Original Research *Highlight article*

Telmisartan improves myocardial remodeling by inhibiting leptin autocrine activity and activating PPARγ

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Impact statement

This study shows the crosstalk between local myocardial RAAS and leptin in hypertensive LVH rats; that Ang II induces myocardial remodeling by stimulating leptin autocrine activity by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway; and that telmisartan improves myocardial remodeling by inhibiting local Ang II-induced leptin autocrine activity and by inhibiting the leptin downstream signal STAT3 phosphorylation by activating PPAR-γ. These findings reveal novel molecular mechanisms by which telmisartan improves myocardial remodeling and could help to identify therapeutic targets for hypertensive LVH.

Abstract

The mechanism responsible for myocardial remodeling in hypertensive left ventricular hypertrophy (LVH) is complex. This study was designed to investigate the role of telmisartan in improving myocardial remodeling in hypertensive LVH and to explore the molecular mechanisms underlying the effects of telmisartan on hypertensive LVH. Hypertensive LVH was established in eight-week-old male Sprague–Dawley (SD) rats by abdominal aortic constriction. Telmisartan was intragastrically administered six weeks after surgery. Telmisartan improved cardiac dysfunction and myocardial fibrosis and reduced myocardial renin-angiotensin-aldosterone system (RAAS) activity and leptin levels in hypertensive LVH rats. To assess the mechanism underlying hypertensive LVH, cardiac fibroblasts were treated in vitro with angiotensin II (Ang II) or leptin, plus various inhibitors. Ang II stimulated leptin synthesis and secretion in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway. Leptin induced collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 pathway. Telmisartan improved collagen metabolism disorder by inhibiting leptin induced by local Ang II in an autocrine manner. Telmisartan also improved Ang II-induced collagen metabolism disorder by inhibiting STAT3 phosphorylation, a leptin downstream signal, by activating PPAR-γ. Telmisartan therefore improved myocardial remodeling in hypertensive LVH rats by acting as an AT1R antagonist, inhibiting leptin autocrine activity induced by local Ang II and by acting as a PPAR-γ agonist, inhibiting downstream leptin activation of STAT3 phosphorylation. These findings indicate the crosstalk between local myocardial RAAS and leptin and suggest a molecular mechanism by which telmisartan improves myocardial remodeling in hypertensive LVH.

Keywords: Hypertensive left ventricular hypertrophy, myocardial remodeling, angiotensin II, leptin, autocrine, telmisartan

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Introduction

Left ventricular hypertrophy (LVH), a type of target organ damage induced by hypertension, can gradually develop to cardiac insufficiency or even congestive heart failure. The mechanism of hypertensive LVH is complex, involving various factors, such as hemodynamic disorders, the renin-angiotensin-aldosterone system (RAAS), and insulin resistance.[1–3] Leptin, an adipokine targeting multiple organs, can activate JAK2/STAT3, MAPK, PI3K, and other signal transduction pathways, exerting a wide range of biological effects in various tissues and organs.[4] Several clinical and experimental studies have shown that leptin is also closely associated with hypertensive LVH and can induce cardiac remodeling by activating the JAK or MAPK signaling pathway.[5–8]

Angiotensin II (Ang II), the primary active molecule in the RAAS, plays an important role in the development of hypertensive LVH.[9,10] Ang II can bind to the Ang II type 1

receptor (AT1-R), inducing a conformational change in the latter, with the active format of AT1-R stimulating various signaling pathways, including those involving extracellular signal-regulated kinase (ERK) and Janus family kinase (Jak). These pathways induce the expression of immediate-early genes (e.g. c-fos, c-jun) and the later-induced fetal-type genes (e.g. TGF-β1 smad3), followed by the induction of protein synthesis and cardiac remodeling.[11–13] Ang II has also been shown to induce leptin synthesis in cardiomyocytes.[14] The leptin signaling pathway was found to be essential for the Ang II-induced development of atrial fibrosis.[15] Leptin antagonists could attenuate Ang II-induced cardiac remodeling.[16] These findings suggested that leptin may be a key bridging molecule in the Ang II-induced development of myocardial remodeling in hypertensive LVH, and that Ang II may promote myocardial remodeling by enhancing leptin autocrine activity.

Telmisartan, an AT1R antagonist with partial PPAR-γ agonist activity, is commonly used to treat hypertension. Telmisartan can also reverse myocardial remodeling and improve LVH in hypertension.[17–19] Moreover, our previous results suggested that telmisartan could reduce perirenal adipose tissue-derived leptin paracrine activity and exert an indirect renoprotective effect on MetS rats by restoring the balance between angiotensin II-AT1R and angiotensin-converting enzyme 2-angiotensin (1–7)-Mas axes.[20] The present study therefore investigated whether telmisartan can reverse myocardial remodeling by reducing leptin autocrine activity induced by local Ang II in rats with hypertensive LVH, and assessed the potential molecular mechanisms underlying the effects of telmisartan on hypertensive LVH.

