

QiShenYiQi Pill Improves Myocardial Hypertrophy Caused by Pressure Overload in Rats

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Research

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Abstract

Background: QiShenYiQi pill (QSYQ), a traditional Chinese medicine, is widely used in the treatment of cardiovascular diseases, but its specific mechanism of action is still unclear. In the current study, we investigated the effect of QSYQ on myocardial hypertrophy in rats by partial abdominal aortic coarctation, and explored its mechanism of action.

Methods: Wistar rats underwent the partial abdominal aortic coarctation were randomized into three groups: model, valsartan and QSYQ groups. And we treated rats which were sham operation as control group. Rats were euthanized at 4 and 8 weeks, and we weighed rat body mass, heart mass, and left ventricular mass. Myocardium sections were stained with hematoxylin and eosin (H&E) and Masson trichrome. Myocardial TGF- beta 1 and CTGF protein expression was detected by immunohistochemistry, and myocardial TGF-b1 and CTGF mRNA expression was detected by real-time qPCR.

Results: QSYQ reduced HMI, LVMI and CVF, improved the changes of myocardial pathology, and reduced the degree of myocardial hypertrophy. After 4 weeks, QSYQ inhibited the mRNA and protein expression of TGF- beta 1 and CTGF. In addition, after 8 weeks, QSYQ reduced the positive area of TGF- beta 1 protein, and its effect is better than that of valsartan.

Conclusions: QSYQ can effectively improve the degree of myocardial hypertrophy in the pressure overload rats, which is related to the mechanism of regulation of TGF- beta 1 and CTGF.

Background

Myocardial hypertrophy is the cardiomyocyte hypertrophy and myocardial fibrosis, which is a compensatory and adaptive response to the pressure overload ^[1]. However, the myocardium usually manifests as overload and thus has maladaptive reactions, which can be used as an independent risk factor for various cardiovascular diseases such as heart failure, arrhythmia, and even sudden death ^[2]. Pathological left ventricular hypertrophy (LVH) can be seen in hypertension, aortic stenosis and other cardiovascular diseases. Hypertension is the main cause of pressure overload myocardial hypertrophy, and the prevalence is 20%~30% ^[3]. At the same time, hypertension combined with LVH can increase the incidence of acute myocardial infarction, congestive heart failure, sudden death, and other cardiovascular events by 6~8 times ^[4,5]. Reconstruction of cardiac structure caused by hypertension is mainly LVH. LVH is an adaptive change in the heart's chronic pressure and volume overload, and is a common complication of hypertension. The early compensatory performance was centripetal hypertrophy, and the late decompensation showed centrifugal hypertrophy, and then the heart function decreased and eventually led to heart failure ^[6]. At present, the mechanism of LVH has not been fully elucidated, its pathogenesis involves hemodynamic factors, neurohumoral regulation factors, cardiovascular tissue autocrine and paracrine factors and genetic factors ^[3]. All kinds of drugs may improve LVH in varying degrees through effective hypotension, but the mechanisms and effects of different drugs on LVH are different. Studies have shown that the effects of five antihypertensive drugs in reversing LVH are

different: angiotensin II receptor antagonists, calcium antagonists, angiotensin-converting enzyme inhibitor (ACEI), diuretics, and β -blockers can make the left ventricular quality of patients the index decreased by 13%, 11%, 10%, 8%, and 6% respectively [7]. Modern medicine for LVH is mainly directed at renin angiotensin aldosterone system, of which ACEI, angiotensin receptor blocker and aldosterone antagonists are the most studied, but only for single pathological links. Traditional Chinese medicine has a variety of ingredients, which can act on multiple targets at the same time to regulate different action links, and has the advantage of comprehensive regulation for the treatment of LVH.

QiShenYiQi pill (QSYQ) is made of effective components of *Radix Astragali*, *Radix Salviae Miltiorrhizae*, *Radix Notoginseng*, and *Lignum Dalbergiae Odoriferae*, and was approved by China State Food and Drug Administration in 2003 for treatment of cardiovascular disease [8,9]. The effective ingredients of traditional Chinese medicine QSYQ are extracted by modern preparation techniques, which have a stable dosage form and controllable quality [10,11]. Research shows that QSYQ can significantly improve the degree of myocardial fibrosis in rats with abdominal aorta coarctation [12], reduce myocardial hypertrophy in rats with coarctation of aorta [13,14], relieve left ventricular remodeling in rats with ligation of left anterior descending coronary artery [15,16], and improve cardiac function and myocardial structure in rats with ischemia-reperfusion [17]. In addition, QSYQ can also effectively improve myocardial damage in adriamycin induced cardiomyopathy mice [18,19], and improve cardiac remodeling in rats with autoimmune cardiomyopathy [20]. However, the effect of QSYQ on myocardial hypertrophy is still unclear. In this study, a rat model of cardiac hypertrophy was established through coarctation of abdominal aorta to further explore the effect and mechanism of QSYQ on the intervention of myocardial hypertrophy.

