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Research Paper

Traditional Chinese Medication Qiliqiangxin Protects Against Cardiac Remodeling and Dysfunction in Spontaneously Hypertensive Rats

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Abstract

Qiliqiangxin (QLQX), a traditional Chinese herbs medication, exerted protective effect in chronic heart failure patients in a multicenter randomized double-blind study. QLQX has also been found to improve cardiac function and reduce cardiac fibrosis in spontaneously hypertension animal model. However, the effect of longterm treatment with QLQX in such a condition and the related molecular mechanisms remain largely unknown. In the present study, thirteen-week-old spontaneously hypertensive rats (SHRs) were treated by daily intragastric administration of QLQX or saline for one year. Echocardiography, electron microscopy, and Masson's trichrome staining were used to determine cardiac function, mitochondria ultrastructure, and cardiac fibrosis, respectively. Quantitative reverse transcription polymerase chain reactions (qRT-PCRs) and Western blotting were used to determine gene expressions. We found that QLQX significantly improved cardiac function and reduced gene markers of pathological hypertrophy including ANP, BNP, and Myh7. QLQX also attenuated cardiac fibrosis and apoptosis in SHRs as evidenced by downregulation of α-SMA, collagen I, collagen III, and TGF-β expressions and reduction of Bax to Bcl-2 ratio. Moreover, the damage of mitochondrial ultrastructure was greatly improved and the reduction of PPAR-α, PPAR-γ, and PGC-1α expression levels was significantly restored in SHRs by treatment with QLQX. In conclusion, longterm treatment with QLQX protects against cardiac remodeling and dysfunction in hypertension by increasing PPARs and PGC-1α.

Key words: Qiliqiangxin, cardiac remodeling, fibrosis, spontaneously hypertension, PPAR, PGC-1α.

Introduction

Hypertension represents a common condition that progresses toward severe cardiovascular morbidity. Hypertensive heart disease (HHD), which is characterized by diastolic dysfunction, cardiac remodeling, and cardiac fibrosis, is one of the major clinical phenotypes caused by elevated blood pressure [1]. HHD leads to the worsening of entire cardiac function and results in an increased risk of adverse cardiac events by further damaging systolic function [2]. Preventing cardiac dysfunction and adverse remodeling would be beneficial for patients with hypertension and HHD, though effective

therapeutic strategies are still limited [3]. Therefore, additional therapeutic targets and treatment options are highly required [4].

Qiliqiangxin (QLQX), a traditional Chinese herbs medication, is composed of 11 distinct herbs including astragali radix, ginseng radix et rhizoma, aconiti lateralis radix preparata, salvia miltiorrhiza radix et rhizoma, semen descurainiae lepidii, alismatis rhizoma, cinnamomi ramulus, polygonati odorati rhizoma, carthami flos, periploca cortex, and citri reticulatae pericarpium [5, 6]. QLQX has been reported to attenuate the progression of cardiac remodeling and fibrosis after acute myocardial infarction and inhibit the development of cardiac hypertrophy and failure after transverse aorta constriction [7, 8]. It has also been shown that QLQX can prevent cardiac hypertrophy and dysfunction both *in vitro* and *in vivo* through several pathways such as activation of mTOR and PPAR-γ, regulation of IL-6, IL-10, and TNF-α, and inhibition of angiotensin II [7-13]. Noteworthy, our group previously reported that QLQX exerted protective effects in chronic heart failure patients in a multicenter randomized double-blind study [14]. These protective effects included the improvement of left ventricle ejection fraction, New York Heart Association (NYHA) functional classification, 6-minute walking distance, and quality of life, as well as the reduction of plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) level [14]. Interestingly, QLQX has also been demonstrated to improve cardiac function and reduce cardiac fibrosis in spontaneously hypertensive animal model through inhibition of cardiac chymase [15]. However, the effect of longterm treatment with QLQX in such a condition and the related molecular mechanisms remain largely unknown.

In this study, we investigated the effect of QLQX in spontaneously hypertensive rats (SHRs) and demonstrated that QLQX could attenuate cardiac remodeling and dysfunction likely by inducing peroxisome proliferator-activated receptors (PPARs) and PPAR-γ coactivator-1α (PGC-1α).

