Shenmai Injection Alleviates Cardiomyocyte Apoptosis Induced by Doxorubicin

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Research

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Abstract

Background: Shenmai Injection (SMI) is a patented Chinese medicine extract, has been widely used to treat myocardial damage caused by doxorubicin (DOX), but its underlying mechanisms remain elusive. The study aimed to explore the protective effect of SMI on myocardial injury caused by DOX *in vivo*.

Methods: The male Sprague-Dawley (SD) rats received DOX (2mg/kg) tail vein injection every week for 4 weeks, with or without SMI and miR-30a agomir treatment for 2 weeks. The protective effect of SMI on myocardial injury caused by DOX has been determined by measuring rat body mass and general heart morphology, myocardial pathological changes, and serum markers. The myocardial pathological changes were observed by Van Gieson (VG) staining, the serum marker levels of myocardial injury were detected by ELISA, the myocardial cell apoptosis was observed by TUNEL assay and transmission electron microscope, and the expression of target protein was detected by Western Blot.

Results: SMI treatment significantly reduced rat HMI and LVMI, reduced the levels of serum CK, LDH, cTnT, NT-proBNP, and also reduced the levels of serum sST2 and GDF-15, and reduced the expression of rat myocardial type I and type III collagen, which was effective reduce the fibrosis of myocardial collagen knot tissue and interstitial. The study further found that SMI can increase the expression of Bcl-2 protein, reduce the expression of Bax, Caspase-9, and Caspase-3 protein, and reduce the apoptotic index of cardiomyocytes.

Conclusion: The potential mechanism of SMI on cardiomyocytes from apoptosis induced by the DOX may be attributed to the regulation of miR-30a/beclin 1.

Introduction

Adriamycin (also known as doxorubicin, DOX) is a broad-spectrum and highly effective anti-tumor drug[1]. Since its discovery in the 1960s, it has been widely used in the treatment of various tumors, such as leukemia, breast cancer, and gastric cancer[2, 3]. However, cardiovascular complications limit the wide range of clinical applications of the DOX, manifested as dose-dependent myocardial toxicity, and ultimately lead to irreversible congestive heart failure (CHF)[4]. The mechanisms of myocardial injury induced by the DOX include myocardial cell apoptosis, autophagy, oxidative stress, and so on[4]. However, since cardiomyocytes were terminally differentiated cells and their ability to divide and regenerate after maturation was limited, cardiomyocyte apoptosis was considered to be the main reason for myocardial injury induced by the DOX[5, 6], but its specific molecular mechanism has not been fully elucidated. Further exploring the molecular mechanism of myocardial toxicity induced by the DOX and finding new therapeutic intervention targets is the key link to effectively reduce or avoid the limitations of the DOX clinical application.

Micro RNA (miRNA) is a type of small non-coding RNA that has been widely reported to participate in various biological processes such as cell proliferation, differentiation, apoptosis, and metabolism, and play a regulatory role in cell physiology and pathology[7]. Previous studies have shown that a variety of

miRNAs are involved in the process of regulating cardiomyocyte apoptosis[8]. For example, miR-181c-5p plays an important role in cardiomyocyte damage and apoptosis induced by myocardial ischemia-reperfusion (I/R)[9], miR-133 and miR-124 regulate cardiomyocyte apoptosis in acute myocardial infarction (AMI)[10, 11]. miR-30a was involved in the apoptosis process related to myocardial I/R, left ventricular insufficiency, and heart failure (HF) after AMI[12, 13]. In addition, studies have shown that miR-30a, miR-30c, and miR-30e were down-regulated in the HF induced by the DOX, and treatment with overexpression of miR-30e leads to down-regulation of Beclin 1 expression and protects primary cardiomyocytes from apoptosis[14]. Our previous research basis also found that overexpression of miR-30a in vivo can also down-regulate the expression of Beclin 1[15]. Therefore, the induction of miR-30a overexpression may play an important role in the apoptosis of cardiomyocytes induced by the DOX.