Methods

Experimental animals and treatment

Male Sprague–Dawley (SD) rats (weight: 180–230 g), purchased from the School of Medicine of Xi'an Jiao Tong University (Xi'an, Shaanxi, China), were randomly divided into three groups of eight rats each, a sham-operated group, an LVH group, and an LVH+TEL group. LVH was generated by pressure-overload resulting from abdominal aortal constriction. Beginning six weeks after surgery, rats in the LVH+TEL group were intragastrically administered 10 mg kg^{-1} day^{-1} telmisartan at noon for six weeks. All animal experiments conformed to "The Guidelines for Experimental Animal Care and Use," established by the National Institutes of Health of the United States in 1996, and to the "Experimental Animal Management Regulations", published by the State Science and Technology Commission of China in 2004.

Generation of hypertension in rats

All rats were fasted for 12 h before the operation. Full anesthesia was induced by intraperitoneal injection of 10% chloral hydrate (100–200 mg kg^{-1}), and the abdominal aorta was exposed layer by layer. Eight cm-long 4–0 silk sutures were passed underneath the abdominal aorta between the origins of the right and left renal arteries. A loop 3 mm in diameter was made by a loose double suture knot across the abdominal aorta. An "L"-shaped blunted 22G injection needle was placed inside the loop and parallel to the abdominal aorta. The loose double suture knot was tightened around the abdominal aorta and the needle. The needle was withdrawn immediately, creating a fixed constriction, 0.7 mm in diameter, in the abdominal aorta. Penicillin (400,000 U ml^{-1}) was injected into the abdominal cavity, which was closed layer by layer, followed by daily intraperitoneal injections of penicillin for three consecutive days to prevent infection. The abdominal aorta was not ligated in the sham-operated group, but the remaining steps were the same as those used to generate LVH. Subsequently, systolic blood pressure was measured in the tail artery of each rat three times every two weeks using a BP-2000 tail cuff with a programmed electrosphygmomanometer, and the systolic blood pressure in each rat was averaged. Successful establishment of abdominal aortic coarctation in hypertensive rats was defined as a $>$50 mmHg difference in systolic blood pressure between the surgery and sham-operated groups.

Determination of Ang II, aldosterone, and leptin concentrations

Arterial blood samples were collected and centrifuged at 3000*g* for 20 min, and the supernatants were collected and stored at −80°C. Portions of the left ventricular muscle tissues were obtained, and blood and flushing fluid were absorbed. The tissues were weighed, and each sample was immersed in 1 ml physiological saline containing protease and peptidase inhibitors. The tissues were sufficiently ground, homogenized, and centrifuged at 4°C and 3000 r/min for 10 min. The supernatants were collected and stored at −80°C. The concentrations of Ang II, ALD (aldosterone), and leptin in these samples were determined with radioimmunoassay (RIA) kits (Linco Research, Inc., St. Charles, MO, USA).

Echocardiographic examination

After 12 weeks, the rats were fully anesthetized with 10% chloral hydrate (100–150 mg kg^{-1}). Echocardiographic parameters in the short-axis view at the level of the left ventricular papillary muscle were measured using a PHILIPS 7500 color Doppler ultrasound instrument (Philips, Amsterdam, the Netherlands). Each rat was monitored through three cardiac cycles, and the average values were calculated. Parameters measured included end-diastolic left ventricular posterior wall thickness, end-diastolic interventricular septal thickness, left ventricular end-diastolic diameter, left ventricular ejection fraction, early diastolic peak velocity, late diastolic peak velocity, early diastolic filling peak velocity (E peak), late diastolic filling peak velocity (A peak), and heart rate.

Hemodynamic monitoring

The rats were anesthetized with 2% sodium pentobarbital and were fixed on an animal station. A catheter filled with heparinized saline was connected to the BL-420F biological

and functional experimental system, and inserted into the left ventricle via the right carotid artery. Data recorded included left ventricular systolic pressure (LVSP), the maximum rising rate of LVSP (dp/dtmax), the maximum descent rate of LVSP (dp/dtmin), and left ventricular end-diastolic pressure (LVEDP).

Determination of metabolism-related parameters of collagen fibers

Arterial blood samples were centrifuged at 3000*g* for 20 min, and the supernatants were collected and stored at −80°C. The concentrations of matrix metalloproteinases (MMP)-1, -2, and -9, and tissue inhibitor of metalloproteinase-1 (TIMP-1) were measured using enzyme-linked immunosorbent assay (ELISA) kits obtained from American CUSBIO (Barksdale, DE, USA), whereas the concentrations of procollagen type I carboxyterminal peptide (PICP) and type I collagen carboxyterminal cross-linked peptide were measured using ELISA kits obtained from American R&D (Muskogee, OK, USA). The concentrations of myocardial hydroxyproline (HYP) were measured after alkaline hydrolysis. Because the oxidation products generated by HYP in the presence of oxidants reacted with dimethylaminobenzaldehyde to form a purplish-red color, myocardial HYP content could be calculated based on the depth of color.

