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Theacrine attenuates myocardial fibrosis after myocardial infarction via the SIRT3/β-catenin/PPARγ pathway in estrogen-deficient mice

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Abstract. – OBJECTIVE: To investigate the role of theacrine in the protection of ventricular remodeling and chronic heart failure after myocardial infarction in the estrogen-deficient mice.

MATERIALS AND METHODS: Female C57BL/6 mice aged 8 weeks old were selected and then subjected to bilateral oophorectomy. At 7 days after surgery, the models of the myocardial infarction were established by ligating the anterior descending coronary artery. On the first day after myocardial infarction, Theacrine (20 mg/kg) was administered via gavage for continuous 28 days. Thereafter, the cardiac function in each group of mice was detected via cardiac ultrasonography for small animals at 7, 14, and 28 days after surgery. The mice were sacrificed after 28 days. The infarct size of mice was determined through 2,3,5-triphenyltetrazolium chloride (TTC) and Evan blue double staining assay, while the myocardial fibrosis was assessed via Masson staining assay. The expression levels of collagen-related proteins Collagen I, Collagen III, alpha-smooth muscle actin (α-SMA), and the transforming growth factor-β (TGF-β) were measured by Western blotting (WB). The terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining assay was applied to evaluate the myocardial apoptosis, and the WB was employed to detect apoptosis-associated proteins. The expression level of silent information regulator 2 homologue 3 (SIRT3) protein was detected by immunohistochemistry, and the expression levels of SIRT3, β-catenin and peroxisome proliferator-activated receptor gamma (PPARγ) protein were measured via WB.

RESULTS: Compared with those in the Sham group, the ejection fraction (EF) and fractional shortening (FS) in estrogen-deficient mice were significantly lowered, the myocardial fibrosis and myocardial apoptosis were clearly aggravated, and the SIRT3 expression was decreased at 28 days after myocardial infarction. The theacrine could improve the cardiac function after the myocardial infarction in estrogen-deficient mice and relieve both myocardial fibrosis and myocardial apoptosis during chronic remodeling after myocardial infarction in estrogen-deficient mice. After the intervention with theacrine, the estrogen-deficient mice with myocardial infarction had up-regulated SIRT3 and PPARγ levels and a reduced β-catenin level in the heart.

CONCLUSIONS: Theacrine is able to activate SIRT3 and repress myocardial fibrosis and apoptosis after myocardial infarction in ovariectomized mice, thereby improving the cardiac function of ovariectomized mice with myocardial infarction through the possible downstream signal pathway β-catenin/PPARγ.

Key Words
Theacrine, Myocardial infarction, Estrogen-deficient, Myocardial fibrosis, SIRT3/β-catenin/PPARγ.

Introduction

Acute myocardial infarction is one of the most severe coronary heart diseases[1]. The mortality rate of coronary heart diseases shows a decreasing tendency in the past decade due to changes in evidence-based therapeutic methods and lifestyles, but myocardial infarction is still a global disease seriously endangering human health[2]. Moreover, the incidence and mortality rates of the coronary heart diseases are evidently increased in elderly

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women because of their decreased estrogen levels after menopause[3,4]. Once myocardial infarction is detected, the mortality rate for women is significantly higher than that for men[5,6]. Since the risks for cardiovascular diseases, as well as venous thrombosis and stroke, are increased after estrogen replacement therapy, estrogen is not recommended as a measure to prevent and treat coronary heart diseases in postmenopausal women at present[7,8]. Therefore, it is particularly important to find effective drugs for preventing and treating coronary heart diseases in postmenopausal women.

More attention has been paid to the left the ventricular remodeling after myocardial infarction[9-11]. Left ventricular remodeling severely weakens the left ventricular function in patients with myocardial infarction and significantly increases the complication and the mortality rates in patients[12,13]. Myocardial fibrosis, whose extent is one of the main factors determining the prognosis in patients with cardiovascular diseases, is an important feature of myocardial remodeling[12]. Sirtuins, a class of NAD+-dependent deacetylases, play vital roles in regulating cell growth, metabolism, senescence, stress, and apoptosis[14]. Among them, the silent information regulator 2 homologue 3 (SIRT3) is important for the prevention and treatment of cardiovascular diseases, and it is able to suppress the myocardial apoptosis and delay the cardiac hypertrophy and heart failure[15,16]. Besides, SIRT3 exerts an important protective effect on organ fibrosis[17,18]. The overexpression of SIRT3 protects bleomycin-induced pulmonary fibrosis[19]. SIRT3-KO mice spontaneously have myocardial fibrosis at the 8th week after birth[20]. Resveratrol activates SIRT3 and inhibits myocardial fibrosis by regulating the transforming growth factor-β (TGF-β)/SMAD signaling pathway, thereby improving cardiac function[21].