Materials and Methods

Animals

All rats were purchased and raised at the Experimental Animal Center of Nanjing Medical University (Nanjing, China). All procedures with rats were in accordance with the guidelines on the use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996), and the experimental protocol was reviewed and approved by the ethical committees of Nanjing Medical University.

Thirteen-week-old spontaneously hypertensive rats (SHRs) were randomly divided into two groups: the SHR control group with intragastric administration of saline (SHR+Saline, n=3) and the SHR treatment group with intragastric administration of 1 g/kg/d QLQX (SHR+QLQX, n=3). Moreover, four Wistar-Kyoto (WKY) rats were treated with saline as the normal control group (Wistar+Saline, n=4). The three groups of rats were housed in one room and fed with a standard diet and water. QLQX was provided by Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, Hebei, China). To guarantee the quality and consistency of QLQX, the raw medicinal materials were of a certain variety, and their areas of origin, medicinal parts, and processing methods were kept consistent. The stability of the product was verified by analyzing 10 batches of QLQX and fingerprints. The three groups of rats were treated by daily intragastric administration of QLQX or saline for one year.

Echocardiography

In order to assess the cardiac structure and function, all rats were fixed in hypsokinesis position after anesthesia with 1.5-2.0% isoflurane and echocardiography was performed blinded using Vevo 2100 (VisualSonics Inc, Toronto, Ontario, Canada) with a 30 MHz central frequency scan head. Evaluation indexes include left ventricular internal dimension-diastole (LVIDd), left ventricular internal dimension-systole (LVIDs), left ventricular fractional shortening (FS), and left ventricular ejection fraction (EF). These parameters were measured from M-mode images taken from the parasternal short-axis view at papillary muscle level.

Electron microscopy

Heart samples were fixed in 2.5% glutaraldehyde for 1 h, treated with 1% osmium tetroxide, and then dehydrated and embedded in Durcupan. Heart samples were sectioned into 60 nm and mounted on Cu-grids, then stained with uranyl acetate and lead citrate, and finally examined under an electron microscope (JEM-1010). Images were taken at 15,000× magnification. A total of 3 sections/specimen from 3 rats per group were used for analyses of mitochondria ultrastructure.

Histological analysis

After intragastric administration of QLQX or saline for one year, hearts were excised after being perfused with phosphate-buffered saline and then fixed with 4% paraformaldehyde. These sections were processed in paraffin using standard techniques and the degree of cardiac fibrosis was evaluated with Masson's trichrome staining. Images were acquired

from at least 20 fields of each heart section randomly. The degree of cardiac fibrosis was assessed by the ratio of fibrotic area (blue) to total myocardial area using Image J software.

Western blotting

Heart tissues were homogenized in RIPA lysis buffer and then centrifuged to acquire protein supernatants. The equal amounts of total proteins were subjected to SDS-PAGE gels and transferred onto PVDF membranes. The membranes were incubated with primary antibodies, including transforming growth factor-β (TGF-β, 1:1000 dilution; Cell Signaling Technology, Boston, Massachusetts, USA), α-smooth muscle actin (α-SMA, 1:1000 dilution; Cell Signaling Technology), B-cell lymphoma 2 (Bcl-2, 1:1000 dilution; Cell Signaling Technology), Bcl-2-associated X protein (Bax, 1:1000 dilution; Cell Signaling Technology), PPAR-α (1:1000 dilution; Abcam, Cambridge, UK), PPAR-γ (1:400 dilution; Abcam), PGC-1α (1:1000 dilution; NOVUS, Littleton, COLO, USA), Protein kinase B (Akt, 1:1000 dilution; Cell Signaling Technology), p-Akt (Ser473, 1:1000 dilution; Cell Signaling Technology), p-Akt (Thr308, 1:1000 dilution; Cell Signaling Technology), LC3 (Light chain 3, 1:1000 dilution; Cell Signaling Technology), and P62 (EBI 3 associated protein 62, 1:1000 dilution; Cell Signaling Technology). Glyceraldehyde 3-phosphate dehydrogenase antibody (GAPDH, 1:2000 dilution; Kangchen, Shanghai, China) was used as a loading control. After this, the membranes were incubated with secondary antibodies for 2 h at room temperature. Then the signals were visualized using the ECL Plus Western blotting detection reagents (Bio-Rad, Hercules, CA, USA) and the ChemiDoc XRS Plus luminescent image analyzer (Bio-Rad). The densitometry of each protein band was analyzed by Imagelab software (Bio-Rad).

