myocardial tissue was unevenly stained, myocardial fibre arrangement was disordered, the gap between myocardial cells was obviously widened, and some myocardial cells were oedematous with vacuolike degeneration. Compared with the model group, the symptoms of myocardial fibrillar lysis, necrosis and exudation were reduced to different degrees in ZTY and Caspase-1 inhibitor groups, as shown in **Figure 3**.

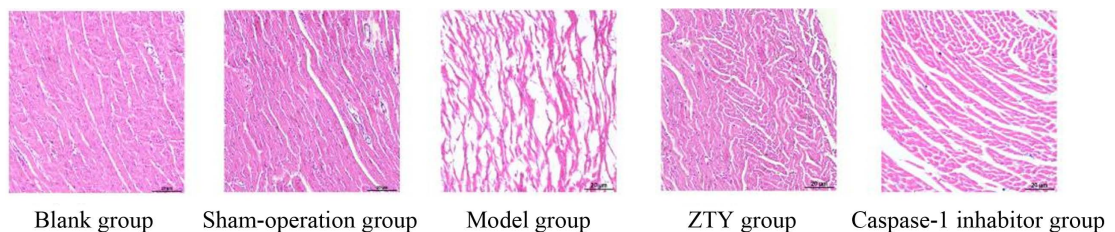
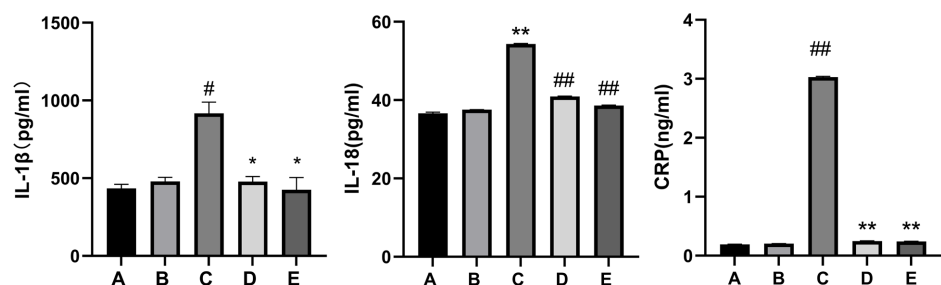


Figure 3. HE staining results of myocardial tissue morphology of rats in each group.

4.4. Serum IL-1 β , IL-18 and CRP Concentrations of Rats in Each Group



Notes: Compared with group A, # indicates $P < 0.05$, ## indicates $P < 0.01$; compared with group C, * indicates $P < 0.05$, ** indicates $P < 0.01$.

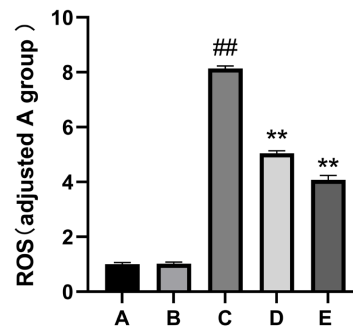
Figure 4. Serum IL-1 β , IL-18 and CRP concentrations of rats in each group. A: Blank group; B: Sham-operation group; C: Model group; D: ZTY group; E: Caspase-1 inhibitor group.

The levels of IL-1 β , IL-18 and CRP in myocardial tissues of rats in the model group were significantly higher than blank group, and the differences were statistically significant ($P < 0.05$). Compared with the model group, the levels of IL-1 β , IL-18

and CRP were significantly reduced in the ZTY group and the Caspase-1 inhibitor group, and the differences were statistically significant ($P < 0.05$), as shown in **Figure 4**.

4.5. Serum ROS Concentrations of Rats in Each Group

The levels of ROS in myocardial tissues of rats in the model group were significantly higher than blank group, and the differences were statistically significant ($P < 0.01$). Compared with the model group, the levels of ROS were significantly reduced in the ZTY group and the Caspase-1 inhibitor group, and the differences were statistically significant ($P < 0.01$), as shown in **Figure 5**.



Notes: Compared with group A, ## indicates $P < 0.01$; compared with group C, ** indicates $P < 0.01$.

Figure 5. Serum ROS concentrations of rats in each group. A: Blank group; B: Sham-operation group; C: Model group; D: ZTY group; E: Caspase-1 inhibitor group.