Theacrine, namely 1,3,7,9-tetramethyluric acid (molecular formula: $C_9H_{12}N_4O_3$), is a purine alkaloid, which has a similar structure to that of caffeine. Studies[22,23] on the pharmacological activity of theacrine mainly focus on its regulatory role in the central nervous system. In recent years, a research[24] has discovered that theacrine activates SIRT3 protein, and is a novel SIRT3 agonist. So far, the effect of theacrine on myocardial infarction is not reported. Therefore, in this work, the models of the myocardial infarction were established in estrogen-deficient mice to explore the influence of theacrine on cardiac function and myocardial remodeling and investigate its possible mechanism of action.

Materials and Methods

Laboratory Animals and Models

The subjects of this study were female C57BL/6 mice aged 8 weeks old and weighing 18-20 g, purchased from the Model Animal Research Center of Weifang Medical University. They were housed in the Specific Pathogen Free (SPF)-level animal room. Experimentation on animals was approved by the Animal Ethics Committee of Weifang Medical University. Bilateral oophorectomy: a mouse was anesthetized via intraperitoneal injection of 400 mg/kg chloral hydrate and then fixed on an operating table, followed by the disinfection of the skin. Thereafter, with the intersections of the mouse's right and left thigh root connection lines and the median line on the back as starting points, the surgical incisions (about 1 cm) directing to the head were made. Taking the right ovary as an example, the skin incision was pulled to the right side by about 0.5 cm. The ovary is located in the fat mass next to the lower kidney. Then, the muscle layer on the surface of the fat mass was bluntly separated, and the ovary was resected. Next, the left ovary was removed in the same way. After that, the skin was sutured, and the mouse was placed on a warm table for recovery. Establishment of myocardial infarction models: a mouse was intraperitoneally injected with 400 mg/kg chloral hydrate for anesthetization, and then fixed on the operating table in the supine position. After that, peroral non-invasive endotracheal intubation was conducted using a self-made trachea cannula, and a ventilator was connected to control breathing (frequency: 90-100 breaths/min, tidal volume: 0.4-0.5 mL). Thereafter, the surgical area was disinfected, and a transverse incision was made on the skin in the left fourth intercostal space, followed by blunt dissection of the pectoralis major and anterior serratus to expose the heart. Next, the pericardium was cut to expose the left auricle and the left ventricle, and a 7-0 non-invasive surgical suture fine needle with thread was used to suture and ligature the coronary artery at 2-3 mm below the lower edge of the left auricle. After the ligation succeeded, it could be observed that the cardiac muscle in the ligated area became white. The chest was closed layer by layer, and the surgical incision was applied externally with erythromycin eye ointment, followed by observation. The tracheal intubation was removed after waking up, and then the mouse was put back to the rearing cage. On the first day after myocardial infarction, Theacrine (20 mg/kg) was administered via gavage for continuous 28 days.

Determination of Cardiac Structure and Cardiac Function in Mice via Echocardiography

Cardiac function was detected using a Vevo 2100 high-resolution ultrasound imaging system for small animals (probe frequency: 35 Hz, penetration depth: 10-15 mm). A mouse was anesthetized with 2% isoflurane, fixed on a constant temperature heating plate, and subjected to depilation of the left chest and then ultrasonic examination. The probe was placed on the left chest of the mouse, 2D ultrasound displayed the left ventricular short axis view, M mode ultrasound was employed to record the left ventricular motion at the papillary muscle level and measure heart rate (HR), left ventricular end diastolic diameter (LVEDd), left ventricular end systolic diameter (LVESd), and interventricular septum (IVS) and left ventricular posterior wall (LVPW) thicknesses, and the system automatically calculated the ejection fraction (EF) and fractional shortening (FS).

Masson Staining

The paraffin sections were deparaffinized, washed, and stained with hematoxylin staining solution for 5-10 min. Next, the sections were stained with Masson Ponceau acid fuchsin solution for 5-10 min, followed by immersion cleaning in 2% glacial acetic acid aqueous solution for a while, differentiation with 1% phosphomolybdic acid aqueous solution for 3-5 min, and staining with aniline blue or light green solution for 5 min. Lastly, the sections were blocked with neutral gum and observed using a microscope.