Shenmai Injection (SMI) is a patented Chinese medicine approved by the China Food and Drug Administration (CFDA). It is an extract containing *Panax ginseng* C.A.Mey and *Ophiopogon japonicus* (Linn.f.) Ker-Gawl[16]. Clinically, SMI was often used in the prevention and treatment of coronary atherosclerotic heart disease, chronic pulmonary heart disease, and viral myocarditis[17, 18], and when used in combination with chemotherapy drugs, it can reduce the damage of non-cancerous tissues[16]. A study showed that SMI can induce mitochondrial phagocytosis, regulate mitochondrial dynamics, inhibit excessive mitochondrial fission, and increase mitochondrial fusion to reduce myocardial I/R damage[19]. In addition, SMI can prevent cardiac dysfunction and apoptosis caused by the DOX by maintaining mitochondrial homeostasis, inhibiting mitochondrial oxidative stress, and mitochondrial fragmentation[16]. Our previous studies have shown that SMI can significantly promote the expression of miR-30a in vivo and reduced DOX-induced myocardial damage by inhibiting excessive autophagy of the myocardium[15], but its effect on the DOX-induced myocardial cell apoptosis needs to be further studied.

Materials And Methods

Experimental animals

Male Sprague-Dawley (SD) rats weighing 250 ± 20 g were purchased from the Spareford Biotechnology Co., Ltd. (Beijing, China, certificate number SCXK2019-0010). Animal experiments were performed and were approved by the Animals Research Committee at the Tianjin Medical University Cancer Institute and Hospital (No.LLSP2019-083). The animals received humane care throughout all the procedures following the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (Publication No. 85–23, revised 1996). The rats were allowed a 5 days acclimatization period before entry into any experimental protocol.

Drugs and Reagents

Doxorubicin hydrochloride for injection (Shenzhen Main Luck Pharmaceuticals Inc., Shenzhen, China, batch number of H44024359, DOX diluted with 0.9% normal saline at a ratio of 1:2 to a mass concentration of 0.5 mg/mL, and fully dissolved each time for use). Shenmai Injection (Chiatai Qingchunbao Pharmaceutical Co., Ltd., Hangzhou, China, Lot number: Z33020019). miR-30a agomir

freeze-dried powder (Synthesized by Suzhou GenePharma Co., Ltd., Jiangsu, China. miR-30a agomir freeze-dried powder was prepared with DEPC water to a 20 μ M stock solution for use). Van Gieson staining kit(Beijing leagene biotech.co.,ltd, China, DC0047),BCA protein concentration test kit (Boster Biological Technology Co., Ltd., Wuhan, China, AR0146), ECL Chemiluminescence Kit (Proteintech Group, Inc, USA, B500022), Bax antibody (Proteintech Group, Inc, USA, 50599-2-Ig), Bcl-2 antibody (Proteintech Group, Inc, USA, 12789-1-AP) Caspase-9 antibody (Proteintech Group, Inc, USA, 10380-1-AP) Caspase-3 antibody (Proteintech Group, Inc, USA, 19677-1-AP) Collagen I antibody (Proteintech Group, Inc, USA, 14695-1-A) Collagen III antibody (Proteintech Group, Inc, USA, 22734-1-AP) Horseradish Peroxidase AffiniPure Goat Anti-Rabbit IgG (H + L) (Proteintech Group, Inc, USA, SA00001-2). TUNEL kit (Roche Group, Inc, Switzerland, 11684817910).

Animal treatment

After the adaptation period, the rats were weighed and randomly divided into control group, model group, miR-30a agomir group, Shenmai injection low-dose group (SMI-L), and Shenmai injection high-dose group (SMI-H), each group has 8 animals, a total of 40 animals. Except for the control group, the rats in the other groups were a rat model of cardiotoxicity induced by tail vein injection of doxorubicin (DOX, tail vein injection, 2.5 mg/kg, once a week for 4 consecutive weeks)[20]. In the third week of modeling, each treatment group received the following treatments: the miR-30a agomir group, 20 nmol of miR-30a agomir was injected into the tail vein twice a week for 2 weeks; SMI-L group received intraperitoneal injection of 4.5ml/kg/time of the SMI, 6 times/week for 2 weeks; SMI-H group received 9ml/kg/times intraperitoneal injection of the SMI, 6 times/week for 2 weeks; the control group and model group were intraperitoneally injected with an equal volume of normal saline, and the experimental dosing regimen was as shown in the Fig. 1A.

