Shengmai Yin formula promotes ventricular remodeling in chronic heart failure rats by inhibiting excessive autophagy via activating AKT/mTOR pathway

Ye Li¹, Qiang Xu¹, Wei Zhou¹, Mei-Juan Lu¹, Jian-Zhong Pang¹, Juan Wang¹, Tian Li³, Xiao-Yue Cai¹, Yan-Kun Liu¹

¹Department of Cardiology, The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300250, China.

*Corresponding to: Ye Li, Department of Cardiology, The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, No. 69, Zengchang Road, Hebei District, Tianjin 300250, China. E-mail: lizapple@126.com.

Author contributions
Ye Li, Wei Zhou, MeiJian Lu, Jianzhong Pang, Juan Wang, Xiaoyue Cai, Tian Li and Yankun Liu contributed to the experimental design, data analysis, writing-original draft for this study. Professor Qiang Xu contributed to the experimental design, data analysis, and manuscript preparation. All authors read and approved the final manuscript.

Competing interests
The authors declare no conflicts of interest.

Acknowledgments
This research was financially supported by Foundation of Tianjin Municipal Education Commission (2019KJ058). We thank Professor Qiang Xu for his valuable suggestions and constructive comments on the manuscript.

Peer review information
TMR Integrative Medicine thanks all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations
CHF, chronic heart failure; SMY, Shengmai Yin; LC3, light chain 3; NT-proBNP, N-terminal pro-B-type natriuretic peptide; STZ, stromelysin-2; ELISA, enzyme linked immunosorbent assay; PBS, phosphate-buffered saline.

Citation

Abstract
Background: Shengmai Yin (SMY) formula, a traditional Chinese medicine, shows a definite therapeutic effect on chronic heart failure (CHF) in clinical practice, but the molecular mechanism remains largely unknown. The PI3K/Akt/mTOR pathway is a classic pathway of autophagy and plays a pivotal role in the occurrence and development of CHF. Here, we aimed to investigate whether SMY formula treat CHF rats by inhibiting excessive autophagy.

Methods: Echocardiography was conducted to evaluate cardiac function. Transmission electron microscopy was used to observe the arrangement of myocardial cells. Enzyme linked immunosorbent assay analysis was performed to quantitative detecting the content of N-terminal pro-B-type natriuretic peptide, stromelysin-2, TNF-α in rat serum. Western blotting was used to detect the expression of AKT, p-AKT, mTOR, p-mTOR, light chain 3 (LC3), and p62. In vitro, myocardial cells were treated with hypoxia reoxygenation and then intervened with SMY. Cell counting kit-8 method was used to measure the cell viability. The immunofluorescence expression of LC3 protein were also examined. Results: In vivo, SMY intervention assisted in the ventricular remodeling, reduced the levels of N-terminal pro-B-type natriuretic peptide, stromelysin-2, TNF-α in serum, and recovered myocardial cell structure in CHF rats. Treatment with SMY significantly promoted the ratio of p-AKT/AKT, p-mTOR/mTOR, and down-regulated the expression level of p62 and the ratio of LC3-II/LC3-I. In vitro, when the concentration of SMY containing serum reached 40% in the medium, the activity of myocardial cells reached the highest at 135.14%. SMY inhibited the expression of LC3 in hypoxic-reoxygenation embryonic ventricular myocytes cells. When the hypoxic reoxygenation cells treated with p-mTOR inhibitor, rapamycin, or p-AKT inhibitor, API-1, SMY could also down-regulate the LC3 expression level. Conclusions: SMY formula functions in restoring cardiac function and promoting myocardial ultrastructural recovery by reducing autophagy activity through up-regulating the ratio of p-AKT/ AKT, p-mTOR/ mTOR, and down-regulating the expression level of p62 and the ratio of LC3-II/LC3-I in CHF rats.