Rat left ventricular myocardial pathology

Left ventricular myocardial specimens obtained from each group of rats were sectioned transversely parallel to the coronary sulcus. The degree of myocardial fibrosis was determined by Masson staining, with the myocardial cells being red and collagen being blue. Five sections of each specimen were randomly selected, and four views of each section situated at a distance from the papillary muscle and perivascular collagen were randomly chosen. The myocardial collagen volume fraction of each sample was calculated using Image J photographic software (National Institutes of Health). All microscopic examinations and quantifications were performed by two investigators in a blinded manner.

Immunohistochemistry

Samples were immunohistochemically stained using a DAKO Catalyzed Signal Amplification (CSA) system (DAKO, Carpinteria, CA, USA), according to the manufacturer's instructions. Tissue cross-sections (4 μm), including the left ventricle and interventricular septum, were stained with rabbit polyclonal anti-leptin antibody (cat. no. ab3583, 1:100) (Abcam, MA, USA) in Tris-buffered saline for 15 min at room temperature, followed by counterstaining with hematoxylin. Five sections per specimen were randomly selected, and four views of each section situated at a distance from the papillary muscle and perivascular collagen were randomly chosen. The integral optical density of the positively stained area of each specimen was calculated using Image J photographic software (National Institutes of Health). All microscopic examinations and quantifications were performed by two investigators in a blinded manner.

Cell culture and treatments

Rat primary cardiac fibroblasts from neonatal SD rats (aged 1–3 days), with >95% being vimentin-positive and α-smooth muscle actin-negative, were purchased from Jiangsu Qi's Biological Co. Ltd. The cells were cultured in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C, in an atmosphere containing 5% CO_2 and saturated humidity. Cell growth state was monitored daily, and the medium changed every two days. Cells reaching 80–90% confluence were sub-cultured at a 1:3 dilution for further proliferation, and cells from passages 2–3 were used in all experiments. Cells attaining 50–60% confluence were cultured in serum-free medium for 12 h prior to intervention. The cells were pretreated for 1 h with the leptin antagonist Leptin tA (PROSPEC, Israel), the AT1R antagonist telmisartan, the ROS scavenger NAC, the ROS-producing agent Dp44mT, pioglitazone (Sigma, St. Louis, MO, USA), the ERK1/2 inhibitor PD98059, the ERK1/2 agonist C6 ceramide, the p38 inhibitor SB205580, the JNK inhibitor SP600125, the AP-1 inhibitor SR11302, the JAK2 inhibitor AG-490, the STAT3 inhibitor S3I-201, or the PPAR-γ inhibitor GW9662 (Selleck Chemicals LLC, USA) prior to treatment with Ang II (Sigma) or leptin (PeproTech, Rocky, USA) for 48 h.

Determination of proteins secreted by cardiac fibroblasts

Cardiac fibroblasts were cultured in serum-free DMEM containing 50 μg/ml ascorbic acid. After the experimental treatment was performed, the supernatants were obtained, and the concentrations of secreted type I collagen (BosterBiological Technology, Wuhan, China) and TGF-β1 (American R&D, Muskogee, OK, USA) were measured by ELISA.

siRNA transfection

Rat ERK siRNA sequences and negative control siRNA sequences were designed and synthesized by Shanghai Jike Gene Biotechnology Co., Ltd. Myocardial fibroblasts were seeded in six-well plates, and transfection with siRNA in X-tremeGENE siRNA Transfection Reagent (Roche Diagnostics, Indianapolis, IN, USA) started when they reached 70–80% confluence. Briefly, reaction solution A, consisting of 5 μl CON siRNA/ERK siRNA and 250 μl serum-free DMEM, was completely mixed with reaction solution B, consisting of 5 μl transfection reagent and 250 μl serum-free DMEM, and the mixture was incubated at room temperature for 5 min. The cells were subsequently transfected with the mixed transfection liquid in 1 ml fresh culture medium per well, followed by further culture for 24 h prior to treatment with Ang II for 48 h.

AP-1 transcriptional activity

The luciferase reporter assay was used to detect the AP-1 transcriptional activity. Cardiac fibroblasts were

co-transfected with luciferase reporter gene (AP-1) constructs and β-galactosidase using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, MA, USA) for 24 h according to the manufacturer's protocol. Following incubation in the presence or absence of inhibitors for 1 h, the cells were treated with 10^{-5} M Ang II for 48 h at 37°C. Luciferase activity was measured using a luciferase assay kit (Promega, Madison, WI, USA).