Materials And Methods

Animals

Male Wistar rats, weighing 180-200g, were provided by the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China; certificate number SCXK (Jun) 2012-0004). Wistar male rats were raised in a cage with a constant temperature of $22\pm 2^{\circ}\text{C}$, a 12-hour light-dark cycle, free drinking water, and a standard diet. All research procedures conformed to Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1996), and approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine, China (No. TCM-LAEC2016016).

Animal model of pathological myocardial hypertrophy

The abdominal aorta constriction method was used to establish a pressure overload rat myocardia hypertrophy model [21]. In short, a rat was anesthetized with sodium pentobarbital (45 mg/kg, intraperitoneal injection), then fixed on the operating table supine, and a longitudinal incision in the abdominal cavity was performed under aseptic conditions. The abdominal aorta was exposed and the abdominal aorta above the right renal artery branch was separated. A size 7 needle was placed parallel to the abdominal aorta, the two were ligated with a size 4 surgical suture, the needle was removed, and

200,000 U of penicillin were instilled to prevent infection, and the abdominal cavity was closed. At the same time, a small amount of picric acid solution was applied around the incision to prevent wound infection in rats. After the operation, the rats were fed with sugar-salt solution and continuously injected intramuscularly with 200,000 U/d penicillin for 3 days to prevent infection. Rats in the sham surgery group were operated and the suture line was placed without ligation. The remaining procedure was similar to that in the surgery group.

Groups and dosing

4 weeks after the operations, sham and the aortic constriction surgery rats were randomly divided into the Sham-operated control group (intragastric infusion with equal volume of distilled water) model group (intragastric infusion with equal volume of distilled water), the valsartan group (intragastric infusion at 7.2 mg/kg; Novartis Pharma Ltd., Beijing, China), and the QSYQ group (intragastric infusion at 135 mg/kg; Tasly Pharmaceutical CO., LTD., Tianjin, China). The interventions were administered at 2 time points (4-week intervention and 8-week intervention), and 8 rats were present in each group at each time point. The rats in each group were dosed once daily in the morning.

Morphological and histological analysis

The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (45 mg/kg) after drug intervention for 4 weeks and 8 weeks. The heart tissue was removed, and the heart mass index (HMI, the ratio of heart weight to body weight) and the left ventricular mass index (LVMI, the ratio of left ventricular weight to body weight) were measured and calculated. Subsequently, the heart was fixed in 4% paraformaldehyde overnight, routinely dehydrated, transparent, embedded, and sectioned, 5 μm thick; deparaffinized, dehydrated using an ethanol gradient, and subjected to hematoxylin-eosin (H&E) staining and Masson staining (all Baihao Biological Technology CO., LTD., Tianjin, China). Dehydrate with ethanol gradient, wash with xylene, fix with neutral resin, and observe under an optical microscope. H&E staining was used to estimate cardiac hypertrophy, Masson staining to measure the area of collagen. The image-Pro Plus image analysis software was used to randomly select 5 fields of view from each slide to measure the area of collagen. The average was calculated as the collagen volume factor (CVF) for this myocardial tissue. $\text{CVF} = \text{area of myocardial collagen fibers} / \text{total area of the image}$.

Real-time quantitative polymerase chain reaction

Ultrapure RNA extraction kit (CWbio Co. Ltd., *Cat#CW0581*) was used to extract total RNA from the myocardial tissue. The HiFi-MMLV cDNA first-strand synthesis kit (CWbio Co. Ltd., *Cat#CW0744*) was used for reverse transcription and the UltraSYBR Mixture with Rox (CWbio Co. Ltd., *Cat#CW0956*) was used for amplification. The primers for the target genes and household genes were purchased from Guangzhou Fulen Gen Co., Ltd., including TGF-beta 1 (GeneCopoeiaTM; *Cat# RQP050181*), CTGF (GeneCopoeiaTM, *Cat# RQP050397*), and glyceraldehyde phosphate dehydrogenase (GAPDH) (GeneCopoeiaTM, *Cat# RQP049537*). The $2^{-\Delta\Delta\text{Ct}}$ method was used for relative quantitative analysis of the raw data of RT-qPCR determination.