2,3,5-Triphenyltetrazolium Chloride (TTC) and EVENS Blue Double Staining

After injecting 2-3 mL 3% EVENS blue staining solution into the jugular vein, the heart was removed, rinsed and weighed. Next, it was frozen at -20°C for 20 min and cut into 5 sections (about 1-2 mm in thickness). Thereafter, the sections were subjected to water bath in 1% TTC phosphate buffer (pH 7.4) at 37°C for 15 min (protected from light and shaken) and fixed with 10% formalin. Lastly, a fluorescence microscope was utilized for photographing.

Terminal Dexynucleotidyl Transferase (TdT)-Mediated dUTP Nick End Labeling (TUNEL) Staining

A TUNEL assay kit (Roche, Basel, Switzerland) was used for staining as follows: the sections were washed with xylene twice (5 min/time), immersed with gradient ethanol for 3 min, and treated with Proteinase K working solution for 15-30 min. Then, the TUNEL reaction mixture was prepared. Next, 50 μL TUNEL reaction mixture was added to the sample, followed by reaction in a dark wet box at 37°C for 60 min. Lastly, the sections were blocked with a drop of glycerin and observed using an optical microscope.

Immunohistochemistry

The mice were anesthetized and then subjected to perfusion fixation with 4% paraformaldehyde. Next, the heart was taken out, dehydrated with saccharose, embedded with OTC, and sliced using a freezing microtome (the thickness of sections was 8 μm). After that, the sections were diluted with primary antibody at 1:150, followed by immunohistochemical staining (DAB assay). The pictures of immunohistochemical results were collected and subjected to quantitative analysis using a Mode Images Advanced 3.2 image analysis system.

Western Blotting (WB)

The total proteins were extracted using a whole protein extraction kit (KeyGen Biotech, Nanjing, China) from myocardial tissues, and the concentration was detected using a bicinchoninic acid (BCA) protein concentration detection kit (Thermo Fisher, Waltham, MA, USA). Then, the proteins were added with loading buffer and boiled in boiling water for 5 min for denaturation. Next, 10 μL protein sample was taken for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the protein sample was transferred onto a membrane at a constant current of 300 mA, blocked with 0.5% skim milk at room temperature for 2 h, and incubated with target protein primary antibody at 4°C overnight. On the next day, the membrane was taken out, washed with Tris-Buffered Saline Tween 20 (TBST) for 3 times (5 min/time), incubated with secondary antibody at room temperature for 2 h, washed with TBST again for 3 times (5 min/time), and exposed.

Statistical Analysis

All experimental results were repeated at least three times, with more than 3 parallel values set in each group, analyzed using Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 software (La Jolla, CA, USA), and expressed as mean ± standard deviation. The unpaired *t*-test was employed for two sets of statistics. The comparison between multiple groups was done using One-way

ANOVA test followed by the post-hoc test (Least Significant Difference). The Bonferroni test was used for multiple sets of statistics. $p<0.05$ suggested that the difference was statistically significant.

Results

Theacrine Improved Cardiac Function in Estrogen-Deficient Mice With Myocardial Infarction

The changes in systolic function were detected using the high-resolution ultrasound imaging system for small animals at 7, 14, and 28 days after surgery, and it was found that at 7 days after surgery, the mice in the myocardial infarction group significantly increased LVESd and LVEDd and clearly reduced FS and EF, compared with those in the Sham group. At 28 days after surgery the Sham group had slightly lowered LVESd and LVEDd and no significantly changed FS and EF compared with the previous values, while the myocardial infarction group showed continuously increased LVESd and LVEDd and decreased FS and EF. After 7 days of theacrine intervention, the LVESd and LVEDd in mice were evidently decreased, and the FS and EF were overtly increased compared with those in the myocardial infarction group, showing statistically significant differences. After 28 d of theacrine intervention, compared with those in the myocardial infarction group, the LVESd and LVEDd in mice continued to decrease, and the FS and EF displayed continuous increases (Figures 1A-1D). At 28 days after myocardial infarction, the TTC and Evan blue double staining assay were performed to detect the infarct size of mice, with blue for viable myocardium, red for post-ligation danger area, and white for infarct area. The results showed that after 28 days of intervention with theacrine, the myocardial infarct size was not significantly different from that in myocardial infarction group, suggesting that theacrine intervention has no significant effect on myocardial infarct size (Figure 1E).