RNA preparation and quantitative RT-PCR

Total RNA was extracted from cardiac fibroblasts using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), and each 500 ng aliquot of RNA was reverse transcripted to cDNA using PrimeScript™ RT Master Mix (Takara, Tokyo, Japan). SYBR Green quantitative PCR assays were performed using the MX3000P Multiplex Quantitative PCR System (Agilent Technologies, Palo Alto, CA, USA), the Brilliant SYBR Green QPCR Master Mix Kit (Agilent Technologies), and primers specific for rat leptin (forward, 5′-CCAAAACCCTCATCAAGACC-3′; reverse, 5′-GTCCAACTGTTGAAGAATGTCCC-3′) and rat GAPDH (forward, 5′-AACGACCCCTTCATTGAC-3′; reverse, 5′-TCCACGACATACTCAGCAC-3′). The real-time PCR conditions for both genes were 5 min at 95°C, 50 cycles with 30 s at 95°C, 1 min at 63°C, 1 min at 72°C, and a final elongation step for 10 min at 72°C. The relative quantities of cDNA were calculated from cycle thresholds (Ct) and were normalized to GAPDH gene using the $2^{-\Delta\Delta Ct}$ method.

Protein extraction and Western blotting

Left ventricular muscle tissues and cardiac fibroblasts protein extracts were prepared using RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and various protease and phosphatase inhibitors: sodium vanadate, leupeptin, and PMSF (Heart Biological Technology, Xian, China). The samples were incubated at 4°C for 15 min and then centrifuged at 14,000 r/min for 15 min. To prepare cytoplasmic and nuclear protein extracts, 1×10^7 cardiac fibroblasts were washed once with PBS and re-suspended in 500 µl Buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 5 mM PMSF, pH 7.9). After 10 min, the nuclei were collected by centrifugation for 10 min at 12,000 r/min at 4°C, with the supernatants defined as cytoplasmic proteins. Nuclear proteins were extracted by incubating nuclei with 500 µl buffer B (10 mM HEPES, 100 mM NaCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 5 mM PMSF, pH 7.9) for 20 min at 4°C. The samples were centrifuged at 12,000 r/min at 4°C for 10 min, with the supernatants defined as nuclear proteins. Protein concentrations were measured by the bicinchoninic acid method.

For Western blotting, 50 µg of protein samples were loaded onto 8–12% Mini-PROTEAN TGX Stain Free protein gels (Bio-Rad, Hercules, CA, USA); following electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes. The membranes were blocked by incubation with 5% nonfat milk for 2 h, followed by incubation overnight at 4°C with specific primary antibodies against AP-1 (c-JUN) (cat. no. #9165, 1:1000), p-Smad3 (cat. no. #9520, 1:1000), Smad3 (cat. no. #9523, 1:1000), p-JAK2 (cat. no. #3776, 1:1000), JAK2 (cat. no. #3230, 1:1000), p-STAT3 (cat. no. #9134, 1:1000), STAT3 (cat. no. #4904, 1:2000) (all from Cell Signaling Technology, Beverly, MA, USA), type 1 collagen (COL 1A1) (cat. no. sc-293182, 1:1000), TGF-β1 (cat. no. sc-65378, 1:1000), PPAR-γ (cat. no. sc-7273, 1:500) (all from Santa Cruz Biotechnologies, CA, USA), MMP-1 (cat. no. ab137332, 1:2000), TIMP-1 (cat. no. ab38978, 1:2000), leptin (cat. no. ab3583, 1:500), PCNA (cat. no. ab92552, 1:5000), and β-actin (cat. no. ab8227, 1:5000) (all from Abcam, MA, USA). The membranes were washed thrice and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (cat. no. ab205718, 1:10,000) or (cat. no. ab205719, 1:10,000) (both from Abcam) for 1 h at room temperature. After three washes, protein was detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore Sigma, MO, USA). Western blotting images were quantified using Image J photographic software (National Institutes of Health).

Statistical analysis

All measured data are presented as means ± SEM. Differences between two groups were compared by independent t-tests, and differences among >2 groups by one way ANOVA, followed by Scheffe's multiple comparison test. All experiments were performed at least three times to ensure that their results were consistent and reproducible. A P-value <0.05 was considered statistically significant.

Results

Telmisartan improves cardiac function and myocardial fibrosis in hypertensive LVH rats

Four weeks after abdominal aortic coarctation, the mean systolic blood pressure of the tail artery was 50 mmHg higher in the LVH than in the sham-operated group ($P < 0.05$), suggesting that surgery induced hypertension in these rats (Supplementary Figure 1). As expected, the AT1R antagonist telmisartan significantly reduced the blood pressure elevation resulting from abdominal aortic coarctation. Moreover, heart mass (HM), left ventricular mass (LVM), heart mass index (HMI), and left ventricular mass index (LVMI) were significantly higher in the rats that had undergone abdominal aortic coarctation than in the sham-operated group at the end of 12 weeks ($P < 0.05$ each), suggesting that surgery resulted in hypertensive LVH (Supplementary Table 1). Telmisartan treatment was found to improve LVH in hypertensive rats.