Quantitative reverse transcription polymerase chain reactions (qRT-PCRs)

Total RNA was extracted from left ventricular tissues of rats with miRNeasy Mini Kit (Qiagen, Hilden, Germany) and reverse transcribed into cDNA using Bio-Rad iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instruction. The cDNA was then subjected to 40 cycles of quantitative PCR in the 7900HT Fast Real-Time PCR System with SYBR green (Takara, Tokyo, Japan). The relative expression levels of mRNA were calculated with GAPDH as the control gene. The primer sequences are as follows (forward and reverse, 5'-3').

GAPDH, GGCACAGTCAAGGCTGAGAATG and ATGGTGGTGAAGACGCCAGTA.

ANP, CTCCCAGGCCATATTGGAG and TCCAGGTGGTCTAGCAGGTT.

BNP, TGGGAAGTCCTAGCCAGTCTC and TCTGAGCCATTTCCTCTGAC.

MyH7, GGATGACGTTACCTCCAACA and GTGTCTCCTCAGCCTTGCTC.

α-SMA, GTCCCAGACATCAGGGAGTAA and TCGGATACTTCAGCGTCAGGA.

Collagen I, GAGCGGAGAGTACTGGATCGA and CTGACCTGTCTCCATGTTGCA.

Collagen III, TGCCATTGCTGGAGTTGGA and GAAGACATGATCTCCTCAGTGTTGA.

Statistical analysis

All data were shown as mean±SEM. The one-way ANOVA followed by Bonferroni's post-hoc test was used to compare the one-way layout data. A *P* value less than 0.05 was considered to be statistically significant. All analyses were performed using SPSS and presented with GraphPad Prism 5.

Results

QLQX improves cardiac function in SHRs

Cardiac function was measured by echocardiography in SHRs after one year treatment with QLQX. As shown in Figure 1A, QLQX significantly improved cardiac function, including EF, FS, and LVIDs. Moreover, QLQX prevented cardiac hypertrophy as evidenced by the reduction of the ratio of left ventricle weight to body weight and the ratio of left ventricle weight to tibia length (Figure 1B). In addition, the gene markers of pathological hypertrophy including ANP, BNP, and Myh7 were also reduced by treatment with QLQX (Figure 1C).

QLQX attenuates cardiac fibrosis and apoptosis in SHRs

Based on histological analysis of cardiac tissue sections, we found that the degree of cardiac fibrosis in the SHR+QLQX group was significantly alleviated when compared with the SHR+Saline group (Figure 2A), which was further supported by the decreased expression levels of α-SMA, collagen I, and collagen III as determined by qRT-PCRs (Figure 2B) and Western blotting (Figure 2C). Besides, the expression level of TGF-β, a key mediator responsible for cardiac fibrosis and remodeling [19, 20], was also reduced by treatment with QLQX (Figure 2C). Meanwhile, the ratio of proapoptotic protein Bax to antiapoptotic protein Bcl-2 was increased in SHRs, while reduced by QLQX treatment, indicating that cardiac apoptosis was also attenuated (Figure 3A). However, the autophagy was not altered in SHRs regardless of QLQX treatment, as indicated by no alteration of the expression levels of P62 and LC3 (Figure 3B).