Theacrine Attenuated Myocardial Fibrosis During Chronic Remodeling After Myocardial Infarction in Estrogen-Deficient Mice

The mice were sacrificed at 28 days after myocardial infarction surgery. Then, the whole heart was taken, fixed, and embedded. Next, the whole heart was sectioned from the surgical ligation plane

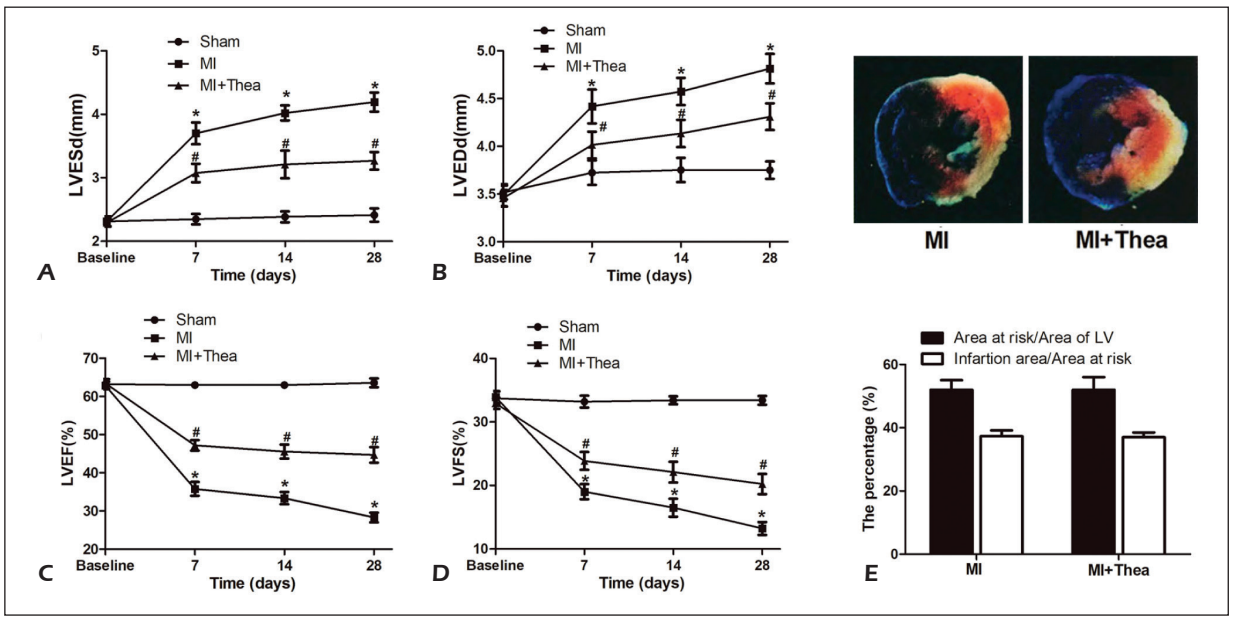

Figure 1. Theacrine improved cardiac function in estrogen-deficient mice with myocardial infarction. **A**, Analysis of left ventricular end-systolic dimension (LVESd) in estrogen-deficient mice with MI. **B**, Analysis of left ventricular end-diastolic dimension (LVEDd) in estrogen-deficient mice with MI. **C**, Analysis of left ventricular ejection fraction (LVEF) in estrogen-deficient mice with MI. **D**, Analysis of left ventricular fractional shortening (LVFS) in estrogen-deficient mice with MI. **E**, TTC and Evan blue double staining assay showed that theacrine has no significant effect on myocardial infarct size (magnification: 10×). *$p<0.05$ *vs.* Sham group, $^{\#}p<0.05$ *vs.* MI group.

to the cardiac apex. The myocardial fibrosis was detected via Masson staining, with blue for fibrosis (Figure 2A). ImageJ was used to measure the fibrosis area and the left ventricular area, and the ratio of fibrosis area to the left ventricular area was utilized to evaluate the severity of fibrosis (Figure 2B). It was discovered that theacrine relieved the myocardial fibrosis after myocardial infarction. The proteins were extracted from tissues around the infarct. WB was carried out to detect the collagen deposition-related markers Collagen I, Collagen III, and alpha-smooth muscle actin (α-SMA), and the results revealed that the Collagen I, Collagen III, and α-SMA were significantly higher in the myocardial infarction group than those in the Sham group, while they were decreased after theacrine intervention, proving that theacrine is capable of alleviating collagen deposition during chronic remodeling after myocardial infarction. The determination of fibrosis-associated protein TGF-β revealed that the expression level of TGF-β in mice was increased in myocardial infarction group compared with that in the Sham group, while such an increase was notably inhibited in the theacrine treatment group (Figures 2C, 2D).