Compared with the sham-operated group, left ventricular diastolic dysfunction was significantly depressed in hypertensive LVH rats, as shown by reductions in dp/dtmin, Ve/Va, and E/A, and increased left ventricular end-diastolic pressure (all $P < 0.05$) (Table 1). In addition, hypertensive LVH rats showed a collagen metabolic imbalance, as shown by reduced degradation of normal collagen (MMP-1), increased degradation of denatured collagen (MMP-2 and MMP-9), and increases in tissue inhibitor of metalloproteinase (TIMP), collagen fiber synthesis (PICP),

and total collagen metabolism (HYP) (all $P < 0.05$) (Table 2). Telmisartan treatment, however, significantly improved left ventricular diastolic dysfunction and balanced collagen metabolism in rats with hypertensive LVH.

To explore the possible links between RAAS and leptin, the concentrations of systemic and myocardial Ang II, ALD, and leptin were measured in hypertensive LVH rats. Compared with the sham-operated group, rats in the hypertensive LVH group showed significant increases in systemic and local myocardial Ang II, ALD, and leptin concentrations (all $P < 0.05$) (Table 3). Treatment of hypertensive LVH rats with the AT1R antagonist telmisartan significantly increased serum Ang II while significantly reducing serum ALD and leptin concentrations, as well as significantly reducing local myocardial Ang II, ALD, and leptin concentrations, indicating a close relationship between local myocardial RAAS and leptin. The finding, that telmisartan increased serum Ang II while reducing myocardial Ang II, may have been due to its inhibition of peripheral tissue AT1R, which stimulated the release into serum of Ang II by a feedback mechanism.

The degree of myocardial fibrosis (increased collagen volume fraction) was greater and the myocardial levels of in situ leptin expression were higher in the hypertensive LVH than in the sham-operated group (all $P < 0.05$) (Figure 1(a)). Western blotting showed significant collagen metabolic disorder, including increased type 1 collagen (COL 1A1) and TIMP-1 levels and decreased MMP-1 levels, resulting from the activation of the leptin-JAK2/STAT3-TGF-1/Smad3 signaling pathway, in the myocardium of hypertensive LVH rats (all $P < 0.05$) (Figure 1(b)). Telmisartan, however, significantly inhibited the activation of the leptin-JAK2/STAT3-TGF-1/Smad3 signaling pathway and improved myocardial fibrosis in hypertensive LVH rats.

These in vivo results showed that the AT1R antagonist telmisartan could inhibit local myocardial RAAS and the leptin signaling pathway, as well as improve cardiac function and myocardial remodeling in hypertensive LVH rats. These findings suggest that the close crosstalk between local myocardial RAAS and leptin signaling pathway promotes myocardial remodeling in hypertensive LVH rats. To determine whether telmisartan can improve myocardial remodeling by balancing the link between local myocardial RAAS and leptin, we explored the molecular relationship between myocardial RAAS and leptin in vitro.

Table 1. Effect of telmisartan on cardiac function in hypertensive LVH rats.

	HR (b.p.m.)	LVSP (mm Hg)	dp/dtmax (mm Hgs^{-1})	dp/dtmin (mm Hgs^{-1})	LVEDP (mmHg)	LVPWTd (mm)	IVSTd (mm)	LVEDD (mm)	LVEF (%)	Ve/Va	E/A
Sham	362 ± 21	116.32 ± 4.16	5917.95 ± 411.33	−4513.52 ± 249.24	8.16 ± 3.73	1.42 ± 0.11	1.36 ± 0.08	6.58 ± 0.41	75.12 ± 6.84	1.28 ± 0.25	1.22 ± 0.14
LVH	370 ± 23	168.31 ± 2.75*	5809.70 ± 198.88	−3241.18 ± 169.54*	16.47 ± 3.28*	1.63 ± 0.17*	1.54 ± 0.09*	7.96 ± 0.67*	78.11 ± 5.18	0.74 ± 0.16*	0.56 ± 0.09*
LVH+TEL	358 ± 26	130.41 ± 2.41*,#	6028.56 ± 208.47	−4198.36 ± 188.76*,#	12.49 ± 2.16*,#	1.49 ± 0.14#	1.45 ± 0.12*,#	6.97 ± 0.51*,#	77.76 ± 4.32	0.98 ± 0.17*,#	0.89 ± 0.11*,#

Note: Data reported as mean ± SEM; *$P < 0.05$ vs. sham group; #$P < 0.05$ vs. LVH group.
Sham: sham-operated rats (n = 8); LVH: rats with hypertensive left ventricular hypertrophy (n = 8); LVH+TEL: rats with hypertensive left ventricular hypertrophy treated with telmisartan (n = 8); HR: heart rate; LVSP: left ventricular systolic pressure; dp/dtmax: maximum rising rate of LVSP; dp/dtmin: maximum descent rate of LVSP; LVEDP: left ventricular end diastolic pressure; LVPWTd: end-diastolic left ventricular posterior wall thickness; IVSTd: end-diastolic interventricular septal thickness; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; Ve: early diastolic peak velocity; Va: late diastolic peak velocity; E: early diastolic filling peak velocity (E peak); A: late diastolic filling peak velocity (A peak).