After 2 weeks of intervention, the rats were induced and maintained anesthesia under 4% and 1.5% isoflurane, and the abdominal aorta was quickly exposed to collect blood. The upper serum was collected by centrifugation, transferred to a centrifuge tube, and stored in a refrigerator at -80°C for later use. After the blood was taken, the rats were fixed on the operating table supine, exposed to the chest cavity, and quickly removed the heart. The residual blood in the heart was washed with normal saline at 4°C and dried with filter paper. Thereafter, the shape and surface lesions of the heart were assessed and the heart mass (HM) was weighed after the tissues and large blood vessels around the heart were removed. Then, we removed the atrial appendage and the atrial tissue, except for the right ventricle. Finally, we weigh the left ventricle (left ventricular mass, LVM), and calculate the left ventricular mass index by body mass (BM) according to the following formula: $LVMI = LVM/BM$ (mg/g). We also calculated the heart mass index ($HMI = HM/BM$ (mg/g)). A part of the removed heart tissue sample was fixed in a 4% neutral formaldehyde buffer solution; the sample to be tested by Western Blot was wrapped in tin foil, and then stored in a freezing tube and quickly frozen in dry ice. After the experiment, the serum and heart tissue samples were immediately transferred to a -80°C refrigerator for storage.

Van Gieson (VG) staining of the myocardium

After 2 weeks of intervention, the rat heart was taken out, washed repeatedly with normal saline, fixed with 4% neutral formaldehyde, dehydrated with alcohol, and the heart tissue was routinely embedded in paraffin. The wax block was sliced routinely (thickness 5 μ m) and then stained with Weigert iron hematoxylin, acidic differentiation solution, and VG staining solution. Then, the differentiation was mounted and the myocardial tissue fibrosis was observed under an optical microscope (Myocardial cells can be seen in yellow, while collagen fibers are red).

Enzyme-linked immunosorbent assay (ELISA) to detect serum levels of myocardial injury markers

After 2 weeks of intervention, blood samples of rats were collected and centrifuged at 3000r/min for 15min, and the supernatant was collected. ELISA kit (Fankebio, Shanghai, China) was used to detect the levels of CK, LDH, cTnT, NT-proBNP, sST2, and GDF-15. The detection method was operated strictly following the kit instructions.

Western Blot to detect the expression of target protein in rat myocardium

The appropriate amount of myocardial tissue was homogenized in liquid nitrogen, and the lysate was added to extract the total tissue protein, and the protein concentration was determined by the BCA protein quantitative method. The 12% SDS-PAGE gel was used to separate the target protein, PVDF transfer membrane, 5% skimmed milk blocking solution was used to incubate for 2 h, and the diluted primary antibody Bax (1:2000), Bcl-2 (1:1000), Caspase-9 (1:1000), Caspase-3 (1:1000), Collagen I (1:1000), Collagen III (1:500), and β -actin (1:2000) were added, incubate overnight at 4°C. After washing the membrane, add horseradish peroxidase (HRP) labeled secondary antibody (1:2000), and incubate at 37°C for 1h. The ECL method was used for color development, and the gray value of the band was analyzed with Image-J software, and the gray ratio of the target protein band/ β -actin band was used as the final result.

TUNEL staining to detect the apoptosis of cardiomyocytes

After the rats were sacrificed, the heart samples were separated and fixed in 4% neutral formaldehyde, then embedded in paraffin and sectioned (5 μ m). Terminal deoxynucleotidyl transferase-mediated dextrouracil triphosphate nick end labeling (TUNEL) staining was performed using the TUNEL apoptosis detection kit following the manufacturer's instructions. The apoptotic nucleus was labeled with green fluorescein, and the nucleus of the entire cardiomyocyte was labeled with DAPI. The image of the heart tissue was observed by a fluorescence microscope and the apoptosis index (AI) was calculated (AI = TUNEL positive nucleus/DAPI stained nucleus).

Transmission electron microscope to observe the ultrastructural changes of myocardial tissue

The heart tissue was quickly cut and trimmed into 1mm³ size and immersed in 2.5% glutaraldehyde fixative. The fixed heart tissue was washed with phosphate-buffered saline (PBS), fixed with 1% osmium acid, dehydrated with concentration gradient acetone, embedded, ultra-thin sectioned, treated with uranium staining and lead staining. Finally, the images were observed and collected under the transmission electron microscope for analysis.