Theacrine Relieved Myocardial Apoptosis During Chronic Remodeling After Myocardial Infarction in Estrogen-Deficient Mice

The determination of myocardial apoptosis in the marginal zone of myocardial infarction via TUNEL assay revealed that no myocardial apoptosis was found in estrogen-deficient mice in the Sham group. At 28 days after myocardial infarction in estrogen-deficient mice, the number of apoptotic cardiomyocytes in the marginal zone of myocardial infarction was significantly more than that in the Sham group, and it was remarkably reduced in the theacrine group (Figure 3A). WB was used to detect the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) and the pro-apoptotic protein Bcl-2 associated X protein (Bax), and the results showed that in comparison with the Sham group, the myocardial infarction group exhibited evidently elevated pro-apoptotic protein Bax and overtly declined anti-apoptotic protein Bcl-2 and Bcl-2/Bax ratio (Figures 3B-3D). The above protein changes were reversed after theacrine intervention, indicating that theacrine can alleviate myocardial apoptosis after myocardial infarction.

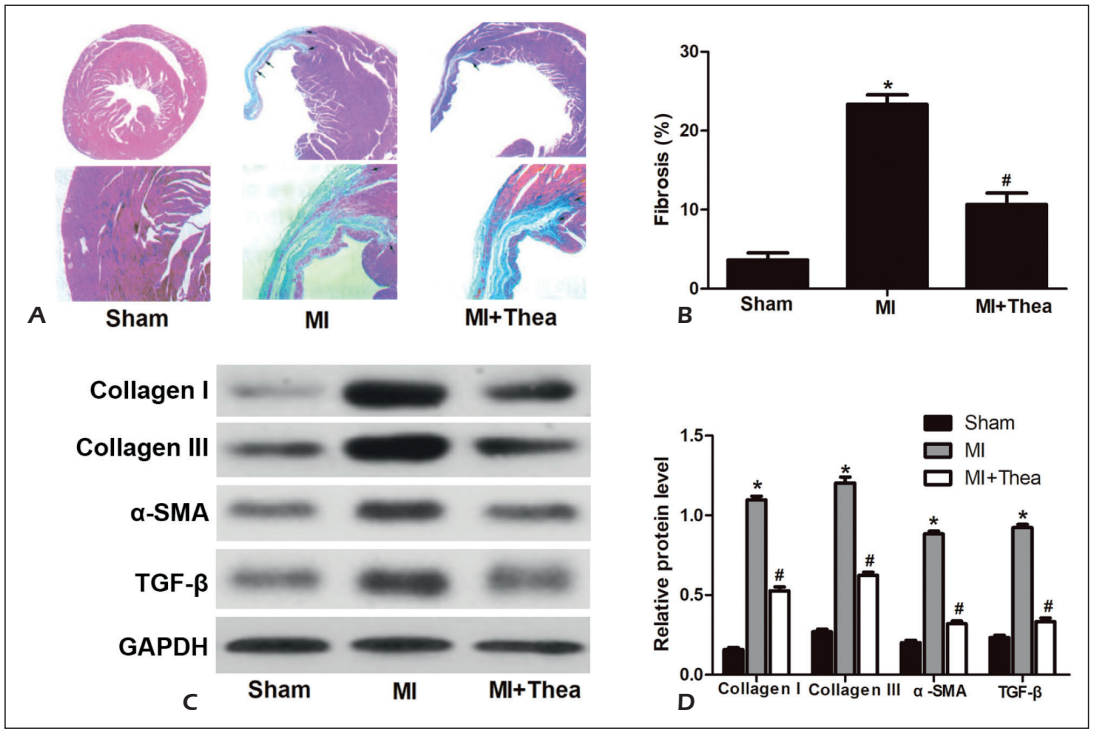

Figure 2. Theacrine attenuated myocardial fibrosis during chronic remodeling after myocardial infarction in estrogen-deficient mice. **A**, Representative images of myocardial fibrosis by Masson staining (magnification: 100×). **B**, Analysis of fibrosis area in different groups. **C**, Western blots analysis indicated the expression of Collagen I, Collagen III, α-SMA, and TGF β in estrogen-deficient mice with MI. **D**, Semi quantitative analysis of Collagen I, Collagen III, α-SMA, and TGF β in estrogen-deficient mice with MI. *$p<0.05$ *vs.* Sham group, $^{\#}p<0.05$ *vs.* MI group.