Immunochemical analysis

The expression of TGF- β 1 protein and CTGF protein in rats were measured by immunochemical assay, the slides were routinely dewaxed by xylene, the slides were hydrated by gradient ethanol, and the antigens were repaired by microwave. Then an appropriate amount of hydrogen peroxide was added to block the endogenous peroxidase activity of the protein. A primary antibody (rabbit anti-rat IgG) was added and incubated at 4°C overnight. Further, the slides were incubated with biotinylated goat anti-rabbit IgG. Finally, DBA was used for color rendering and hematoxylin was used for secondary dyeing (antibody against TGF- β 1, antibody against CTGF, streptavidin – biotin complex with peroxidase (SABC-POD) (rabbit IgG) ready-to-use kits and DAB Chromogenic Reagent Kit were all from Boster Biological Engineering CO., LTD., Wuhan, China). After dehydration, transparent sealing with neutral resin. Determination of results: Cells with a clearly defined structure, having a significantly greater coloration than the background, and brown-yellow granules at the corresponding area were considered to be positive. The cells that did not develop any color or had the same level of coloration as the background were considered to be negative. We randomly selected 5 fields from each slide under an optical microscope of 40 \times magnification. The area ratio of the positively stained matter was determined using the Image-Pro Plus imaging analysis software and the average was calculated.

Statistical Analysis

All experimental results were statistically analyzed using SPSS software (v11.5; SPSS Inc., Chicago, Illinois, USA), and expressed as mean \pm S.D. One-way analysis of variance was used to compare multiple groups, and then the least significant difference (LSD) test was used for multiple comparisons. The value of $P < 0.05$ was considered statistically significant.

Results

Effect of QSYQ on Rat Cardiac Morphology

The model group of abdominal aorta constriction rats had significantly higher HMI and LVMI than the sham-operated control group ($P < 0.01$). Compared with the model group of abdominal aorta constriction, the HMI and LVMI of the two treatment groups of valsartan and QSYQ were significantly decreased ($P < 0.01$), and the trend of reduction increased over time. However, the reduction in HMI and LVMI in the QSYQ group was greater than that in the valsartan group at 8 weeks ($P < 0.05$) (Fig. 1).

Effect of QSYQ on Myocardial Histopathology

H&E staining in myocardial tissue showed that the rat cardiac myocytes were arranged orderly, the cytoplasmic staining was uniform, and there were no necrotic foci in the Sham-operated control group. But the model group showed extensive and multifocal myocardial fibrosis, hypertrophy and swelling of cardiac myocytes, hyperplasia of adjacent fibrous tissue, and inflammatory cell infiltration around the

vessels. Compared with the model group, the above pathological changes were reduced in the Valsartan group and the QSYQ group (Fig. 2).

Masson trichrome staining in myocardial tissue showed that in the Sham-operated control group, there was no obvious blue collagen fibers in the myocardial interstitial, but in the model group, a large amount of collagen was deposited in the myocardial interstitial and around the perivascular, and the blue fiber area was larger. And the collagen volume fraction (CVF) of the model group increased significantly ($P<0.01$), showing a trend of increasing over time. After treatment (valsartan and QYSQ), the area of blue collagen fibers was lower than that of the model group, and the CVF was significantly lower ($P<0.01$), indicating that the degree of myocardial fibrosis was reduced (Fig. 2).

Effect of QSYQ on mRNA expression of TGF- beta 1 and CTGF

At 4 weeks after abdominal aortic coarctation, pressure overload caused an increase in the expression levels of TGF- β 1 and CTGF mRNA in the rat myocardium ($P<0.01$). Valsartan treatment group can reduce the expression of CTGF mRNA in rat myocardium ($P<0.01$), but the expression of TGF- β 1 mRNA has no significant effect ($P>0.05$). While QSYQ group can obviously inhibit the expression of TGF- beta 1 and CTGF mRNA in rat myocardium ($P<0.01$). At 8 weeks, there was no statistical difference in the expression levels of TGF- β 1 and CTGF mRNA each group ($P>0.05$) (Fig. 3).

Effect of QSYQ on protein expression of TGF- beta 1 and CTGF

Immunohistochemical staining of TGF- beta 1 and CTGF in myocardial tissue showed that in the Sham-operated control group there were a small number of color change was weak, but in the model group the brown area had expanded, staining was enhanced, and expression of TGF- beta 1 and CTGF had increased. After the treatments (valsartan and QYSQ), expression of TGF- beta 1 and CTGF was reduced compared with the model group. Semi-quantitative analysis showed that the positive area percentage of TGF- β 1 and CTGF protein in the pressure overload model group was significantly higher than that in the Sham-operated group at 4 weeks ($P<0.01$). Valsartan treatment can reduce the percentage of positive area of TGF- β 1 and CTGF protein ($P<0.05$ or $P<0.01$), while the QSYQ group had a similar result. At 8 weeks, the percentage of positive area of TGF- beta 1 and CTGF protein in the model group was still higher ($P<0.01$). There was no significant difference in the positive area of CTGF protein in each group ($P>0.05$), but valsartan group could reduce the positive area of TGF- beta 1 protein. QSYQ group showed the same effect as valsartan group, and its decrease effect was better than valsartan group ($P<0.01$) (Fig. 4).