Keywords: chronic heart failure; ventricular remodeling

© 2023 By Author(s). Published by TMR Publishing Group Limited. This is an open access article under the CC-BY license. (https://creativecommons.org/licenses/by/4.0/)
**Introduction**

Chronic heart failure (CHF) is a complex clinical syndrome characterized by ventricular systolic and diastolic dysfunction [1, 2]. There are approximately 64 million heart failure patients worldwide, with approximately 8.9 million in China [3, 4]. Heart failure seriously affects the quality of life of patients, with a 5-year survival rate of only 50% [5]. Ventricular remodeling is the fundamental mechanism of heart failure, which produces progressive lesions during the stable and exacerbating stages of the condition, leading to the exacerbation of heart failure. Myocardial cell death and excessive activation of the neuroendocrine system are key factors leading to ventricular remodeling [6, 7].

One of the mechanisms that cause myocardial cell death in the process of CHF is autophagic cell death [8, 9]. Autophagy is a programmed cell death, a protective mechanism that promotes cell survival through lysosomal breakdown and recovery of useful components within cells, supplementing essential metabolic substrates for cells [10–14]. However, excessive autophagy may activate the cytokine system, lead to myocardial cell death and exacerbate the development of heart failure, and ultimately result in patient death [15]. There are two main pathways for autophagosome generation, which relies on and does not rely on mTOR. Autophagy dependent on mTOR regulates the autophagy process by regulating the AKT/mTOR signaling pathway [16]. As a heterodimer, PI3K activation promotes the binding of AKT to PDK1, which phosphorylates AKT protein. AKT activation further phosphorylates and activates downstream target protein mTOR, participating in cell proliferation, differentiation, apoptosis, and energy metabolism [17]. After being successfully activated, the PI3K-AKT-mTOR pathway negatively regulates the autophagy process to a certain extent [17]. Light chain 3 (LC3) is a biomarker produced by autophagosomes in the body [18–20]. After autophagy activity begins, cytoplasmic LC3-I plays its role by enzymatic hydrolysis of some peptides, which then transforms into membrane type LC3-II [21–23]. Clinical studies have shown that the expression level of Beclin1 and LC3-II is significantly increased in the heart tissue of patients with chronic heart failure [24].

Shengmai Yin (SMY) formula originates from the *The Origin of Medicine* (Yuansu Zhang, 1186 C.E.) and is a classic traditional Chinese medicine prescription for treating Qi (Qi refers to the basic substance that constitutes the human body and maintains life activities, and is the unity of substance and function) and Yin (in Chinese philosophy, the female, latent, passive principle, characterized by dark, cold, wetness, passivity, disintegration, etc.) deficiency syndrome [25]. It is composed of three drugs: *Ginseng Radix et Rhizoma Rubra*, *Ophiopogonis Radix*, and *Schisandrae Chinensis Fructus*. Researchers have found that SMY formula improves cardiac function in CHF patients, and delays the progression of myocardial fibrosis [26–28]. However, there is still a lack of understanding the effect of SMY on regulating autophagy pathway in treating CHF.

In this study, we constructed a CHF model rat and analyzed the cardiac function indicators the left ventricular end diastolic dimension, left ventricular end-systolic dimension, left ventricular posterior wall dimensions and left ventricular posterior wall thickness of systolic after intervention with SMY. The levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP), natriuretic peptide (NT-proBNP), ST2, and NF-κB were measured in the serum. The myocardial ultrastructure was observed after intervention with SMY, and the expression levels of key proteins mTOR, AKT, p62, and LC3-I/II in the autophagy pathway were detected. The rat embryonic ventricular myocytes were also cultured and immunofluorescence analysis was performed on the expression level of LC3 in the autophagy pathway after drug intervention.