4.6. ASC, Nlrp3, Caspase-1 and GSDMD mRNA Expression of Rat Myocardial Tissues in Each Group

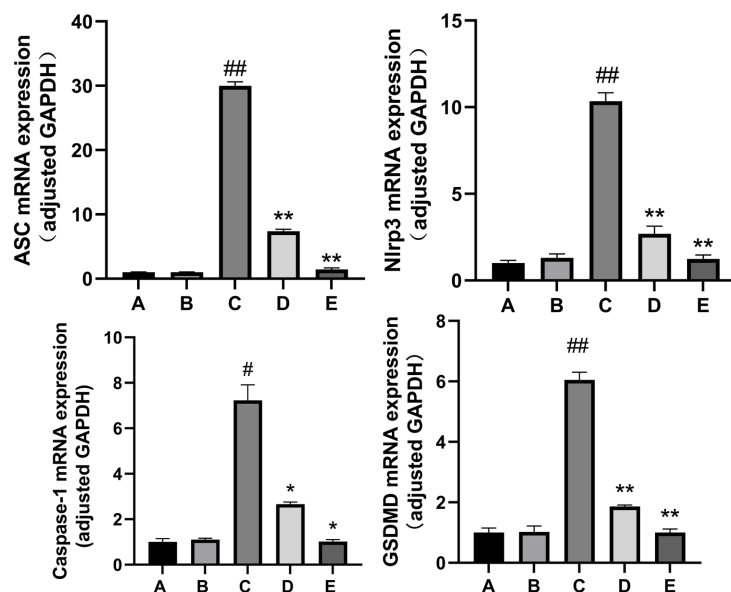


Figure 6. ASC, Nlrp3, Caspase-1 and GSDMD mRNA expression of rat myocardial tissues in each group. (Notes as above).

The expression of ASC, Nlrp3, Caspase-1 and GSDMD mRNA was significantly higher in the model group than the blank group ($P < 0.05$ or $P < 0.01$); the expression of ASC, Nlrp3, Caspase-1 and GSDMD mRNA were lower in the ZTY group and Caspase-1 inhibitor group in comparison with the model group ($P < 0.05$ or $P < 0.01$), as shown in **Figure 6**.

4.7. Caspase-1 and GSDMD Protein Expression of Rat Myocardial Tissues in Each Group

The expression of Caspase-1 and GSDMD was significantly higher in the model group than in the blank group and the sham-operation group ($P < 0.01$); the expression of Caspase-1 and GSDMD was lower in the ZTY group and Caspase-1 inhibitor group in comparison with the model group ($P < 0.05$ or $P < 0.01$), as shown in **Figure 7**.