Discussion

Hypertension is the most common chronic noncommunicable disease, and its morbidity, disability and mortality are high. Although awareness rate, treatment rate and control rate of hypertension are on the rise, but they are still at a low level [22]. In clinical, more than 30% of hypertension may have LVH, and the incidence of LVH is positively correlated with the severity of hypertension [23]. Long term pressure

overload leads to excessive deposition of cardiac collagen fibers, increased collagen concentration, imbalance of collagen proportion and disorder of arrangement, resulting in altered cardiac function and structure, thereby increasing the incidence of cardiovascular events. A meta analysis of 2449 patients also showed that the risk of total cardiovascular events in patients with left ventricular hypertrophy reversed / persistently normal hypertension decreased by 46% [24]. Therefore, the prevention / reversal of LVH can significantly reduce the risk of cardiovascular events and death, and has become one of the hot topics in the field of cardiovascular disease [25,26].

The collagen content (collagen volume fraction, CVF) in normal myocardial tissue is about 3%~5% in rats. When CVF increases to 8%~12%, the diastolic function of the myocardium is damaged and the systolic function is maintained. However, when the CVF rises to more than 20%, the systolic function of rat myocardium becomes impaired [27]. In the pressure overload rats, with the prolongation of the intervention time, QSYQ can further reduce HMI, LVMI and CVF in rats, and reduce the degree of myocardial hypertrophy. It is suggested that QSYQ can effectively improve the myocardial hypertrophy of rats with pressure overload, and it has the effect of resisting myocardial hypertrophy.

Long term high load work of the heart will lead to changes in cardiac hypertrophy, interstitial fibrosis, and microvascular, which ultimately damage the cardiac contractile function and lead to heart failure [28]. TGF- beta 1 is closely related to extracellular matrix deposition, and it is recognized as a target for the treatment of organ fibrosis [29]. TGF- beta 1 has a variety of physiological functions, which can inhibit inflammation and cell proliferation during normal expression, and overexpression can lead to adverse consequences, such as fibrosis [30]. A variety of stimuli, such as pressure overload, myocardial infarction, and immune injury, can activate TGF- beta 1 signal and initiate fibrosis, resulting in a large amount of collagen deposition [31]. Clinical studies have shown that the increase of plasma TGF- beta 1 levels in hypertensive patients is closely related to hypertension and its target organ damage [32]. Compared with normal blood pressure rats, the expression of TGF- beta 1 in the blood vessels of spontaneously hypertensive rats increased, while the increased expression of TGF- beta 1 could induce the proliferation of vascular smooth muscle cells [33]. Under the condition of pressure overload, hypoxia inducible factor -1 (HIF-1) protects the heart and aorta of mice by down regulating the expression of TGF- 1-smad2/3 and TGF- beta 1-ERKI/2 [34]. In addition, fluvastatin could inhibit TGF- beta 1 and induce Smad7 expression in a dose-dependent manner, thereby delaying myocardial hypertrophy and myocardial fibrosis in spontaneously hypertensive rats [35]. The results showed that QSYQ could significantly inhibit the expression of TGF- beta 1 mRNA and reduce the positive area of TGF- beta 1 protein in rat myocardium at 4 weeks after intervention, while reduce the positive area of TGF- beta 1 protein in rat myocardium at 8 weeks. It is suggested that the effect of QSYQ on the anti-hypertrophy of myocardium is related to the expression of TGF- beta 1.

Connective tissue growth factor (CTGF) is a downstream effect factor of TGF- beta 1, which only mediates the negative effect of TGF- beta 1. Therefore, inhibition of the expression of CTGF may be a new target for the treatment of myocardial hypertrophy. CTGF, as a fibrotic factor, is closely related to the

development of multiple organ fibrosis. It is confirmed that TGF- β can induce the up regulation of CTGF expression in cardiac myocytes and cardiac fibroblasts, and the up regulation of CTGF is associated with increased expression of fibronectin, type I collagen and type III collagen^[36]. The expression of TGF- β 1 and CTGF in myocardial infarction model rats showed that TGF- β 1 was mainly expressed in the onset of myocardial infarction, acute inflammatory reaction and post myocardial infarction repair phase, while CTGF played a role in the development stage of cardiac fibrosis^[37]. TGF- β 1 can induce CTGF expression, while CTGF enhances TGF- β 1 signaling pathway. They interact with each other and enter the vicious cycle, which leads to accumulation of extracellular matrix and eventually develops into cardiac hypertrophy. In addition, the myocardial fibrosis in mdx mice began with the increase of CTGF expression, and the up regulation of CTGF was consistent with the increase of TIMP-1 expression^[38]. CTGF promotes the development of myocardial fibrosis by promoting collagen synthesis and inhibiting its degradation.