**Materials and methods**

**Reagents**

Isoflurane (Shandong Luguang Biotechnology, Shandong, China); Captopril tablets (Changzhou Pharmaceutical Factory, Changzhou, China), 30% acrylamide (Solarbio, Beijing, China), 1.5M Tris-HCl pH 8.8 (Solarbio, Beijing, China), 1 M Tris-HCl pH 6.8 (Solarbio, Beijing, China), TEMED (Solarbio, Beijing, China), 5 × Tris-Glycine running buffer (Solarbio, Beijing, China). Rat TNF-α Elisa Kit (Shanghai Kejing Trading Co., Ltd., Shanghai, China), Rat NT-proBNP Elisa Kit (Shanghai Kejing Trading Co., Ltd., Shanghai, China), Rat ST2 Elisa Kit (Shanghai Kejing Trading Co., Ltd., Shanghai, China). Mouse Anti-β-tubulin mAb (ZSGR-Bio, Beijing, China), AKT Polyclonal antibody (Proteintech, Des Plaines, IL, USA), Anti-LC3B antibody (Cell Signaling Technology, Danvers, MA, USA), Anti-mTOR Antibody (BOSTER, Pleasanton, CA, USA), SQSTM1/p62 (D1Q5S) Rabbit mAb (Cell Signaling Technology, Danvers, MA, USA), Phospho-AKT (Ser473) Monoclonal antibody (Proteintech, Des Plaines, IL, USA). Fast Blocking Western (YESSEN, Shanghai, China).

The adult dosage of SMY raw medicine is 9 g of *Ginseng Radix et Rhizoma Rubra*, 2 g of *Ophiopogonis Radix*, and 6 g of *Schisandrae Chinensis Fructus*, provided by the pharmacy of the Second affiliated Hospital of Tianjin University of Traditional Chinese Medicine.

**Animals**

Feeding. Sprague Dawley rats (160–180 g, 80 males) were purchased from Beijing Hualuafang Biotechnology Co., Ltd. The production license number for experimental animals is SCXK (Beijing) 2019-0008. All rats were raised in the specific pathogen-free animal room of Yishengyuan Gene Technology (Tianjin) Co., Ltd. The laboratory animal use license number is SYZK (Tianjin) 2021-0003, and the laboratory animal qualification certificate number is 1103222201030277S. This research was conducted in accordance with international principles for laboratory animal use and care and the guidelines of the Animal Care Committee at the Faculty of Medicine, Yishengyuan Gene Technology (Tianjin) Co., Ltd., and was approved by the Animal Care Committee of the Faculty of Medicine, Yishengyuan Gene Technology (Tianjin) Co., Ltd. All animals were free to eat and drink water, and raised at room temperature and relative humidity of 40–70%.

**Modeling.** A chronic heart failure rat model was established after myocardial infarction through ligation the anterior descending branch of the coronary artery. After anesthesia of the rats, tracheal intubation was performed, a ventilator was connected, and the heart was exposed through thoracotomy between 2–3 ribs on the left side. Then the pericardium was punctured, and the anterior descending branch of the left coronary artery was ligated 2 mm below the left atrial appendage. The chest was gently compressed to expel air and closed.

**Groups.** Rats were randomly divided into 5 groups: negative control group, model group, captopril (positive drug) group, SMY high-dose group, and SMY low-dose group. There were 10 rats in each group. Rats were continuously administered for 4 weeks and their cardiac function was measured once a week. For the negative control group, rats were not subjected to drug intervention and were raised routinely. For the model group, rats were given daily gavage of the same volume of physiological saline as the treatment group. For the positive control group, rats were administered captopril at a dose of 50 mg/kg/d by gavage. For SMY high-dose group, rats were administered SMY granules at a dose of 1.6 g/kg/d. For SMY low-dose group, rats were treated with SMY granules at a dose of 0.8 g/kg/d for 28 days.

**Echocardiography**

The M-mode echocardiography system (GE HealthCare, Chicago, IL, USA) and 13 MHz ultrasonic probe (GE HealthCare, Chicago, IL, USA) were used to evaluate cardiac function in rats. Record echocardiography on the short axis of the left ventricle to measure left ventricular ejection fraction and fraction shortening.