Statistics analysis

All the data are expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (One-Way ANOVA) was used for comparison between multiple groups. Values of $P < 0.05$ were considered statistically significant. In addition, all statistical analyses were performed using GraphPad Prism 8.3 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Effect of SMI on the body mass and general morphology of the heart of rats

The rats in the model group were lethargic, slow to respond, and their body weight was significantly lower than that in the control group ($P < 0.01$). The rats in the miR-30a agomir and SMI treatment groups had better mental status, sensitive responses, and no significant changes in body weight compared with the model group ($P > 0.05$), but the bodyweight of treatment groups had an upward trend, as shown in the Fig. 1B. The heart of rats in the model group was larger than that of the control group, and the HMI and LVMI were significantly increased ($P < 0.01$); the miR-30a agomir and SMI groups had less heart disease, and the HMI and LVMI were reduced ($P < 0.01$ or $P < 0.05$), as shown in the Fig. 1C-E.

Effects of SMI on serum CK, LDH, cTnT, NT-proBNP in rats

The levels of CK, LDH, cTnT, and NT-proBNP in the model group were higher than those in the control group ($P < 0.01$), suggesting that the rats in the model group have a myocardial injury and cardiac dysfunction. The miR-30a agomir and SMI can improve the heart function of rats, reduce myocardial damage, reduce the levels of CK, LDH, cTnT, and NT-proBNP ($P < 0.01$ or $P < 0.05$), and have a myocardial protective effect. Moreover, the SMI-L in the Chinese medicine group was significant, as shown in the Fig. 2.

Effect of SMI on myocardial histology in rats

Through observation under an optical microscope, we found that the myocardium of the control group had fewer collagen fibers, while the myocardium of the model group had a significant increase in red collagen fibers than the control group, suggesting that the DOX can cause myocardial collagen knot tissue hyperplasia and interstitial fibrosis in rats. The miR-30a agomir and SMI treatment can alleviate the cardiomyopathy caused by the DOX, and the Chinese medicine group has a significant effect at low doses, as shown in the Fig. 3.

Effect of SMI on myocardial collagen in rats

The level of serum sST2 and GDF-15 in the model group increased, and the expression of myocardial collagen I and collagen III increased significantly ($P < 0.01$). After treatment with miR-30a agomir and SMI, the levels of serum sST2 and GDF-15 in rats decreased, and the expression of the collagen I and III decreased ($P < 0.01$ or $P < 0.05$). Moreover, the effect of traditional Chinese medicine is significant with SMI-L, suggesting that miR-30a agomir and SMI treatment can reduce collagen deposition in rats and have a myocardial protective effect. And the effect of SMI-L is more significant in the Chinese medicine group, as shown in the Fig. 4. It shows that miR-30a agomir and SMI treatment can reduce the deposition of collagen in rats and have a cardioprotective effect.

Effect of SMI on cardiomyocyte apoptosis in rats

A small amount of normal cardiomyocyte apoptosis can be seen in normal rats, and the apoptotic index is at a low level. In the model group treated by the DOX, a large number of myocardial cell apoptosis was found, the myocardial arrangement was disordered, and the nucleus was round or granular, suggesting that the nuclear chromatin was concentrated, marginalized, isolated, and scattered, and the apoptosis index higher ($P < 0.01$). After miR-30a agomir and SMI treatment, the above-mentioned myocardial changes were all relieved, and the myocardial cell apoptosis index decreased ($P < 0.01$ or $P < 0.05$). And the effect of SMI-L is more significant in the Chinese medicine group, as shown in the Fig. 5A-B.

Transmission electron microscopy showed that the nucleus of the control group was in a long spindle shape, the nuclear membrane was intact, the chromatin was evenly arranged, some nucleoli were visible, and there was no apoptosis. Compared with the control group, the nucleus of the model group was irregular, the chromatin was marginalized, and the nuclear membrane was partially separated, showing a state of apoptosis. The nucleus morphology of the miR-30a agomir group and SMI-L group was relatively regular, and chromatin margination was significantly reduced, and nuclear membrane separation was not observed. The nuclear membrane separation state of the SMI-H group was weakened and apoptosis was still seen, as shown in the Fig. 5C.