Conclusion

QSYQ can effectively improve the degree of myocardial hypertrophy in the pressure overload rats and it had a tendency to have a greater effect with longer treatment duration, which is related to the mechanism of regulation of TGF- β 1 and CTGF.

Abbreviations

QSYQ: QiShenYiQi pill; H&E: hematoxylin and eosin; LVH: left ventricular hypertrophy; ACEI: angiotensin-converting enzyme inhibitor; HMI: heart mass index; LVMI: left ventricular mass index; CVF: collagen volume factor; CTGF: connective tissue growth factor; TGF- β : transforming growth factor- β .

Declarations

Ethics approval and consent to participate

All research procedures conformed to Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86–23, revised 1996), and approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine, China (No. TCM-LAEC2016016).

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SCL, QW, MFW, ML, XJW and LX performed the experiments. SCL wrote the manuscript. QW, MFW and ML analyzed data. XJW and LX searched and reviewed literature. JPZ conceived and designed the study. All authors have read and approved the final manuscript.

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Figures

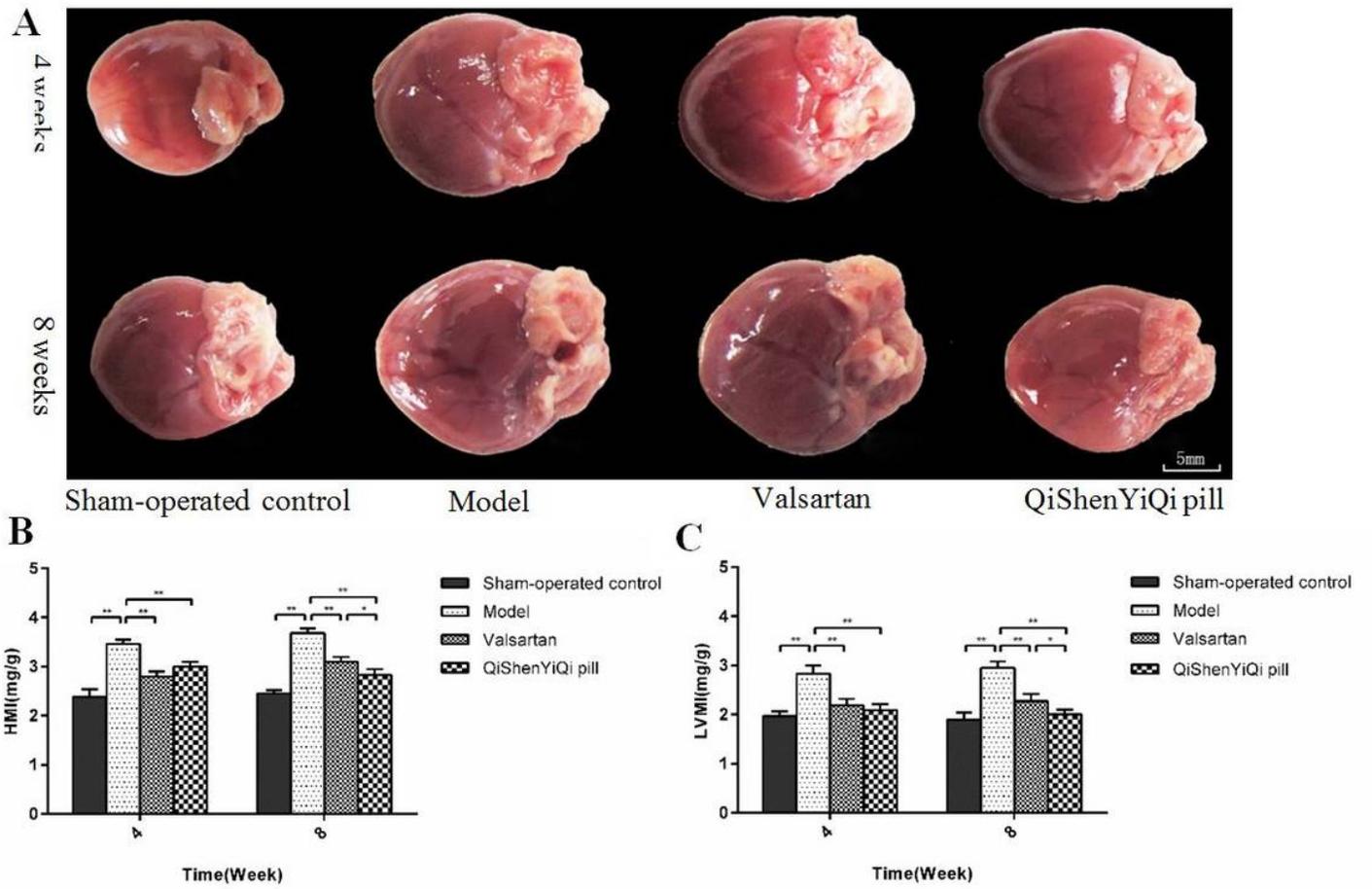


Figure 1

Effects of QSYQ on HMI and LVMI. (A) The morphological changes in the heart of rats. (B) HMI of each group. (C) LVMI of each group. Data are expressed as mean \pm SD. * $P < 0.05$, ** $P < 0.01$.