**Enzyme linked immunosorbent assay (ELISA) analysis**

The levels of NT-proBNP, ST2 and TNF-α in the serum were examined by ELISA analysis. The process was conducted according to the methods reported by Song et al. [29].
Transmission electron microscopy detection
The stained sections of rat myocardium were observed using transmission electron microscopy (H-7650B; Hitachi, Tokyo, Japan) according to the methods reported by Kang et al. [30].

Western blot
Western blot analysis was conducted according to the methods reported by Kang et al. [31]. The exposure was made and the grayscale values were analyzed using image J software.

Cell culture and grouping
Cell culture. The rat embryonic ventricular myocytes, H9c2 cell line were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). At 37 °C and 5% CO₂ cells were cultured in high glucose Dulbecco’s modified eagle medium. The concentration of fetal bovine serum is 10%. The medium was supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

Cell grouping. Negative control group: myocardial cells were incubated for 17 hours.

Hypoxia reoxygenation group: myocardial cells adhered to the wall for 48 hours, followed by hypoxia for 12 hours and reoxygenation for 4 hours.

SMY + Hypoxia reoxygenation group: after 48 hours of myocardial cell adhesion, 40% SMY containing serum was added for pretreatment for 0.5 hours, followed by 12 hours of hypoxia and 4 hours of reoxygenation.

Rapamycin + hypoxia reoxygenation group: after myocardial cells adhere to the wall, they are pre-treated with rapamycin for 0.5 hours, followed by hypoxia for 12 hours and reoxygenation for 4 hours.

Rapamycin + SMY + hypoxia reoxygenation group: myocardial cells were pretreated with rapamycin for 0.5 hours, treated with drug-containing serum for 0.5 hours, and then subjected to hypoxia for 12 hours and reoxygenation for 4 hours.

API-1 + hypoxia reoxygenation group: after 48 hours of myocardial cell adhesion, API-2 pretreatment was added for 0.5 hours, followed by 12 hours of hypoxia reoxygenation for 4 hours.

API-1 + SMY + hypoxia reoxygenation group: myocardial cells adhered to the wall for 48 hours and were pretreated with API-2 for 0.5 hours, followed by treatment with drug-containing serum for 0.5 hours, followed by hypoxia for 12 hours and reoxygenation for 4 hours.

Immunofluorescence
Fixation and permeation: a cover glass slide of the already crawled cells in the culture plate was washed with 1 × phosphate-buffered saline (PBS) 3 times. Then the climbing plate was fixed with 4% paraformaldehyde for 15 minutes. The glass slides were washed with 1 × PBS three times, each time for 3 minutes. Absorbent paper absorbs 1 × PBS, add 5% normal serum on a glass slide and seal at room temperature for 1 hour. Absorb the sealing solution with absorbent paper, enough diluted primary antibody was added to each slide and placed in a wet box. Then it was incubated overnight at 4 °C. The climbing tablets were soaked with 1 × PBST three times, and 3 minutes for each time. After absorbing excess liquid on the climbing tablets with absorbent paper, the diluted fluorescent secondary antibody was dripped. The tablets were incubated at 37 °C in a wet box for 1 hour, and they were soaked with 1 × PBST three times, each time for 3 minutes. Incubate with DAPI dropwise in dark for 5 minutes, stain the sample with nuclei. 1 × PBST was used to wash off excess DAPI for four times. Dry the plate, and seal the plate with anti-fluorescence quenching agent. The images were observed and collected by the laser confocal microscope (LSM710; Carl Zeiss, Oberkochen, Germany).

Statistical analysis
All data were presented as mean ± standard deviation and analyzed using GraphPad Prism 8.0. The significance of the data was evaluated according to the one-way analysis of variance test or two-way analysis of variance. P < 0.05, **P < 0.01.