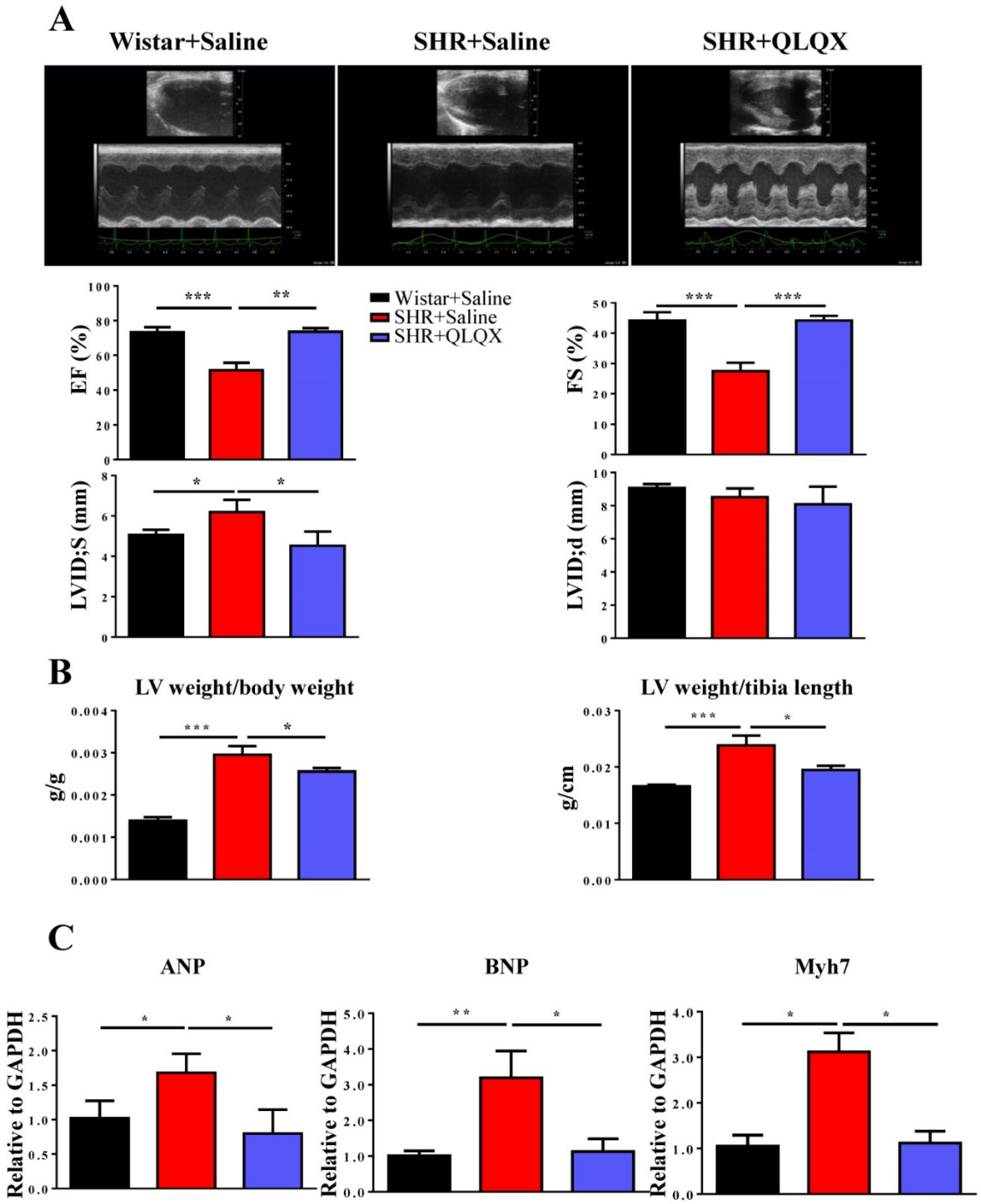

Figure 1. QLQX improves cardiac function in SHRs (A) QLQX significantly improved cardiac function in SHRs, by preserving ejection fraction (EF), fractional shortening (FS), and left ventricular internal dimension-systole (LVIDs). (B) QLQX reduced the ratio of left ventricle weight to body weight and the ratio of left ventricle weight to tibia length in SHRs. (C) QLQX reduced the gene markers of pathological hypertrophy in SHRs. N=3-4 per group. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

QLQX attenuates mitochondrial ultrastructure damage and restores downregulation of PPAR-α and PPAR-γ in SHRs

Using transmission electron microscopy (TEM), we demonstrated that mitochondria ultrastructure of cardiac myocytes showed more swelling and vacuolization in SHRs, which could be greatly improved by QLQX treatment (Figure 4A). The peroxisome proliferator-activated receptors (PPARs) are involved in various aspects of cardiac energy metabolism and we previously reported that QLQX could increase PPAR-α, PPAR-γ, and PGC-1α in acute

myocardial infarction [7]. Here we also found that PPAR-α, PPAR-γ, and PGC-1α were decreased in SHRs and QLQX could elevate all of them (Figure 4B), which is consistent with our previous report [7]. Similarly, the Akt signaling pathway was not changed in SHRs regardless of QLQX treatment (Figure 4C). Thus, we speculate that QLQX might probably improve cardiac energy metabolism in SHRs via increasing PPARs and PGC-1α.