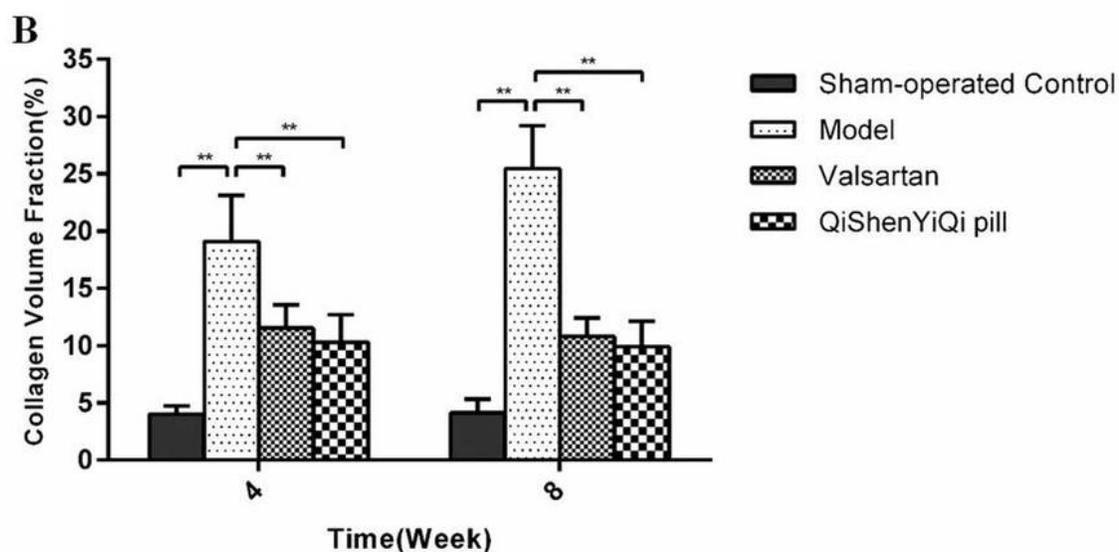
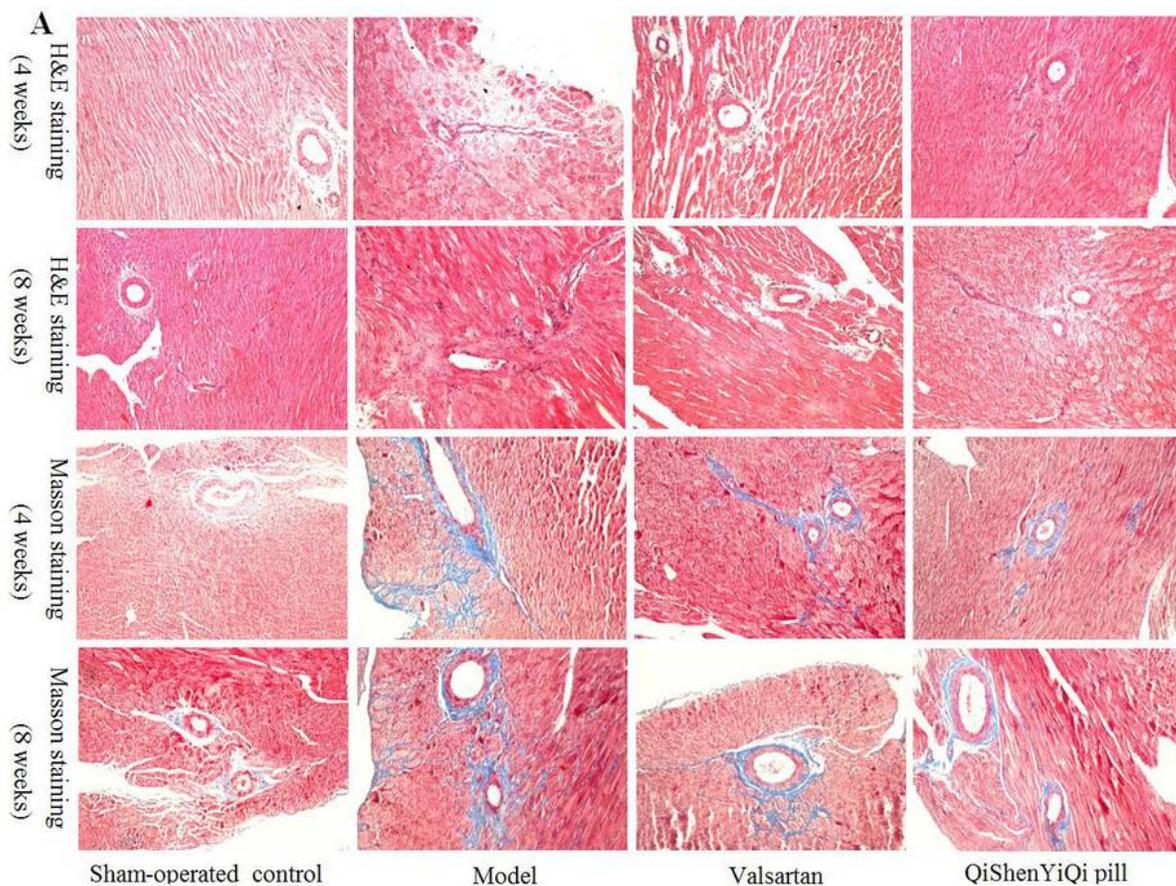