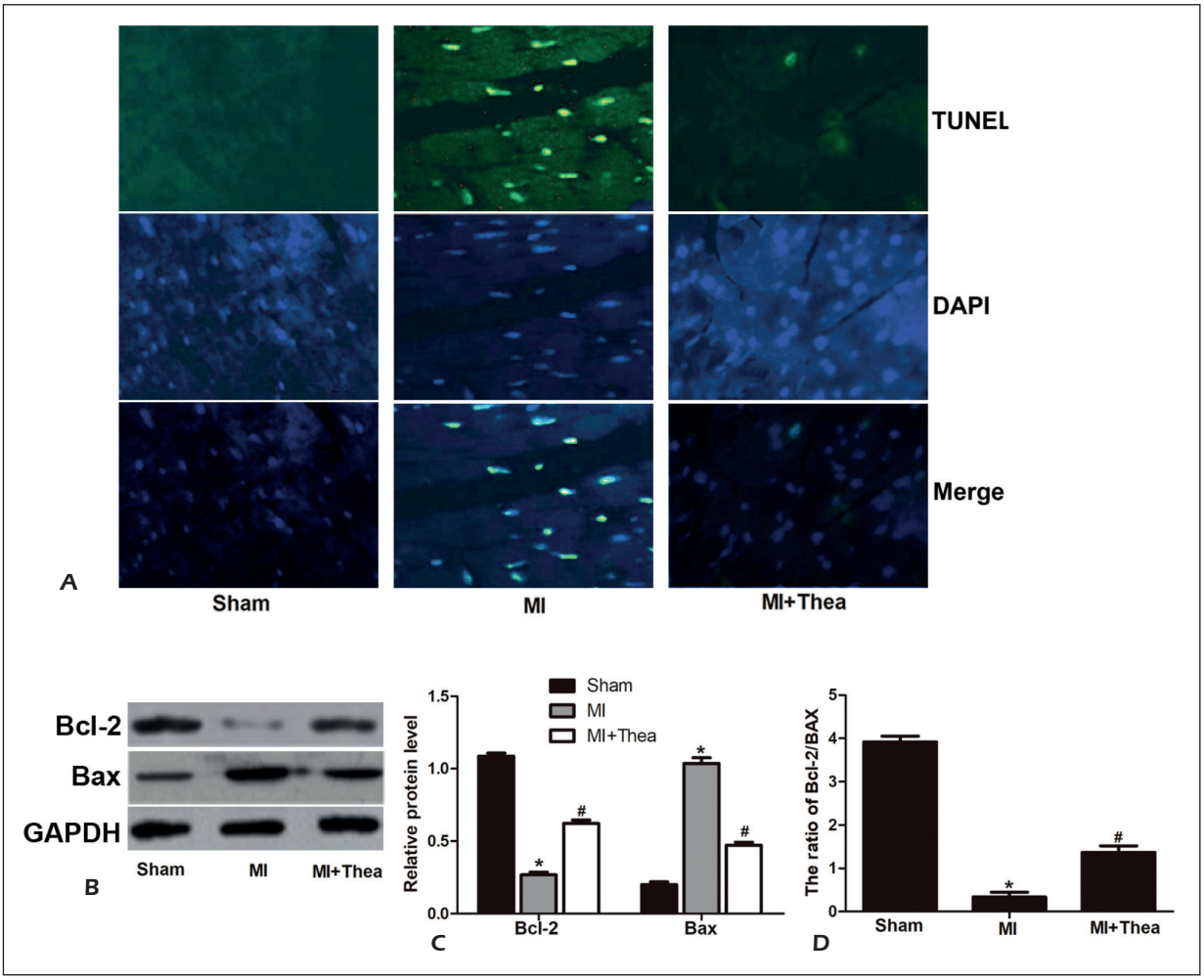

Figure 3. Theacrine relieved myocardial apoptosis during chronic remodeling after myocardial infarction in estrogen-deficient mice. **A**, Representative images of myocardial apoptosis by TUNEL staining (magnification: 40×). **B**, The Western blots analysis indicated the expression of Bcl-2 and Bax in estrogen-deficient mice with MI. **C**, Semi quantitative analysis of Bcl-2 and Bax in estrogen-deficient mice with MI. **D**, Analysis of the ratio of Bcl-2/Bax. *$p<0.05$ *vs.* Sham group, $^{\#}p<0.05$ *vs.* MI group.

Theacrine Up-Regulated SIRT3 Level, Decreased β-Catenin Level and Increased Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) Level in the Heart of Estrogen-Deficient Mice With Myocardial Infarction

The results of immunohistochemistry revealed that the expression of SIRT3 protein in myocardial cells in the marginal zone of myocardial infarction was significantly decreased compared with that in the Sham group at 28 days after myocardial infarction, while it was notably elevated after theacrine intervention (Figure 4A). WB was conducted to detect the expressions of SIRT3 protein, β-catenin, and PPARγ in myocardial cells in the marginal zone of myocardial infarction, and the results showed that in comparison with the Sham group, the myocardial infarction group had significantly decreased expression of SIRT3, significantly increased expression of β-catenin, and overtly reduced PPARγ expression in myocardial cells in the marginal zone of myocardial infarction, while the theacrine group showed clearly elevated SIRT3 protein expression, evidently lowered β-catenin expression, and remarkably up-regulated PPARγ expression in myocardial cells in the marginal zone of the myocardial infarction (Figures 4B, 4C), implying that theacrine can up-regulate the SIRT3 level in the heart in estrogen-deficient mice with myocardial infarction, and may suppress fibrosis after myocardial infarction in the estrogen-deficient mice through the β-catenin/PPARγ signaling pathway.