Effects of SMI on the expression of Bax, Bcl-2, Caspase-9, and Caspase-3 apoptosis-related proteins in rat myocardium

The expression of pro-apoptotic protein Bax in the model group was up-regulated ($P < 0.01$), the expression of anti-apoptotic protein Bcl-2 was down-regulated ($P < 0.05$), and the expression of apoptotic effector proteins Caspase-9 and Caspase-3 increased compared with the control group ($P < 0.01$), suggesting that apoptosis was activated. miR-30a agomir and SMI group can down-regulate the expression of Bax protein ($P < 0.01$ or $P < 0.05$), up-regulate the expression of Bcl-2 protein ($P < 0.01$ or $P < 0.05$), and inhibit the expression of Caspase-9 and Caspase-3 proteins ($P < 0.01$ or $P < 0.05$), suggesting

that the SMI group can inhibit myocardial cell apoptosis, and SMI-L group has a significant effect in the Chinese medicine, as shown in the Fig. 6.

Discussion

DOX is currently one of the most commonly used anthracycline anti-tumor drugs and is widely used to treat various malignant tumors, including hematological malignancies and solid tumors. However, the use of DOX was often restricted due to left ventricular dysfunction, and even cardiotoxic reactions such as dilated cardiomyopathy and CHF[21]. At present, the pathogenesis of cardiotoxicity caused by the DOX is not fully understood. Traditional studies believed that the DOX produces semiquinone free radicals due to metabolism in the body, which in turn leads to oxidative damage to cardiomyocytes[22]. However, recent studies have found that apoptosis is still an important cause of myocardial damage caused by the DOX[5, 6]. Therefore, it is urgent to find drugs that can alleviate the apoptotic damage of cardiomyocytes caused by the DOX without affecting the use of anthracyclines. It is also a new strategy to improve the survival rate of cancer patients and improve the long-term quality of life of patients.

SMI is a form of modern Chinese medicine, derived from the classic prescription Shengmai San[23]. In recent years, it has been proven that SMI can improve the efficacy of anti-tumor drugs and reduce their toxicity[24]. Our previous studies have also shown that SMI has the effect of reducing the myocardial damage caused by the DOX. It mainly promotes the expression of miR-30a, which leads to the decrease of Beclin 1 expression, inhibits the excessive autophagy of the myocardium, and protects the myocardium from the damage of the DOX[15]. Beclin 1 is an important target downstream of miR-30a[25]. As we all know, Beclin 1 is a key node in the crosstalk between autophagy and apoptosis. On the one hand, Beclin 1 is a component of the class III PI3K/Vps34 complex and is essential for the formation of autophagic vesicles. On the other hand, Beclin 1 binds to the anti-apoptotic protein Bcl-2, leading to the dissociation of the pro-apoptotic protein Bax, which initiates the apoptosis cascade[26, 27]. Therefore, the down-regulation of Beclin 1 expression is consistent with previous related research results and helps protect cardiomyocytes from apoptosis[14]. And a related study has also shown that SMI can alleviate myocardial endoplasmic reticulum stress and caspase 12 dependent apoptosis, and play a role in protecting the myocardium[23]. However, whether SMI also exerts an anti-cardiomyocyte apoptosis effect through miR-30a/Beclin 1 in cardiotoxicity induced by the DOX remains to be further studied.

To verify the protective mechanism of SMI on the myocardial injury induced by the DOX, we used rat tail vein injection of the DOX to replicate the DOX cardiotoxicity model. Since anthracycline anti-tumor drugs can damage myocardial cell membranes and cause myocardial cell damage, the markers of myocardial injury are of great significance for diagnosing the cardiotoxicity of anthracyclines. Cardiac enzymes, troponin, and brain natriuretic peptides were traditional biomarkers for detecting myocardial injury[28]. Creatine kinase (CK) and lactate dehydrogenase (LDH) were also widely used in the detection of cardiotoxicity of anticancer drugs[29, 30]. Troponin can be used as an independent predictor of cardiotoxicity after chemotherapy, troponin T (cTnT), and N-terminal pro-brain natriuretic peptide (NT-proBNP) can be used as an important marker for the evaluation of early myocardial dysfunction in

cardiotoxicity induced by the anthracyclines[31]. However, studies have shown that myocardial enzymes, cTnT, and NT-proBNP still have certain differences in predicting and diagnosing cardiotoxicity[32, 33], and the combined application can further clarify the existence of cardiotoxicity. In this study, we found that the serum CK, LDH, cTnT, and NT-proBNP levels of rats in the model group increased, while miR-30a agomir and SMI treatment groups could reduce the rat serum CK, LDH, cTnT and NT-proBNP levels. Moreover, the SMI-L effect of the Chinese medicine treatment group was significant. The above results suggest that SMI can reduce the myocardial damage caused by DOX and improve the heart function of rats.