Table 2. Effect of telmisartan on metabolism-related parameters of collagen fibers in hypertensive LVH rats.

	MMP-1 (pg/ml)	MMP-2 (ng/ml)	MMP-9 (pg/ml)	TIMP-1 (ng/ml)	MMP9/TIMP1	PICP (ng/ml)	ICTP (ng/ml)	PICP/ICTP	HYP (μg/g)
Sham	40.94 ± 9.13	13.02 ± 2.43	261.44 ± 14.51	3.84 ± 1.16	0.066 ± 0.016	19.51 ± 1.12	3.88 ± 0.77	5.12± 0.58	165.78 ± 19.44
LVH	28.19 ± 6.47*	15.94 ± 2.44*	354.75 ± 14.13*	10.01 ± 2.21*	0.035 ± 0.008*	32.17 ± 8.87*	4.11 ± 0.78	7.91 ± 0.91*	634.74 ± 40.17*
LVH+TEL	35.69 ± 8.11*,#	12.98 ± 2.73#	308.75 ± 19.48*,#	6.01 ± 1.87*,#	0.052 ± 0.011*,#	25.34 ± 5.76*,#	3.91 ± 0.84	6.52 ± 0.87*,#	378.68 ± 30.14*,#

Note: Data reported as mean ± SEM; *$P < 0.05$ vs. sham group; #$P < 0.05$ vs. LVH group.
Sham: sham operated rats (n = 8); LVH: rats with hypertensive left ventricular hypertrophy (n = 8); LVH+TEL: rats with hypertensive left ventricular hypertrophy treated with telmisartan (n = 8); MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase; PICP: procollagen type I carboxy-terminal peptide; ICTP: type I collagen carboxy-terminal cross-linked peptide; HYP: hydroxyproline.

Table 3. Effect of telmisartan on systemic and myocardial RAAS and leptin in hypertensive LVH rats.

	Serum			Myocardial tissue		
	Ang II (pg/ml)	ALD (pg/ml)	Leptin (ng/ml)	Ang II (pg/ml)	ALD (pg/ml)	Leptin (ng/ml)
Sham	150.12 ± 40.15	710.23 ± 84.12	2.96 ± 0.75	70.12 ± 8.74	33.48 ± 5.79	0.97 ± 0.05
LVH	408.74 ± 80.11*	988.15 ± 96.14*	10.71 ± 1.12*	150.44 ± 13.19*	75.69 ± 9.66*	3.19 ± 0.16*
LVH+TEL	474.58 ± 60.58*,#	746.87 ± 71.23#	4.53 ± 0.99*,#	80.17 ± 10.19#	41.16 ± 6.74#	2.06 ± 0.37*,#

Note: Data reported as mean ± SEM; *$P < 0.05$ vs. sham group; #$P < 0.05$ vs. LVH group.
Sham: sham operated rats (n = 8); LVH: rats with hypertensive left ventricular hypertrophy (n = 8); LVH+TEL: rats with hypertensive left ventricular hypertrophy treated with telmisartan (n = 8); Ang II: angiotensin II; ALD: aldosterone.