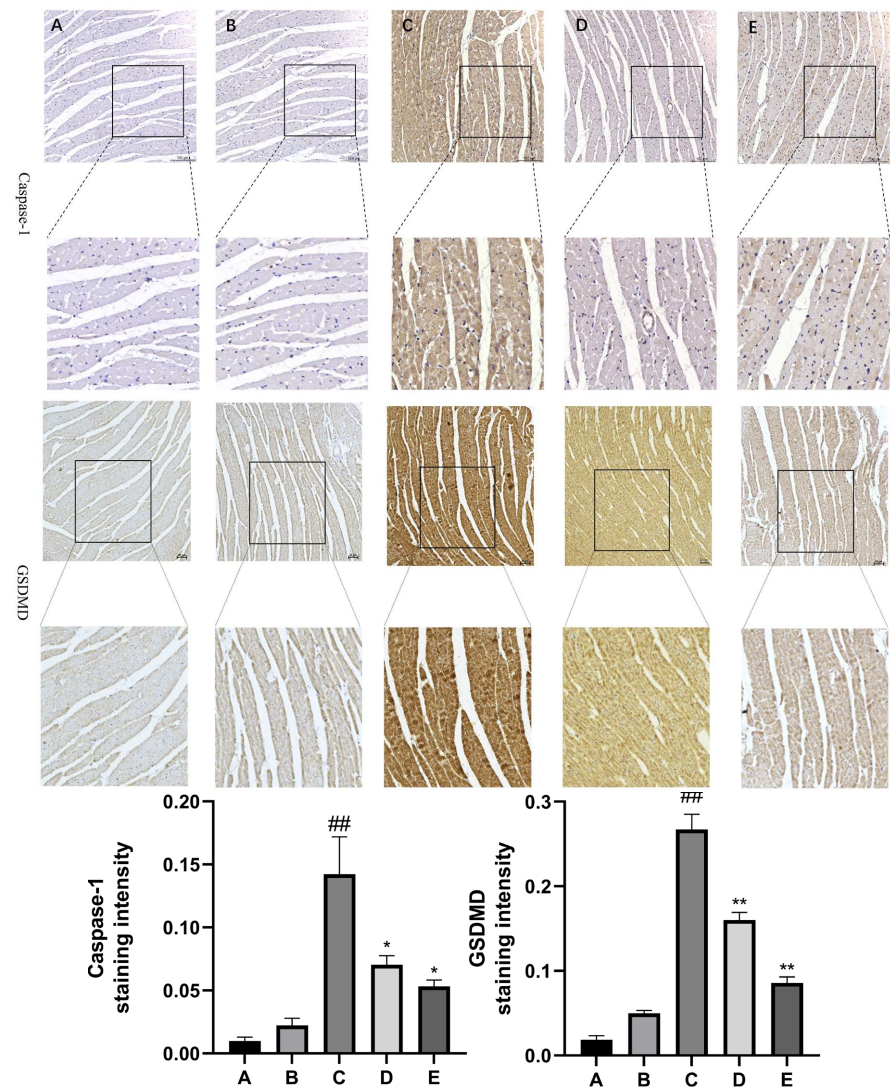


Figure 7. Caspase-1 and GSDMD protein expression of rat myocardial tissues in each group. (Notes as above).

5. Discussion

Coronary heart disease belongs to the category of “thoracic paralysis” and “cardiac pain” in Chinese medicine research [25] [26]. The disease mechanism is usually thought of root deficiency and branch excess, with deficiency of qi as the root deficiency and phlegm and blood stasis as the branch excess [27]. Deficiency of yang qi and qi deficiency makes Qi transformation disordered, resulting in dysfunction of fluid transport, causing phlegm and blood stasis [6]. Stagnation of blood and phlegm in the meridians and blockage of qi, traps the body in a vicious cycle.

While in the theory of Zhuang medicine, coronary heart disease belongs to the Zhuang medicine “a boring”. “A boring” refers to the “dragon road” blockage [28] and it can lead to sudden pain in the anterior region of the heart. Even the pain can lead to the back of the shoulder, wheezing, and can not lie down, etc.

Cellular pyroptosis is a pro-inflammatory programmed death mediated by inflammatory cysteine asparaginase, accompanied by the release of large amounts of pro-inflammatory factors that cause inflammatory injury [29].

Inflammatory mediators belong to the category of “toxicity” in traditional Chinese medicine. “Toxicity, the evil gas is not resolved”. It is assumed that inflammatory mediators (toxicity) can cause visceral dysfunction and disorder of fluid metabolism [30]. The toxicity is transformed into phlegm turbidity, stagnation of phlegm turbidity transforming into heat, and it violates the blood to form stasis, phlegm and stasis paralysis in the blood, which leads to a series of pathological changes such as qi and yin deficiencies, phlegm and stasis in the blood, and so on [31] [32]. Hence, it is proposed that cellular pyroptosis may be a reflection of the microscopic pathology that phlegm, stasis and toxicity are obstructions in the development process of blood vessels [33].

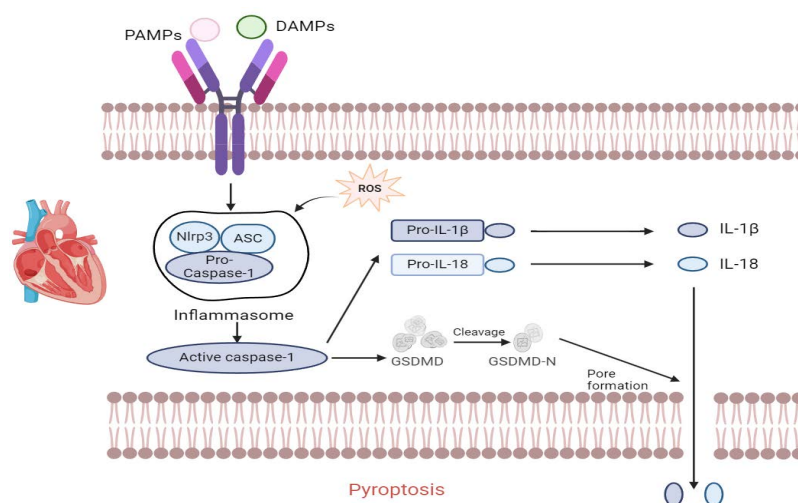


Figure 8. The pathogenesis of pyroptosis in CHD.