Results
SMY intervention assisted in the ventricular remodeling in chronic heart failure rats
In order to confirm the effect of SMY intervention on ventricular remodeling in CHF rats, echocardiography analysis was conducted. After 4 weeks of SMY treatment, compared with the control group, the left ventricle end-diastolic dimension, left ventricular end-systolic dimension, left ventricular posterior wall dimensions, left ventricular posterior wall thickness of systolic markedly increased in the model group, while the ejection fraction showed a significant decrease. When compared with the model group, the SMY high-dose group and the Captopril (positive control) group showed a significant decrease in left ventricular end-systolic dimension and a markedly increased in ejection fraction (Table 1). This indicates that SMY high-dose effectively restored the cardiac function of CHF rats.

SMY intervention reduced the levels of NT-proBNP, ST2 and TNF-α in CHF rats
In clinical practice, the NT-proBNP is considered as an indicator for evaluating heart failure. In addition, growth ST2 is associated with TNF-α involved in the development process of CHF. To detect whether SMY intervention could function on the above proteins, the expression level of NT-proBNP, ST2 and TNF-α in the serum of CHF rats was examined with ELISA assay. The results showed that compared with the control group, the levels of NT-proBNP, ST2, and TNF-α in the model group were quite high. While in the positive control group and SMY high-dose group, the expression level of the three factors significantly decreased (Figure 1). This result confirmed that SMY could effectively alleviate the progression of heart failure in CHF rats.
The effect of SMY intervention on myocardial cell structure in chronic heart failure rats

To investigate the effect of SMY intervention on the structure of myocardial cells, transmission electron microscopy observations were conducted. It showed that in the negative control group, the myofibril of myocardial cells was arranged neatly, the myofilaments were compact, the structure of light and dark bands was clear, and the number of mitochondria was large and orderly. In the model group, the myofibril of myocardial cells was disorder, the myofilaments were loose and broken, the structure of light and dark zones was unclear, most of the myofibril were dissolved and disappeared, the number of mitochondria was significantly reduced, and most of the mitochondrial cristae disappeared with the rupture of the inner and outer membranes. After the intervention of positive control and SMY high-dose, the arrangement of myofibril of myocardial cells was still neat, the myofilaments were slightly loose without obvious fracture, and the structure of sarcomere of light and dark zone was clear. Meanwhile, the number of mitochondria significantly increased, the arrangement was relatively neat, the structure was clear, the mitochondrial cristae partially disappeared, and the inner and outer membranes were relatively intact (Figure 2).

These results indicated that SMY intervention effectively restored the collagen fiber morphology and mitochondrial quantity of myocardial cells.

SMY acted on the Akt/mTOR pathway to downregulate autophagy levels in chronic heart failure rats

The Akt/mTOR pathway regulates the occurrence and development of autophagy process. To explore whether SMY regulates the autophagy process of myocardial cells through the Akt/mTOR pathway, western blot was conducted to detect the expression levels of AKT, mTOR, p62, and LC3 I/II in heart failure rats. The results showed that compared with the negative control group, the p-Akt/Akt ratio was significantly increased in the SMY high-dose and positive control group (P < 0.05). Similarly, compared with the control group, the proportion of p-mTOR/mTOR increased in the SMY high-dose and positive control group (P < 0.05). Moreover, the proportion of LC3-II/LC3-I significantly reduced among the SMY low-dose, SMY high-dose and positive control group. For the expression of p62 protein, it decreased in the SMY low-dose, SMY high-dose and positive control group. And both of the SMY high- and low-dose groups performed better than that of the positive control group (P < 0.05) (Figure 3). The above results indicate that SMY intervention inhibits autophagy level by increasing the ratios of p-Akt/Akt and p-mTOR/mTOR.