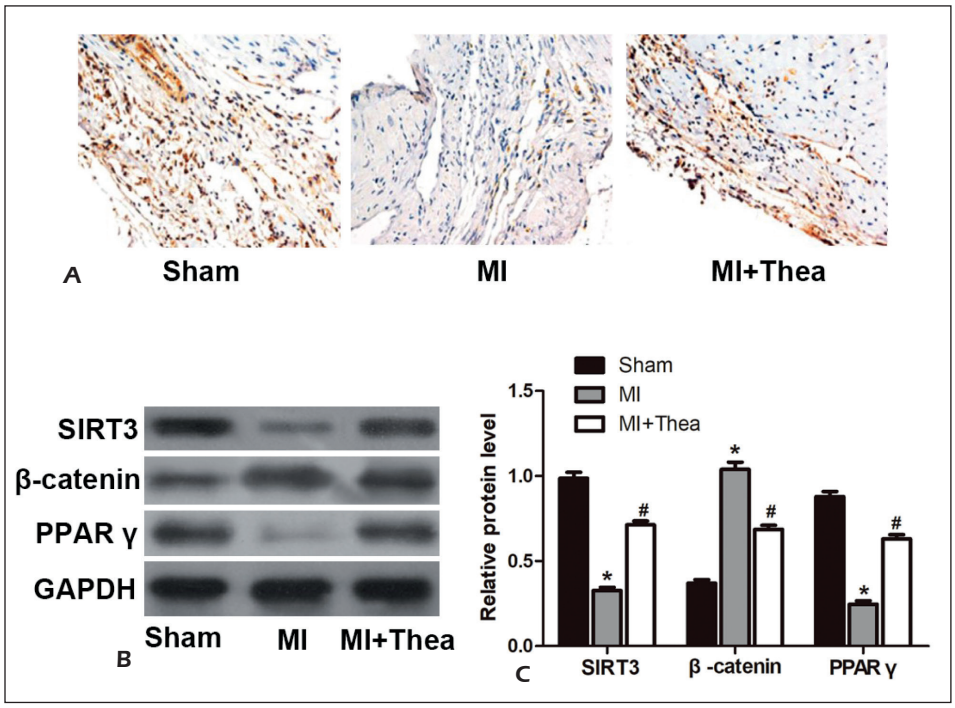

Figure 4. Theacrine up-regulated SIRT3 level, decreased β-catenin level, and increased peroxisome proliferator-activated receptor gamma (PPARγ) level in the heart of estrogen-deficient mice with myocardial infarction. **A**, The representative images of the expression of SIRT3 by immunohistochemistry staining (magnification: 100×). **B**, The Western blots analysis indicated the expression of SIRT3, β-catenin, and PPARγ in estrogen-deficient mice with MI. **C**, Semi quantitative analysis of SIRT3, β-catenin, and PPARγ in estrogen-deficient mice with MI. *$p<0.05$ *vs.* Sham group, $^{\#}p<0.05$ *vs.* MI group.

Discussion

After the menopause, the protective effect of estrogen on the cardiovascular system in women disappears, so the incidence rate of coronary heart diseases in postmenopausal women is significantly higher than that in men of the same age and premenopausal women, with relatively poor prognosis in case of myocardial infarction[3,6]. Myocardial remodeling after myocardial infarction severely impairs the left ventricular function in patients with myocardial infarction, thus elevating complication rate and evidently increasing the mortality rate[25,26]. Myocardial fibrosis is an important feature of myocardial remodeling[12]. Excessive accumulation of collagen results in aggravated myocardial stiffness and decreased compliance of the ventricular wall, thereby leading to ventricular dysfunction and heart failure[27,28]. Therefore, the severity of myocardial fibrosis is one of the main factors determining the prognosis of patients with cardiovascular diseases[12]. It was found in this study that within 4 weeks after myocardial infarction, ovariectomized mice had continuously elevated LVESd and LVEDd and decreased FS and EF, as well as cardiac function. At 28 days after myocardial infarction, evident fibrosis was detected in the area around the infarct in ovariectomized mice, with overtly increased expressions of collagen deposition-related markers Collagen I, Collagen III, and α-SMA and fibrosis-associated protein TGF-β. Besides, in the area around the infarct, both the number of apoptotic cells and the pro-apoptotic protein expression were significantly increased, while the anti-apoptotic protein expression was notably reduced.