Zhuangtongyin is an empirical formula that Zhuang folk doctors based on “Three routes and two roads are not accessible” theory of the disease mechanism

[34]. The whole formula is combined with the common Guangxi herbs *Euonymus fortunei*, *Polygala fallax* and pseudo-ginseng, which has fewer herbs but more refined. There is the function of blood circulation, dissipate blood stasis and supplement the deficiency, turbid lipid lowering, dredging the three routes and two roads [35].

Caspase-1 is a member of the cysteoaspartic enzyme family and makes a difference to the pyroptosis pathway by promoting the maturation of inflammatory factors such as IL-1 β and IL-18, as well as cleaving the pyroptosis substrate GSDMD [6] [36]-[38]. It results in the formation of micropores in the cell membrane, the outflow of intracellular ions and the inward flow of extra-membranous water, which leads to cell swelling and lysis, further leading to the release of numerous inflammatory factors, such as IL-1 β and IL-18, and ultimately inducing cellular pyrolysis [39]-[42], as shown in **Figure 8**.

6. Conclusion

The results of transmission electron microscopy showed that the cell membranes of cardiomyocytes in the ZTY group were more complete without pore formation, and mitochondrial swelling was relieved in comparison with the model group. Staining results showed that fibrillar lysis was reduced in the ZTY group. These results showed that ZTY could protect cardiomyocytes, reduce mitochondrial swelling, relieve inflammation and reduce the death of cardiomyocytes. It was further found that ZTY could inhibit the expression of ASC, Nlrp3, Caspase-1 and GSDMD mRNA in Caspase-1 mediated proptosis pathway. At the transcriptional level, ZTY can also inhibit the expression of Caspase-1 and GSDMD proteins. Pyroptosis is often accompanied by the release of inflammatory factors, while ZTY can reduce the content of inflammatory factors IL-1 β , IL-18 and ROS in the serum of rats with coronary heart disease model. Therefore, the above experimental results indicate that Zhuangtongyin can inhibit the caspase-1 mediated pyroptosis by reducing the expression of ROS, relieving myocardial inflammation and protecting cardiomyocytes. This study can provide more research information for deepening and understanding the specific mechanism of ZTY in treating cardiovascular diseases. At the same time, it also provides research ideas for further development of Guangxi's characteristic Zhuang medicine for treating coronary heart disease.

Authors' Contributions

HQT and SMZ conceived and designed the experiments. SMZ, XFH, and WCW performed the experiments. SMZ and WZW performed data analysis. XQZ and JYW provided experimental guidance. SMZ wrote the manuscript. HQT revised the manuscript. All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animal care and use procedures were approved by the Ethics Committee of

Youjiang Medical University for Nationalities.

Funding

This work is supported by the National Natural Science Foundation of China (project No.8246151084).