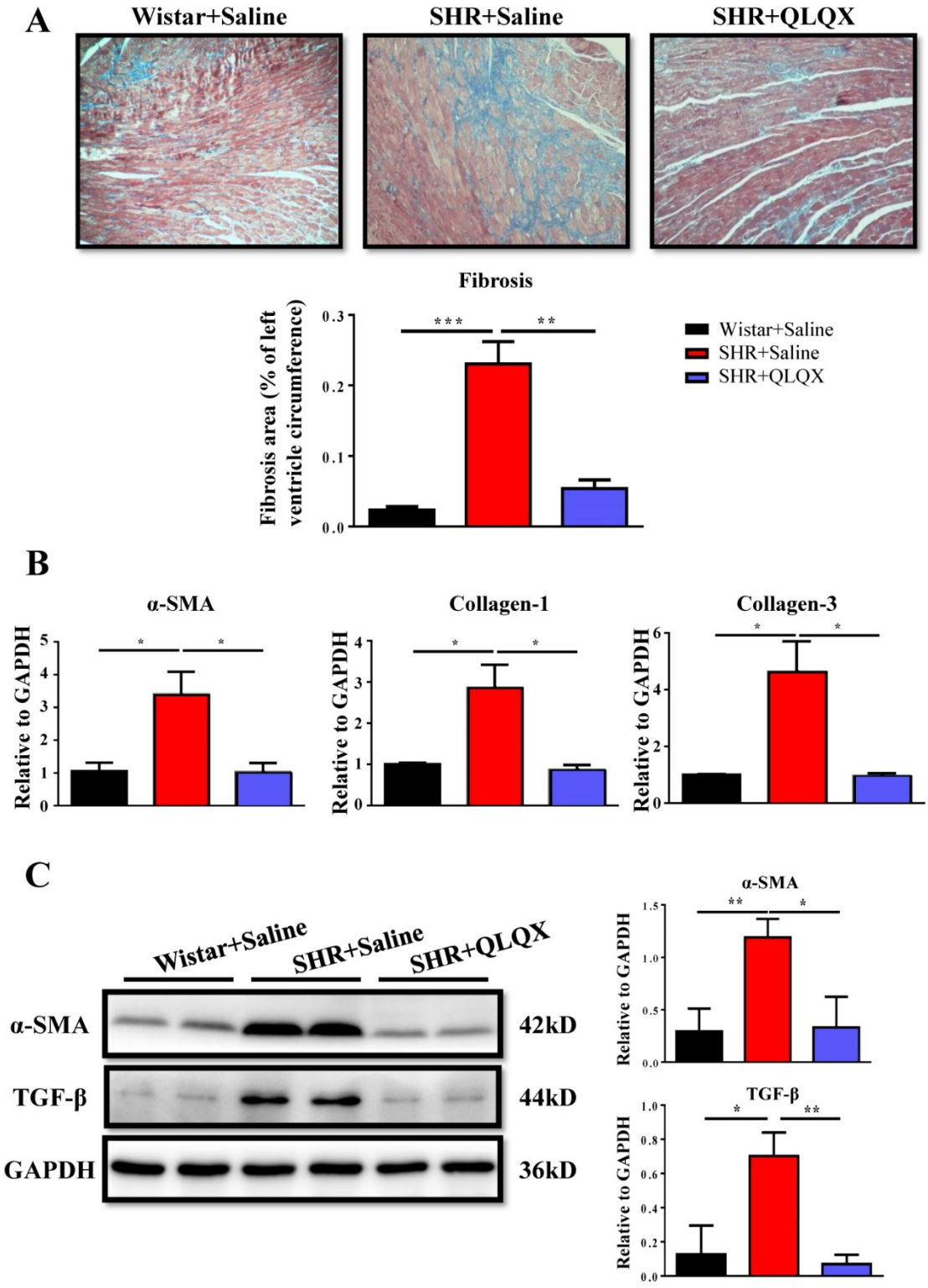

Figure 2. QLQX attenuates cardiac fibrosis in SHRs (A) QLQX decreased cardiac fibrosis in SHRs as determined by Masson's trichrome staining. (B) QLQX downregulated mRNA levels of α-SMA, collagen I, and collagen III in SHRs. (C) QLQX decreased protein levels of α-SMA and TGF-β in SHRs. N=3-4 per group. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