Figure 2

Effects of QSYQ on pathomorphism of myocardium. (A) Representative photomicrograph of hematoxylin and eosin (H&E) and masson trichrome staining of myocardium. (B) The collagen volume fraction for each group. The collagen volume fraction was quantitatively analyzed by using Image-Pro Plus 6.0 (magnification, $\times 100$). Data are expressed as mean \pm SD. Data are expressed as mean \pm SD. ** $P < 0.01$.

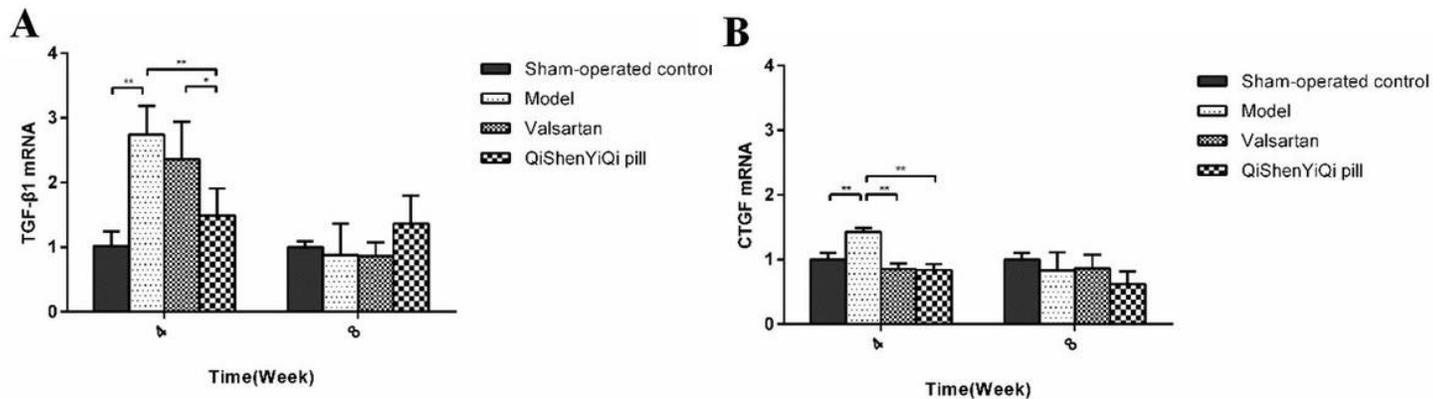


Figure 3

Effects of QSYQ on the mRNA expression of TGF-β1 and CTGF. (A) The TGF-β1 mRNA expression for each group. (B) The CTGF mRNA expression for each group. Data are expressed as mean ±SD. Data are expressed as mean ±SD. * P<0.05, ** P<0.01.