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- [1] Zhang, S.T., Pang, L.L., Tang, H.Q., Huang, X.S., Feng, Y. and Hang, C.H. (2019) Effects of Zhuangtongyin on Cardiac Function and Vasodilatory Function in Rats Modeled with Coronary Heart Disease. *Chinese Pharmacy*, **30**, 2480-2484.
- [2] Zhong, Y., Li, X., Liang, T., Ding, B., Ma, K., Ren, W., *et al.* (2024) Effects of NLRP3 Inflammasome Mediated Pyroptosis on Cardiovascular Diseases and Intervention Mechanism of Chinese Medicine. *Chinese Journal of Integrative Medicine*, **30**, 468-479. <https://doi.org/10.1007/s11655-024-3655-2>
- [3] Chen, W.W., Gao, R.L., Liu, L.S., Zhu, M.L., Wang, W., Wang, Y.J., *et al.* (2018) Summary of the China Cardiovascular Disease Report 2017. *Chinese Journal of Circulation*, **33**, 1-8.
- [4] Ma, L.Y., Wu, Y.Z. and Chen, W.W. (2018) Highlights of the China Cardiovascular Disease Report 2018. *Chinese Journal of Hypertension*, **27**, 712-716.
- [5] Ji, N., Qi, Z., Wang, Y., Yang, X., Yan, Z., Li, M., *et al.* (2021) Pyroptosis: A New Regulating Mechanism in Cardiovascular Disease. *Journal of Inflammation Research*, **14**, 2647-2666. <https://doi.org/10.2147/jir.s308177>
- [6] Lei, Z.Q., Luan, F., Gao, M., Hu, J.W. and Zeng, N. (2023) Relationship between Pyroptosis and Cardiovascular Diseases and Traditional Chinese Medicine Prevention and Treatment Research. *China Journal of Chinese Materia Medica*, **48**, 1779-1791.
- [7] Qiu, Y.Y., Liu H.X., Yu Z.J., *et al.* (2024) Research Progress of Pyroptosis in Atherosclerosis. *Chinese Modern Doctor*, **62**, 115-119.
- [8] Chen, X., Li, Y., Li, J., Liu, T., Jiang, Q., Hong, Y., *et al.* (2022) Qishen Granule (QSG) Exerts Cardioprotective Effects by Inhibiting NLRP3 Inflammasome and Pyroptosis in Myocardial Infarction Rats. *Journal of Ethnopharmacology*, **285**, Article 114841. <https://doi.org/10.1016/j.jep.2021.114841>
- [9] Han, L.X., Zhang, H.X., Zhang, M.Y. *et al.* (2024) Effects of Several Blood-Activating and Blood-Stasis-Removing Chinese Herbal Injections on Vasodilatation and Immune Function-Related Indexes in Patients with Myocardial Ischemia-Reperfusion Injury. *Liaoning Journal of Chinese Medicine*, **50**, 146-149. (In Chinese)
- [10] Ge, Z.S., Xu, Z.M. and Li, P. (2021) Advances in Research on Cardiomyocyte Pyroptosis Mediated by NLRP3 Inflammasome and Traditional Chinese Medicine Intervention. *China Journal of Chinese Materia Medica*, **46**, 5210-5217.
- [11] Liu, Y.P. and Huang C.H. (2012) Progress of Modern Research on the Constituent Drugs of Zhuang Tong Drink, an Empirical Formula of Zhuang Medicine. *Chinese Journal of Traditional Chinese Medicine Information*, **19**, 111-112.
- [12] Feng, Y. (2017) Study on the Mechanism of Action of Ang II and Related Molecules