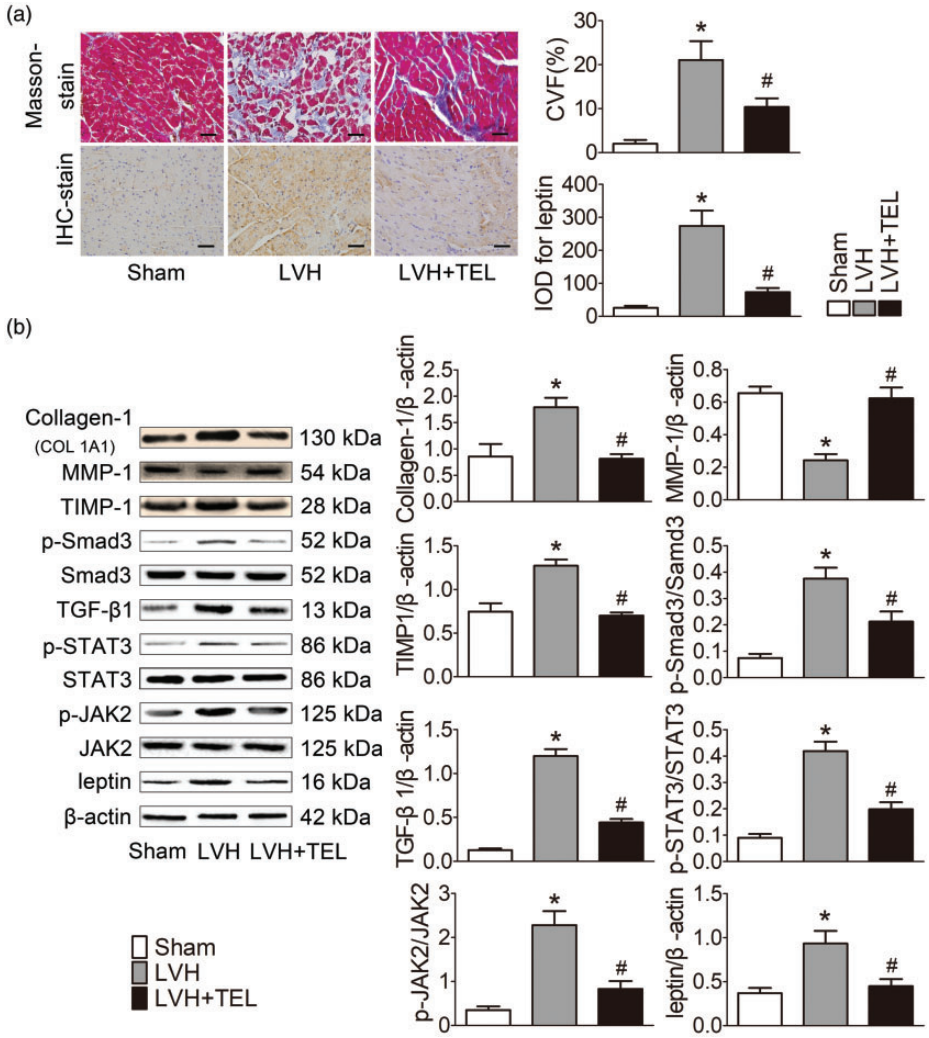

Figure 1. Telmisartan improves myocardial fibrosis and inhibits leptin signaling pathways in the myocardium of hypertensive LVH rats. (a) Masson staining and immunohistochemical staining for leptin of myocardium of the three groups of rats. Results are reported as CVF and IOD for leptin. Original magnification, ×400; bar, 50 μm. (B) Western blotting showing the expression of myocardium tissue collagen-1 (COL 1A1), MMP-1, TIMP-1, leptin/JAK2/STAT3, and TGF-β1/Smad3 signaling pathway proteins, with expression normalized to that of β-actin, JAK2, STAT3, or Smad3 in the same samples. Sham, sham-operated rats (n = 8); LVH, rats with hypertensive left ventricular hypertrophy (n = 8); LVH+TEL, rats with hypertensive left ventricular hypertrophy treated with telmisartan (n = 8). Data are reported as mean ± SEM; *$P < 0.05$ vs. Sham group; #$P < 0.05$ vs. LVH group. (A color version of this figure is available in the online journal.)
IHC: immunohistochemical; CVF: collagen volume fraction; IOD: integral optical density; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase.

Ang II stimulates leptin synthesis and secretion in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway

Using cardiac fibroblasts, we found that Ang II-stimulated leptin synthesis and secretion in a concentration- and time-dependent manner (Supplementary Figure 2). To explore the mechanisms by which Ang II stimulates leptin synthesis and secretion, we used the AT1R antagonist telmisartan, the ROS scavenger NAC, the ERK1/2 inhibitor PD98059, the ROS-producing agent Dp44mT, the ERK1/2 agonist C6 ceramide, and siRNA sequences of ERK 1/2 to inhibit or activate signaling pathways downstream of Ang II in cardiac fibroblasts (Figure 2(a) to (c)). Pretreatment of these cells with telmisartan, NAC, or PD98059 reduced the Ang II-stimulated leptin expression (Figure 2(a)). Only PD98059 was able to inhibit the increased expression of leptin induced by Dp44mT, whereas neither telmisartan nor NAC could inhibit the C6 ceramide-induced increase in leptin expression (Figure 2(b)). These findings indicate that Ang II can stimulate leptin expression in cardiac fibroblasts by activating the AT1R-ROS-ERK1/2 signaling pathway, confirmed by assessing the effects of ERK 1/2 siRNA (Figure 2(c)).