The changes in the rat myocardial tissue were further detected and observed under an optical microscope that the red collagen fibers in the myocardium of the model group increased significantly compared with the control group, indicating that the DOX caused the proliferation of the myocardial collagen knot tissue and interstitial fibrosis in the rat lesions. While miR-30a agomir group and SMI treatment group significantly alleviated myocardial fibrosis induced by the DOX in rats. The soluble ST2 (sST2), a member of the family of interleukin-1 receptors, was produced by cardiomyocytes and cardiac fibroblasts and was associated with structural and functional changes of left ventricular caused by myocardial damage, fibrosis, and poor remodeling, and can be used as an early sensitive predictor of left ventricular dysfunction caused by anthracycline drugs[34, 35]. Growth differentiation factor-15 (GDF-15) was a member of the transforming growth factor- β superfamily and was closely related to collagen renewal, deposition, and the degree of cardiac fibrosis[36]. Under normal circumstances, GDF-15 was weakly expressed by cardiomyocytes, but it was induced to be expressed and secreted under conditions of myocardial ischemia, tissue damage, oxidative stress, and inflammation. It was a predictor of a variety of cardiovascular adverse events and mortality risk assessment and has diagnostic value in oncology drug cardiotoxicity[37–39]. The main characteristics of myocardial pathological response are myocardial fibrosis, collagen deposition, and the increase in the synthesis of type I collagen and type III collagen, leading to cardiac dysfunction[40]. In the cardiotoxicity induced by the DOX, we found that SMI can reduce the levels of serum sST2 and GDF-15 in rats, and reduce the expression of Collagen I and Collagen III, indicating that SMI can reduce the cardiac fibrosis induced by the DOX and reduce collagen deposition.

Cardiomyocyte apoptosis was considered to be an important mechanism of DOX cardiotoxicity[5, 6]. Apoptosis can remove damaged aging or harmful cells to maintain cell homeostasis[41], while cardiomyocytes are non-renewable cells, and cardiomyocyte apoptosis was the main feature of myocardial injury[4]. The gold standard of the transmission electron microscope was used to detect apoptosis[42], and the phenomenon of the disappearance of microvilli, condensation of chromatin, expansion of endoplasmic reticulum, the disappearance of nuclear fragmentation, and formation of crescent apoptotic bodies was observed under the electron microscope[43]. This study found that miR-30a agomir and SMI can alleviate the apoptosis of the rat cardiomyocytes and reduce the apoptosis index of cardiomyocytes. Apoptosis was the result of multi-molecule co-regulation, among which the Bcl-2 family and the Caspase family are two important parts of regulating apoptosis. The Bcl-2 protein family includes both Bax pro-apoptotic members and Bcl-2 anti-apoptotic members, which were closely related

to the Caspase family[44]. Bcl-2 can regulate cell apoptosis through Caspase-9 and Caspase-3 dependent pathways, which means that by increasing Bax and reducing Bcl-2 expression, it can stimulate mitochondria to release cytochrome C, and cytochrome C further recruits Caspase-9 to promote the formation of apoptotic bodies, and then induces the activation of Caspase-3 and triggers the Caspase cascade, which ultimately leads to apoptotic cell death[45, 46]. Bcl-2 can be used as the upstream regulatory mechanism of Caspase. Overexpression of Bcl-2 can effectively inhibit Caspase activation and apoptosis induced by various factors. In this study, we found that SMI can increase the expression of anti-apoptotic protein Bcl-2 and decrease the expression of pro-apoptotic protein Bax, thereby inhibiting the expression of Caspase-9 and Caspase-3, suggesting that SMI can inhibit rat cardiomyocyte apoptosis. In summary, combined with our previous research results, it is suggested that SMI can increase the expression of miR-30a and decrease the expression of Beclin 1. It can not only inhibit the excessive autophagy of the myocardium but also reduce the myocardial damage caused by DOX by inhibiting the apoptosis of myocardial cells.