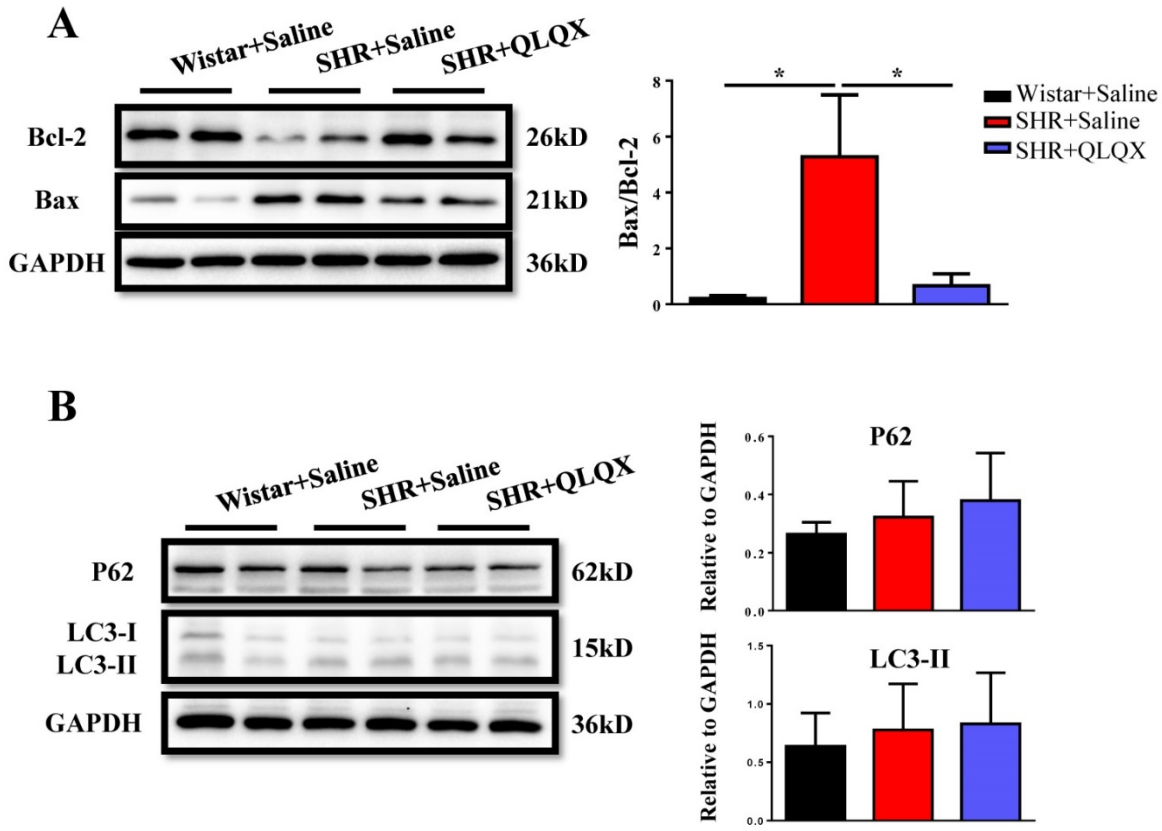

Figure 3. QLQX decreases cardiac apoptosis without affecting autophagy in SHRs QLQX reduced the ratio of Bax to Bcl-2 in SHRs. (B) QLQX did not affect the autophagic proteins including P62 and LC3. N=3-4 per group. *, *P*<0.05.

Discussion

Hypertension, which presents vascular sclerosis and left ventricle hypertrophy, will finally develop to cardiac decompensation if not effectively interfered [16]. To slow the progress of cardiac remodeling and heart failure induced by hypertension, the present therapeutic strategies lay emphasis on inhibiting β-adrenergic receptor, angiotensin II, and aldosterone. However the effects of these treatments are still limited for chronic phase of hypertension. Traditional Chinese medication has a certain foundation in clinic with a long history and may set a new direction for therapeutic strategies. Previous study has indicated that QLQX, a traditional Chinese medication, had beneficial effect on chronic heart failure patients and could prevent the heart from adverse remodeling [14, 17]. Here, in this study, we focused on the protective effect of QLQX in a rat model of genetic hypertension and found that QLQX could improve cardiac function and attenuate cardiac remodeling and fibrosis after one-year treatment.