SIRT3 plays a significant role in repressing myocardial fibrosis[21]. Senescence is one of the risk factors for cardiovascular diseases, and SIRT3 can suppress the expressions of senescence-related proteins P16 and P21 by regulating the release of ROS, thus inhibiting the senescence of human umbilical vein endothelial cells[29,30]. Moreover, SIRT3 deacetylates and activates PGC-1α, delaying the progression of cardiac hypertrophy and heart failure[31]. In addition, SIRT3 plays an important protective role in organ fibrosis[12-13]. SIRT3 alleviates the TGF-β-induced myofibroblast transition in an *in vitro* model and slows down the progression of fibrosis[21]. Furthermore, SIRT3 is capable of activating GSK3, thereby promoting the degradation of β-catenin and alleviating senescence-related fibrosis in mice[32]. Studies[33,34] have manifested that theacrine up-regulates the expression of SIRT3 in the liver, and *in vitro* experiments have also suggested that theacrine improves the expression level of SIRT3 protein in cells, indicating that theacrine may be a potential SIRT3 activator. In this experiment, theacrine was selected as the intervention drug, and it was found that after 7 days of theacrine intervention, LVESd and

LVEDd in mice were significantly decreased, while FS and EF were overtly increased compared with those in the myocardial infarction group. After 28 days, LVESd and LVEDd in mice continued to decline, and FS and EF showed continuous increases, suggesting that theacrine can improve the cardiac function in ovariectomized mice with myocardial infarction. Further study found that after 28 days of theacrine intervention, the myocardial fibrosis after myocardial infarction was significantly relieved, and the expressions of collagen deposition-related markers Collagen I, Collagen III, and α-SMA in the area around the infarct and fibrosis-related protein TGF-β1 were decreased. Meanwhile, after the intervention with theacrine, the pro-apoptotic proteins in myocardial cells in the area around the infarct were clearly reduced, the anti-apoptotic proteins in myocardial cells were evidently increased, and the number of apoptotic myocardial cells was overtly lowered.

PPAR is a ligand-dependent superfamily of nuclear steroid receptors, and PPARγ is a subtype of this family. The activation of PPARγ reduces the myocardial infarct size during the acute phase of ischemia-reperfusion injury in rats[35]. After treated with pioglitazone, a PPARγ agonist, the mice with myocardial infarction have alleviated myocardial hypertrophy, inhibited interstitial fibrosis, and improved ventricular remodeling and cardiac function[36]. Jia et al[37] have shown that SIRT3 degrades the expression of β-catenin in cells by activating and deacetylating GSK3p. β-catenin, a key factor in the Wnt signaling pathway, is able to promote the transition of myocardial fibroblasts, resulting in myocardial fibrosis[38]. The degradation of β-catenin via gene silencing or drugs can inhibit TGF-β-induced trans-differentiation of myocardial fibroblasts[39]. Moreover, there is an interaction effect between β-catenin and PPARγ. Some studies[40,41] has revealed that PPARγ can directly relate to β-catenin, and the T cell/lymphatic enhancement factor binding domain of β-catenin can bind to the catenin domain of PPARγ, thereby interacting with each other. To further explore the mechanism of action of theacrine, the expressions of SIRT3, β-catenin, and PPARγ were detected in this study, and the results showed that theacrine significantly increased the expressions of SIRT3 protein and PPARγ and decreased the expression of β-catenin. Therefore, it is speculated that theacrine can activate SIRT3 and may promote the degradation of β-catenin to further regulate the expression of PPARγ, thus repressing myocardial fibrosis and improving the cardiac function in ovariectomized mice with myocardial infarction. However, the models of removal of both ovaries can clinically simulate postmenopausal women, but there are still differences between the models and postmenopausal women, so the clinical trials are needed to confirm whether theacrine can prevent or treat coronary heart disease and myocardial infarction in postmenopausal women.

Conclusions

We showed that theacrine is able to activate SIRT3 and repress myocardial fibrosis and apoptosis after myocardial infarction in ovariectomized mice, thereby improving the cardiac function of ovariectomized mice with myocardial infarction through the possible downstream signal pathway β-catenin/PPARγ.

Conflict of Interests

The authors declared that they have no conflict of interests.

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