SMY intervention inhibited the expression of LC3 in rat embryonic ventricular myocytes

To confirm the molecular mechanism of SMY in treating heart failure, we used SMY containing serum to intervene in rat embryonic ventricular myocytes, H9c2 cell line, induced by hypoxia. The primary cultured cardiac myocytes were divided into the following 7 groups: (1) negative control group; (2) hypoxia reoxygenation group; (3) SMY-containing serum + hypoxia reoxygenation group; (4) hypoxia reoxygenation group + rapamycin; (5) hypoxia reoxygenation group + rapamycin + SMY-containing serum group; (6) hypoxia reoxygenation group + API-1 group; (7) hypoxia reoxygenation group + API-1 + SMY-containing serum group.

Cell counting kit-8 experiment was then conducted to verify the dose effect of SMY serum intervention to cell viability. The results showed that compared with the control group (0% SMY-containing serum), when the concentration of serum reached 40%, the activity of myocardial cells was the highest at 135.14% (Figure 4). Therefore, in the subsequent experiments, 40% SMY-containing serum was chosen.

Subsequently, SMY intervention was conducted on H9c2 cells, and immunofluorescence analysis was performed on LC3 protein. The stronger the green fluorescence of LC3, the higher the degree of autophagy. As shown in Figure 5A, in the hypoxia reoxygenation group, the expression of LC3 increased, indicating an increase in autophagy level. While in the hypoxia reoxygenation + SMY group, the expression of LC3 was much lower compared with all other groups except negative control group. After treatment with rapamycin alone in the hypoxia reoxygenation group cells, the expression level of LC3 significantly increased; with the addition of SMY containing serum, the expression level of LC3 decreased. Furthermore, when treated with the AKT phosphorylation inhibitor, API-1, in the hypoxic reoxygenation cells, the expression of LC3 increased; while treated with SMY-containing serum, the expression level of LC3 markedly decreased. Furthermore, to investigate the protein expression level of Akt, mTOR, p62 and LC3, western blot analysis was conducted. The results showed that when hypoxia reoxygenation occurs, the ratio of p-Akt/Akt, mTOR/TOR was decreased, while p62 and LC3 II/I was increased. When SMY was added, compared with the hypoxia reoxygenation treated group, the ratio of p-Akt/Akt, p-mTOR/mTOR significantly increased (P < 0.01), and the expression level of p62 and LC3 II/I decreased. When treated with rapamycin or API-1, no significant difference was observed compared with the hypoxia reoxygenation treated group. When SMY was added, an increased ratio of p-Akt/Akt and p-mTOR/mTOR was found, and the expression level of p62 and LC3 II/I decreased (Figure 5B and 5C).

This indicated that SMY reduced autophagy levels in the H9c2 cells by promoting both of the ratio of p-AKT/AKT and p-mTOR/mTOR, and inhibiting p62 and LC3 II/I, which is consistent with the previous results shown in Figure 3.
Figure 2 Observation on the ultrastructure of rat myocardium. After intervention with captopril or SMY low-dose or SMY high-dose for four weeks, the ultrastructure of rat myocardium was collected and observed with transmission electron microscopy. Bar = 2 μm. SMY, Shengmai Yin.

Figure 3 Expression level of the key proteins involved in the AKT-mTOR signal pathway in CHF rats. (A) Western blot assay of AKT, p-AKT, mTOR, p-mTOR, p62 and LC3. (B) Grey intensity analysis of western blot results. The data was presented as mean ± standard deviation and analyzed using GraphPad Prism 8.0. The significance was evaluated according to the one-way analysis of variance test. *P < 0.05, **P < 0.01. CHF, chronic heart failure; SMY, Shengmai Yin; LC3, light chain 3.