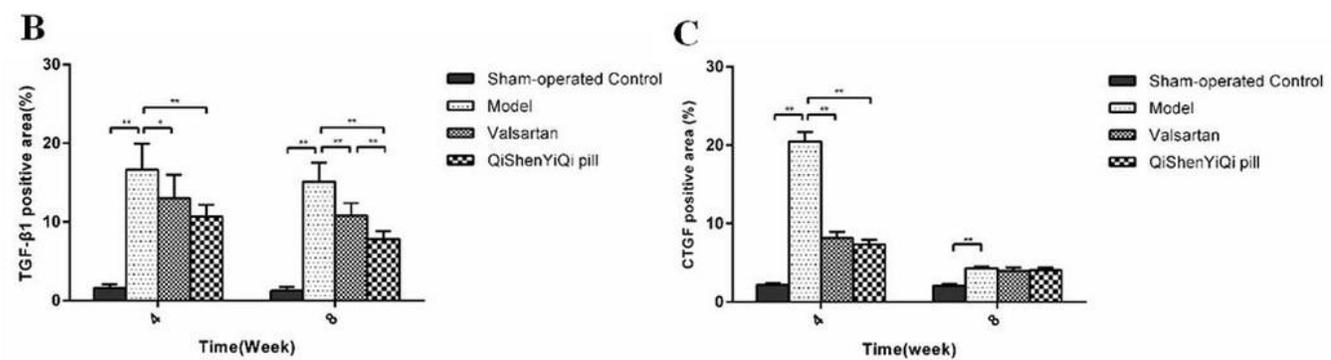
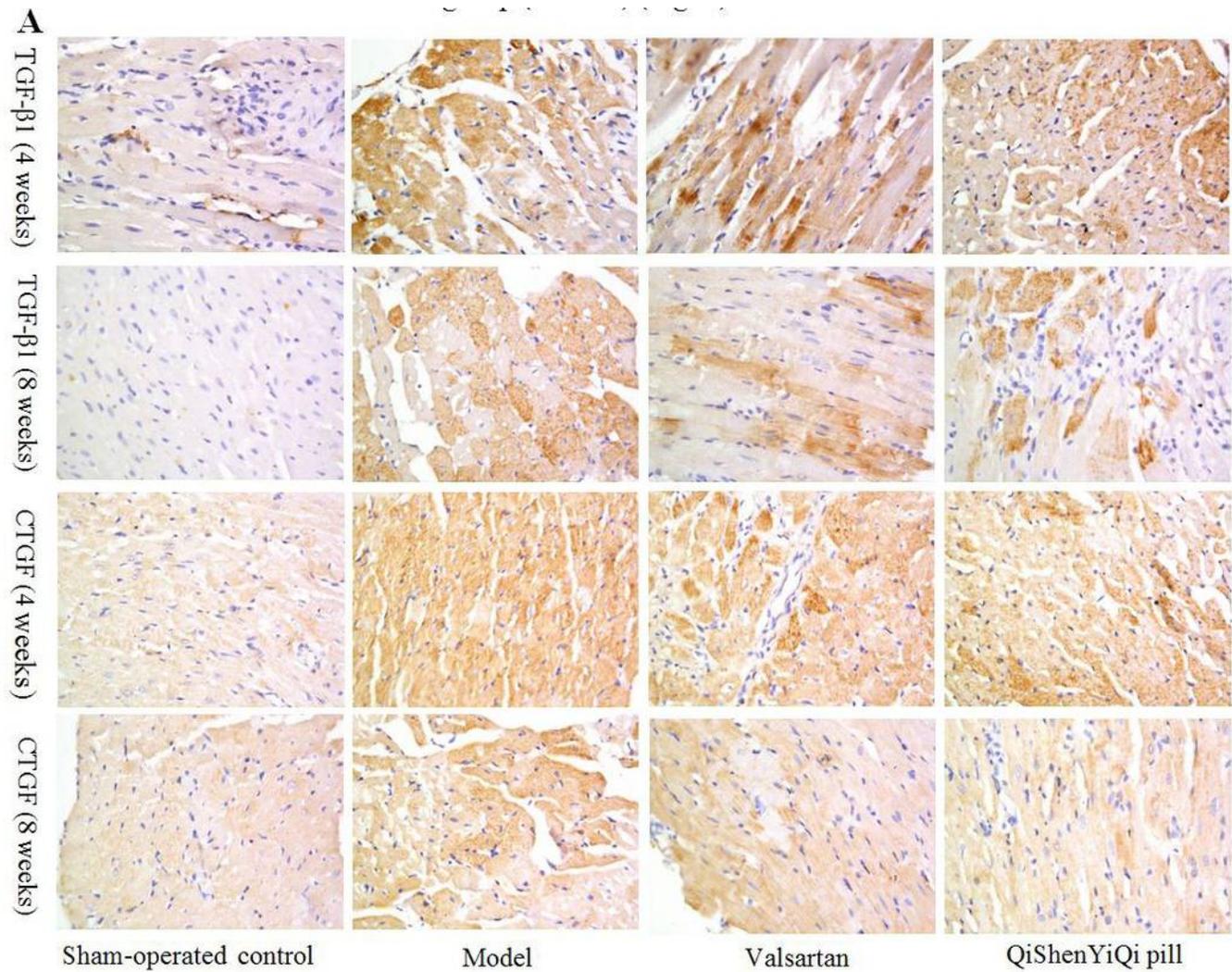


Figure 4

Effects of QSYQ on the protein expression of TGF-β1 and CTGF. (A) Representative photomicrograph of immunohistochemical staining of myocardium. (B) The TGF-β1 positive area for each group. (C) The CTGF positive area for each group. The percentage of immunohistochemical staining area was quantitatively analyzed by using Image-Pro Plus 6.0 (magnification, ×400). Data are expressed as mean ±SD. Data are expressed as mean ±SD. * P<0.05, ** P<0.01.