The major findings of the present study are as follows. Firstly, QLQX could significantly improve cardiac function by preserving EF and FS in SHRs. In addition, the gene markers of pathological hypertrophy including ANP, BNP, and Myh7 were reduced by QLQX treatment. Secondly, QLQX alleviated cardiac fibrosis and apoptosis, and improved mitochondrial ultrastructure of cardiomyocytes in SHRs. Finally, QLQX restored the reduction of PPAR-α, PPAR-γ, and PGC-1α expressions in SHRs. Taken together, these data demonstrated that QLQX could protect against cardiac remodeling and dysfunction in hypertensive rats by increasing PPARs and PGC-1α.

Sustained hypertension progressively leads to left ventricular hypertrophy, cardiac fibrosis, and ultimately heart failure [18]. It was previously reported that QLQX was effective to improve cardiac function and reduce cardiac fibrosis in SHRs [15]. However, the effect of long-term treatment with QLQX in hypertension and the related molecular mechanisms remain largely unknown. Here, we demonstrated that treatment with QLQX for one year was effective to preserve EF and FS in SHRs, and prevent ventricular hypertrophy as well. Moreover, hypertension-induced cardiac fibrosis was greatly alleviated by QLQX, suggesting a potential protective effect of long-term treatment with QLQX on cardiac remodeling and dysfunction in hypertension.