- in RAAS Activation Pathway of Coronary Heart Disease Myocardial Ischemia and Blood Stasis Syndrome Rats with Zhuangtongyin Intervention. Master's Thesis, Guangxi University of Traditional Chinese Medicine.
- [13] Zhao, W.C., He, C.Y. and Wang, F.C. (2020) Screening Potential Chinese Materia Medica and Their Monomers for Treatment Diabetic Nephropathy Based on Caspase-1-Mediated Pyroptosis. *Journal of Southern Medical University*, **40**, 1280-1287.
- [14] Cheng, Z., Li, Z., Gu, L., Li, L., Gao, Q., Zhang, X., *et al.* (2022) Ophiopogonin B Alleviates Cisplatin Resistance of Lung Cancer Cells by Inducing Caspase-1/GSDMD Dependent Pyroptosis. *Journal of Cancer*, **13**, 715-727.
<https://doi.org/10.7150/jca.66432>
- [15] Wei, Z., Gao, R., Sun, Z., Yang, W., He, Q., Wang, C., *et al.* (2023) Baicalin Inhibits Influenza A (H1N1)-Induced Pyroptosis of Lung Alveolar Epithelial Cells via Caspase-3/GSDME Pathway. *Journal of Medical Virology*, **95**, e28790.
<https://doi.org/10.1002/jmv.28790>
- [16] Li, Y., Tu, Z., Chen, F., Yang, X., Deng, R., Su, F., *et al.* (2023) Anti-Inflammatory Effect of Danhong Injection through Inhibition of GSDMD-Mediated Pyroptosis. *Phytomedicine*, **113**, Article 154743. <https://doi.org/10.1016/j.phymed.2023.154743>
- [17] Zhang, S.T. (2019) Study on the Effects of Zhuangtongyin on Inflammation and Neurohumoral Factors Associated with Coronary Heart Disease Myocardial Infarction in Rats. Master's Thesis, Youjiang Medical University for Nationalities.
- [18] Huang, X.S., Zhang, S.T., Tang, H.Q., Huang, C.H., Liu, Y.P., Lan, H.Q., *et al.* (2018) Effects of Pretreatment with Zhuangtongyin on Ang II, at-1R, Cx43 and Their Effects on Reperfusion Injury in Rats with Coronary Heart Disease with Blood Stasis. *Chinese Journal of Experimental Formulas*, **24**, 157-162.
- [19] Zhang, S.T., Pang, L.L., Tang, H.Q. and Huang, C.H. (2017) Comparison and Improvement of Preparation Methods of Rat Myocardial Ischaemia Model. *Chinese Journal of Comparative Medicine*, **27**, 98-101.
- [20] Pang, L.L., Feng, Y., Tang, H.Q., Huang, X.S., Ning, W.L., Zhang, S.T., *et al.* (2018) Study on the Mechanism of Oxidative Stress in Rats with Coronary Heart Disease Myocardial Ischaemia and Blood Stasis by Zhuangtongyin. *Lishizhen Medicine and Materia Medica Research*, **29**, 513-514.
- [21] Huang, X.S. (2019) Protective Effect and Mechanism Study of Zhuangtong Drink Pretreatment Affecting Reperfusion Injury in Rats with Coronary Heart Disease Blood Stasis Evidence Through AngII-(at-1R)-Cx43 Axis. Master's Thesis, Guangxi University of Traditional Chinese Medicine.
- [22] Gu, Z.M., Miao, Z.M. and Li, C.G. (2009) Effects of Caspase-1 Inhibitors on Acute Gouty Arthritis in Rats. *Journal of Qingdao University Medical College*, **45**, 437-440.
- [23] Guo, B., He, X., Yu, Y., Liu, R.F., Wang, M., Li, Y., *et al.* (2024) Effect of Xin Kang Chong Yi on Cardiomyocyte Death in Rats with Chronic Heart Failure. *Chinese Journal of Traditional Chinese Medicine*, **42**, 1-15.
- [24] Sun, X. (2023) Cypa Mediates Cardiomyocyte Focal Death after Ischemia-Reperfusion Injury Via the Caspase-3/GSDME Pathway. Jiangsu University.
- [25] Tan, J.P., Zhong, S., Zhang, Q.Y., Yang, Y., Li, J., Huang, S.C., *et al.* (2023) Exploring the Mechanism of Action of Haifu Yuyu Tang in Treating Blood Stasis in Coronary Heart Disease Based on Mitochondrial Energy Metabolism. *Lishizhen Medicine and Materia Medica Research*, **34**, 288-291.
- [26] Bi, Y., Mao, J., Wang, X., Zhao, Z., Li, B. and Hou, Y. (2015) Expert Survey on the Prevention and Treatment Situation of Traditional Chinese Medicine for Coronary