Conclusions

In summary, this study explored the potential effects and underlying mechanisms of SMI on myocardial injury induced by DOX in rats. Our research results show that SMI can reduce HMI and LVMI, reduce the levels of serum markers of myocardial injury, such as CK, LDH, cTnT, NT-proBNP, sST2 and GDF-15, and effectively reduce the fibrosis of myocardial collagen nodules and interstitium. Further research found that SMI can increase the expression of anti-apoptotic proteins, reduce the expression of pro-apoptotic proteins, and reduce the apoptotic index of cardiomyocytes. Combined with our previous research evidence, SMI is most likely to regulate cardiomyocyte apoptosis through miR-30a/beclin1.

Abbreviations

SMI: Shenmai Injection, DOX: doxorubicin, I/R: ischemia-reperfusion, AMI: myocardial infarction, HF: heart failure, CHF: congestive heart failure, CFDA: China Food and Drug Administration, HM: heart mass, LVM: left ventricular mass, BM: body mass.

Declarations

Acknowledgments

Not applicable.

Authors' contributions

LLT and ZNX designed the study. ZNX and LYY performed the experiments. ZNX, LSC and JQJ contributed to the analysis of data. ZNX wrote the manuscript. ZWQ, ZYP and ZJP reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All of the procedures were reviewed and approved by Animal experiments were performed and were approved by the Animals Research Committee at the Tianjin Medical University Cancer Institute and Hospital (No.LLSP2019-083) before and during the experiment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Effect of Shenmai injection (SMI) on the body mass and general morphology of the heart of rats. (A) Flow chart of the experimental dosing regimen. (B) Changes in body mass of rats in each group. (C) The general morphology of the heart of rats. (D) Heart mass index of rats in each group. (E) Left ventricular mass index of rats in each group. Data are expressed as mean $\pm$ SD, * P <0.01, # P <0.05.

Figure 2

Effects of Shenmai injection (SMI) on serum CK, LDH, cTnT, NT-proBNP in rats. (A) The serum CK content of rats in each group. (B) The serum LDH content of rats in each group. (C) The serum cTnT content of rats in each group. (D) The serum NT-proBNP content of rats in each group. Data are expressed as mean $\pm$ SD, * $P < 0.01$, # $P < 0.05$.

Figure 3

Effect of Shenmai injection (SMI) on pathological changes of the myocardium. Representative photomicrograph of the Van Gieson (VG) staining of the myocardium ($\times 400$).

Figure 4

Effect of Shenmai injection (SMI) on myocardial collagen in Rats. (A) Collagen I and Collagen III expression in the myocardium of rats in each group as detected by western blot. (B) The relative protein level of Collagen I in the myocardium. (C) The relative protein level of Collagen III in the myocardium. (D) The serum sST2 content of rats in each group. (E) The serum GDF-15 content of rats in each group. Data are expressed as mean $\pm$ SD, * P <0.01, # P <0.05.

Figure 5

Effects of Shenmai injection (SMI) on myocardial apoptosis in Rats. (A) Apoptotic morphological changes of rat cardiomyocytes by TUNEL staining ($\times 400$). (B) Apoptotic index (AI) of myocardial cell in each group. (AI=TUNEL positive nucleus/DAPI stained nucleus). Data are expressed as mean $\pm$ SD, $*P<0.01$, $\#P<0.05$. (C) Transmission electron micrographs of rat myocardium in each group, with a scale of $1\mu\text{m}$.

Figure 6

Effects of Shenmai injection (SMI) on Bax, Bcl-2, Caspase-9, and Caspase-3 protein expression. (A) Bax, Bcl-2, Caspase-9, and Caspase-3 expression in the myocardium of rats in each group as detected by western blot. (B) The relative protein level of Bax in the myocardium. (C) The relative protein level of Bcl-2 in the myocardium. (D) The relative protein level of Caspase-9 in the myocardium. (E) The relative protein level of Caspase-3 in the myocardium. Data are expressed as mean $\pm$ SD, * $P < 0.01$, # $P < 0.05$.