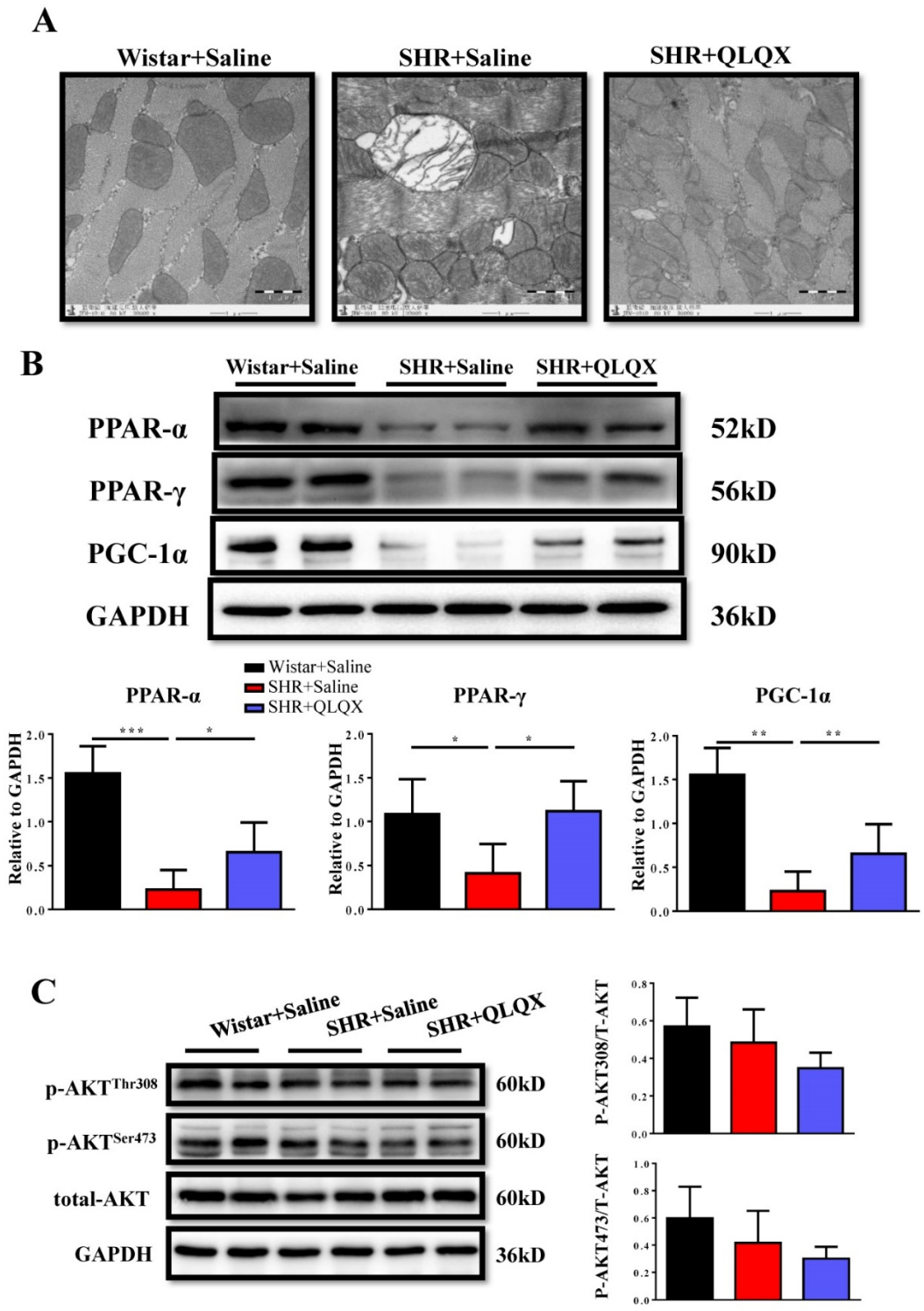

Figure 4. QLQX attenuates the damage of mitochondrial ultrastructure and restores the expression levels of PPAR-α, PPAR-γ, and PGC-1α in SHRs (A) QLQX preserved the mitochondria ultrastructure in SHRs as determined by transmission electron microscopy. (B) QLQX increased PPAR-α, PPAR-γ, and PGC-1α expression levels in SHRs. (C) QLQX did not affect the Akt signaling in SHRs. N=3-4 per group. *, *P*<0.05; **, *P*<0.01; ***, *P* <0.001.

Cardiomyocyte apoptosis is a hallmark induced by a variety of cardiac injuries including hypertension. Here we showed that treatment with QLQX for one year was effective to change the apoptotic proteins in SHRs. Additionally, QLQX was previously reported to reduce cardiomyocyte autophagy during 4 weeks of pressure overload in mice [8]. However, in the present study, the autophagic proteins were not modified in SHRs regardless of QLQX treatment. The differences between this study and ours are that we used different animal models of hypertension and treated

animals with QLQX for much longer period. Thus, the effect of QLQX in cellular autophagy during hypertension may be related to both disease stage and length of treatment.

Cardiac energy metabolism defect is an important mediator of remodeling progression and it also contributes to the development of myocardial diseases [19-21]. The peroxisome proliferator-activated receptor (PPAR) family has been confirmed to regulate cardiac energy metabolism at the gene expression level [22-25]. The PPAR family consists of PPAR-α, PPAR-δ, and PPAR-γ. It has been reported that activation of PPAR-γ prevented lipopolysaccharide-mediated cardiac dysfunction and that PGC-1α was required for QLQX-associated enhancement in cardiomyocyte mitochondrial energy metabolism [26, 27]. In our previous study, we also demonstrated that QLQX could improve cardiac function and attenuate cardiac remodeling after AMI by activating PPAR-γ [7]. Moreover, the regulation of PPAR family was associated with cardiac fibrosis and ventricular remodeling [28, 29]. In line with these studies, the present study found that QLQX treatment significantly attenuated the damage of myocardium mitochondrial ultrastructure in SHRs. Moreover, QLQX treatment upregulated the expression levels of PPAR-α, PPAR-γ, and PGC-1α in SHRs, suggesting that QLQX might deliver the protective effect on cardiac remodeling and dysfunction in SHRs through activation of PPARs and PGC-1α.

Several limitations of the present study should be highlighted. Firstly, it has been reported that activations of PPAR-α and PPAR-γ have beneficial effects on cardiac hypertrophy and fibrosis by regulating fibroblast proliferation and differentiation, however, the direct evidence for the role of PGC-1α in regulating cardiac fibrosis is still lacking [29, 30]. Secondly, though we confirmed the elevation of PPAR-α, PPAR-γ, and PGC-1α with QLQX treatment, it deserves to determine if the activation of PPAR pathway is necessary for the protective effects of QLQX in preventing cardiac remodeling and dysfunction in hypertension. Thirdly, as QLQX contains 11 distinct active components, which compound(s) in QLQX should be responsible for these protective effects observed in this study warrants further investigation.

Conclusion

In conclusion, our study demonstrates that QLQX protects against cardiac remodeling and dysfunction in spontaneously hypertensive rats by activation of PPARs and PGC-1α.

Acknowledgments

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Competing Interests

Dr. Xinli Li received research grants from Shijiazhuang Yiling Pharmaceutical Co., Ltd. All other authors have reported that they have no relationships to disclose.

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