- Artery Disease. *Chinese Medicine*, **6**, 109-114.
<https://doi.org/10.4236/cm.2015.62012>
- [27] Jiang, X., Ma, C., Gao, Y., Cui, H., Zheng, Y., Li, J., *et al.* (2023) Tongxinluo Attenuates Atherosclerosis by Inhibiting ROS/NLRP3/Caspase-1-Mediated Endothelial Cell Pyroptosis. *Journal of Ethnopharmacology*, **304**, Article 116011.
<https://doi.org/10.1016/j.jep.2022.116011>
- [28] Pang, L.L. (2017) Study on the Mechanism of Zhuangtongyin on Oxidative Stress in Rats with Myocardial Ischemia and Blood Stasis Syndrome of Coronary Heart Disease. Guangxi University of Traditional Chinese Medicine.
- [29] Yang, X., He, T., Han, S., Zhang, X., Sun, Y., Xing, Y., *et al.* (2019) The Role of Traditional Chinese Medicine in the Regulation of Oxidative Stress in Treating Coronary Heart Disease. *Oxidative Medicine and Cellular Longevity*, **2019**, 1-13.
<https://doi.org/10.1155/2019/3231424>
- [30] Qu, J., Zou, Y. H. and Zhi, N. (2012) Progress of Modern Research on the Doctrine of Poisonous Evil. *Chinese Medical Emergencies*, **21**, 1629-1631.
- [31] Yu, N., Jia, L.Q., Song, N. and Yang, G.L. (2019) Exploring the Relationship between Pyroptosis and Atherosclerosis Based on the Pathogenesis of “Phlegm, Blood Stasis and Toxicity”. *Chinese Archives of Traditional Chinese Medicine*, **37**, 2186-2188.
- [32] Zhao, X., Ma, J.Y., Song, Z.G. and Zhao, S. (2024) Analysis of the Relationship Between Pyroptosis and Coronary Heart Disease Based on the Theory of “Phlegm, Blood Stasis, and Toxicity”. *Acta Chinese Medicine*, **39**, 290-296.
- [33] Huang, W.S., Xue, H.F., Gu, Z.X., Liu, R., Tian, Z., Liu, F.C.S., *et al.* (2019) Progress and Review of Chinese Medicine Research on Cellular Focal Death. *Lishizhen Medicine and Materia Medica Research*, **30**, 1961-1963.
- [34] Li, J., Zhou, X.L., Yang, R.X. and Shi, M. (2020) Progress of Research on the Prevention and Treatment of Cardiovascular Diseases by Zhuangtongyin. *Electronic Journal of Integrative Cardiovascular Disease of Chinese and Western Medicine*, **8**, 11-12.
- [35] Wang, L.L. (2023) Study on the Mechanism of Action of Xinfeng Capsule on Ra Immunoinflammation Based on NLRP3/GSDMD Pathway-Mediated Cellular Pyroptosis. Anhui University of Traditional Chinese Medicine.
- [36] Wei, S., Feng, M. and Zhang, S. (2022) Molecular Characteristics of Cell Pyroptosis and Its Inhibitors: A Review of Activation, Regulation, and Inhibitors. *International Journal of Molecular Sciences*, **23**, Article 16115.
<https://doi.org/10.3390/ijms232416115>
- [37] Yan, H., Luo, B., Wu, X., Guan, F., Yu, X., Zhao, L., *et al.* (2021) Cisplatin Induces Pyroptosis via Activation of MEG3/NLRP3/Caspase-1/GSDMD Pathway in Triple-Negative Breast Cancer. *International Journal of Biological Sciences*, **17**, 2606-2621.
<https://doi.org/10.7150/ijbs.60292>
- [38] Huan, P., Sun, X., He, Z., Yang, S., Wang, X., Xie, H., *et al.* (2023) Qiji Shujiang Granules Alleviates Dopaminergic Neuronal Injury of Parkinson's Disease by Inhibiting NLRP3/Caspase-1 Pathway Mediated Pyroptosis. *Phytomedicine*, **120**, Article 155019.
<https://doi.org/10.1016/j.phymed.2023.155019>
- [39] Zhang, X., Qu, H., Yang, T., Liu, Q. and Zhou, H. (2022) Astragaloside IV Attenuate Mi-Induced Myocardial Fibrosis and Cardiac Remodeling by Inhibiting ROS/Caspase-1/GSDMD Signaling Pathway. *Cell Cycle*, **21**, 2309-2322.
<https://doi.org/10.1080/15384101.2022.2093598>
- [40] Ye, Z., Li, L., Li, Y., Hu, Y., Wu, M., Yu, H., *et al.* (2023) Tou Nong Powder Obstructs Ulcerative Colitis through the Regulation of Nf- κ B/NLRP3/Caspase-1/GSDMD

Inflammasome Pyroptotic Pathway. *Journal of Ethnopharmacology*, **317**, Article 116846. <https://doi.org/10.1016/j.jep.2023.116846>

- [41] Hou, Z., Li, F., Chen, J., Liu, Y., He, C., Wang, M., *et al.* (2019) Beneficial Effects of Sagacious Confucius' Pillow Elixir on Cognitive Function in Senescence-Accelerated P8 Mice (SAMP8) via the NLRP3/Caspase-1 Pathway. *Evidence-Based Complementary and Alternative Medicine*, **2019**, 1-13. <https://doi.org/10.1155/2019/3097923>
- [42] Burdette, B.E., Esparza, A.N., Zhu, H. and Wang, S. (2021) Gasdermin D in pyroptosis. *Acta Pharmaceutica Sinica B*, **11**, 2768-2782. <https://doi.org/10.1016/j.apsb.2021.02.006>