Figure 4 Cell viability test with the treatment of dosage of SMY containing-serum. 0, 5%, 10%, 20%, 40% and 60% SMY containing-serum were added into the cell medium, respectively. The data was presented as mean ± standard deviation and analyzed using GraphPad Prism 8.0. CHF, chronic heart failure; SMY, Shengmai Yin.
Figure 5 The protein expression of Akt/mTOR signaling pathway. (A) Immunofluorescence examination of LC3 protein. The rat embryonic ventricular myocytes, H9c2 cell line were divided into 7 groups. The negative control group, the hypoxia reoxygenation group (hypoxia for 12 h and reoxygenation for 4 h), SMY + hypoxia reoxygenation group (40% SMY serum pretreatment for 0.5 h, followed by 12 h of hypoxia and 4 h of reoxygenation), rapamycin + hypoxia reoxygenation group (pre-treated with rapamycin for 0.5 h, followed by hypoxia for 12 h and reoxygenation for 4 h), rapamycin + SMY + hypoxia reoxygenation group (cells were pretreated with rapamycin for 0.5 hours, treated with SMY serum for 0.5 h, and then subjected to hypoxia for 12 h and reoxygenation for 4 h), API-1 + hypoxia reoxygenation group (API-2 pretreatment for 0.5 h, followed by 12 h of hypoxia and reoxygenation for 4 h), and API-1 + SMY + hypoxia reoxygenation group (cells were pretreated with API-1 for 0.5 hours, treated with SMY serum for 0.5 h, and then subjected to hypoxia for 12 h and reoxygenation for 4 h). (B) Western blot analysis of the protein expression of Akt/mTOR signaling pathway. (C) Grey intensity analysis of western blot results. The data was presented as mean ± standard deviation and analyzed using GraphPad Prism 8.0. The significance was evaluated according to the one-way analysis of variance test. *P < 0.05, **P < 0.01, ***P < 0.001. CHF, chronic heart failure; SMY, Shengmai Yin; C3, light chain 3; RAPA, rapamycin; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate.

Discussion

Due to its high incidence and mortality rate, CHF poses a serious threat to human life and health [32]. SMY, composed of Ginseng Radix et Rhi zona Rubra, Ophiopogonis Radix, and Schisandrae Chinensis Fructus, is a classic formula for treating Qi and Yin deficiency syndrome. It is frequently used for the treatment of CHF in clinical practice in China. Our work shows that SMY promotes the ratio between p-AKT/AKT and p-mTOR/mTOR in myocardial tissue, down-regulates the expression ratio of LC3II/LC3I and the level of p62, inhibits excessive autophagy, promotes the recovery of myocardial ultrastructure, and restores rat cardiac function.

The Western medications used clinically for treating CHF include diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, calcium channel blockers, aldosterone antagonists, and angiotensin receptor-neprilysin inhibitors. However, side effects of the above drugs include bradycardia, low blood pressure, and worsening heart function, bringing new risks to CHF patients. Traditional Chinese medicine shows good effects in the treatment of CHF in both animal experiments and clinical aspects, while the side effects are relatively few.

The PI3K/Akt/mTOR signaling pathway could be activated due to stimuli such as injury and oxidative stress [33], which is one of the effective means to alleviate CHF. In addition, the activation of the PI3K/Akt/mTOR signaling pathway is closely related to the occurrence of autophagy [34]. When CHF occurs, PI3K is activated and upregulates key regulatory factors of autophagy, including Akt. The Thr308 and Ser473 sites of PI3K phosphorylation of Akt activate Akt [35, 36], while activated Akt phosphorylates mTOR [37]. mTOR is a key regulator of cell growth and metabolism, and its mTORC1 subtype is also an upstream active factor that plays a regulatory role in autophagy [38]. Phosphorylated mTOR can inhibit transient autophagy induced by hypoxia through the PI3K/Akt/mTOR signaling pathway, thereby alleviating CHF. The results of our study show that the expression of p-PI3K, p-Akt, and p-mTOR in the SMY group is significantly increased, indicating that the PI3K/Akt/mTOR signaling pathway is activated.

LC3 exists in autophagosomes and plays an important role in the formation of autophagosomes [39]. When autophagy occurs, a large amount of LC3I is converted into LC3II, and the LC3II/LC3I ratio increases. In this study, the LC3 II/LC3 I ratio significantly decreased after SMY treatment, indicating that SMY reversed the trend of increased autophagy related protein expression caused by CHF and had a certain inhibitory effect on autophagy. As one of the classic biomarkers of the downstream stage of autophagy, p62 can also reflect changes in autophagy. P62 can bind to ubiquitination substrates and LC3 on the inner membrane of autophagosomes, and is degraded by forming autophagosomes in the lysosomal system [40]. When autophagy is inhibited, the expression of p62 protein increases [41]. The results of this study showed that after SMY treatment, the expression of p62 protein significantly increased. Combined with the changes in LC3 II/I, it is speculated that the binding between autophagosomes and lysosomes is blocked, and autophagic flow is inhibited.

Li et al. reported that Qishen Tiaohong granules down-regulated the autophagy of myocardial cells in heart failure mice and weakened the injurious effect of excessive autophagy on myocardial cells 4 weeks after surgery, during the decompensated phase of heart failure [42].
Fan et al. found that Qili Qiangxin capsules could inhibit autophagic activity, and activate the AKT/mTOR pathway in vivo. It protected against myocardial injury through decreasing excessive autophagy-mediated cell death in vitro. Blocking of AKT signaling by Capivaserib significantly reduced the effect of Qili Qiangxin capsules on inhibiting autophagy-mediated cell death, indicating the AKT protein may be one of the targets of Qili Qiangxin capsules [43]. SMI is composed of Ginseng Radix et Rhizoma Rubra, Schisandrae Chinensis Fructus, and Ophiopogonis Radix. Both Ginseng Radix et Rhizoma Rubra and Ophiopogon japonicus have been reported to have inhibitory effects on autophagy activity. Lim et al. reported that Korean Ginseng Radix et Rhizoma Rubra treatment downregulated the expression of LC3-II, beclin-1, and the number of autophagic vacuoles, which attenuated chronic cyclosporine-induced excessive autophagosome formation and autophagic aggregates [44]. Wu et al. found that under specific circumstances, Shenmai injection, which consists of Ginseng Radix et Rhizoma Rubra and Ophiopogonis Radix inhibited autophagic process by decreasing the Beclin 1-Bcl-2 complex dissociation induced by doxorubicin [45]. Park et al. reported that autophagy induced by doxorubicin has a protective function in hepatocellular carcinoma, and Korea Ginseng Radix et Rhizoma Rubra extract significantly inhibited autophagy at a late stage and sensitized hepatocellular carcinoma cells to doxorubicin-induced cell death [46]. Furthermore, Bian et al. demonstrated that 20(S)-Ginsenoside Rg3 blocked the late stage of autophagy, including increased LC3-I/II conversion, accumulation of LC3, degradation of p62, and loss of the balance between autophagy and apoptosis, all these lead to the happening of apoptosis process [47]. Ophiopogonin D is a steroidal glycoside and an active component extracted from Ophiopogonis Radix. It could improve antioxidative protection of the cardiovascular system through decreasing the number of autophagic vacuoles, LC3-II/LC3-I, and down-regulating the LC3 expression level in H9c2 cells. Moreover, Ophiopogonin D could reduce the content of reactive oxygen species generated from mitochondrial damage and inhibit autophagic activity, due to its protective effects in the hearts against doxorubicin-induced toxicity [48].

It is unclear whether Schisandrae Chinensis Fructus has the effect of inhibiting autophagy activity. And the functional small molecule components in SMI, especially those that target and promote AKT and mTOR phosphorylation. Due to the impact of the epidemic, we did not conduct a detailed study on the functions of each drug in this study. In future work, we will verify the functions of individual medicinal herbs in traditional Chinese medicine prescriptions and small molecule targeting AKT and mTOR, providing a clear understanding of the mechanism of SMI in treating CHF.

Conclusion

SMY functions in restoring cardiac function and promoting myocardial ultrastructural recovery by reducing autophagy activity through up-regulating the ratio of p-AKT/AKT, p-mTOR/mTOR, and down-regulating the expression level of p62 and the ratio of LC3-II/LC3-I in the treatment of CHF.

References