To further explore the mechanism by which Ang II increases leptin synthesis of cardiac fibroblasts, we assessed the effects of Ang II on the expression of the classical transcriptional activator, activation protein 1 (AP-1; assessed by the levels of one of its subunits c-JUN). Ang II increased the expression of AP-1 (c-JUN) in cardiac fibroblasts, but this expression was inhibited by the ROS scavenger NAC and the ERK1/2 inhibitor PD98059, but not by the p38 inhibitor SB205580 or the JNK inhibitor SP600125 (Figure 2(d)). Ang II enhanced AP-1 (c-JUN) expression in cell nuclei, but this enhancement was inhibited by NAC and PD98059. By contrast, Ang II had no effect on AP-1 (c-JUN) expression in the cytoplasm (Figure 2(e)). Ang II

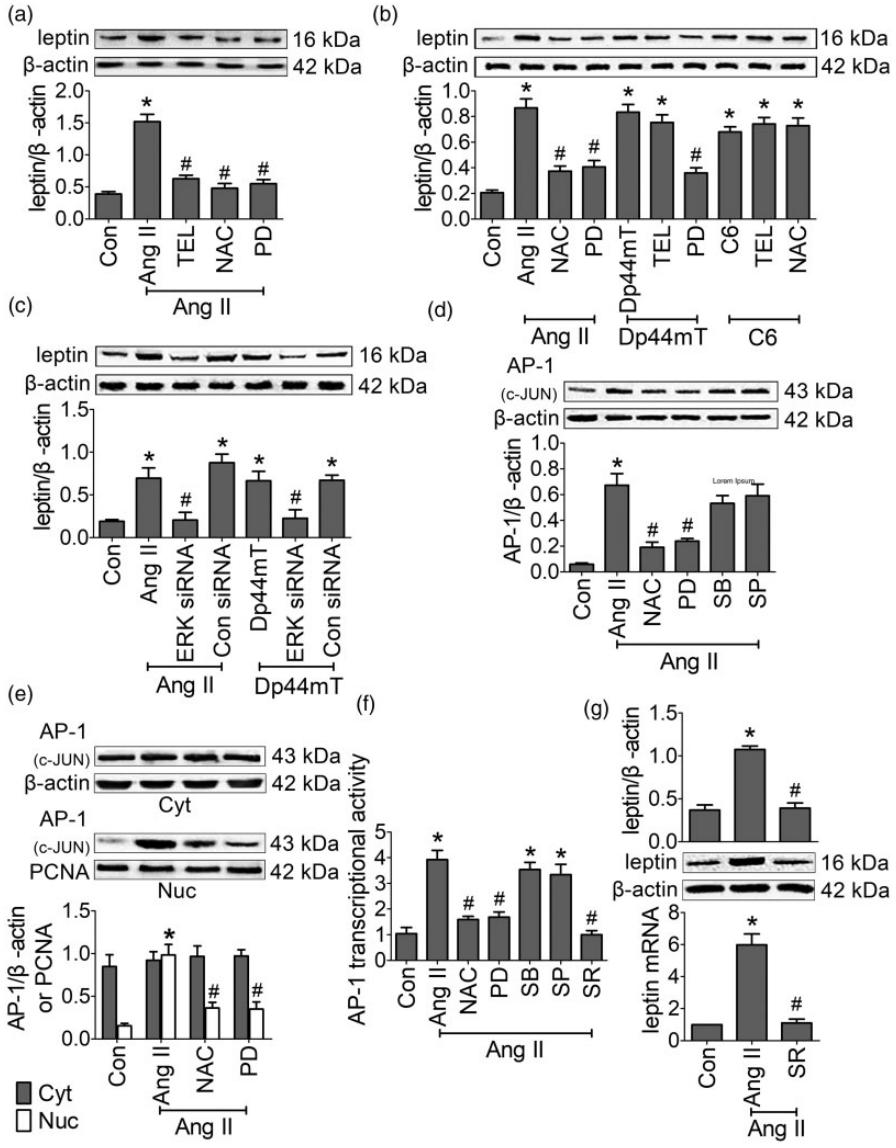

Figure 2. Ang II stimulates leptin synthesis and secretion in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway. (a to c) Western blotting showing the expression of leptin in cardiac fibroblasts normalized to expression of β-actin in the same samples. Cardiac fibroblasts were pretreated with the AT1R antagonist telmisartan, the ROS scavenger NAC, or the ERK1/2 inhibitor PD98059 for 1 h or with siRNA for 24 h, before treatment with Ang II, the ROS-producing agent Dp44mT, or the ERK1/2 agonist C6 ceramide for 48 h. (d) Western blotting of AP-1 (c-JUN) by cardiac fibroblasts normalized to β-actin. Cardiac fibroblasts were pretreated with the ROS scavenger NAC, the ERK1/2 inhibitor PD98059, the p38 inhibitor SB205580, or the JNK inhibitor SP600125 for 1 h before treatment with Ang II for 48 h. (e) Western blotting of AP-1 (c-JUN) expression in the cytoplasm and nuclei of cardiac fibroblasts normalized to expression of β-actin or PCNA, respectively. Cardiac fibroblasts were pretreated with the ROS scavenger NAC or the ERK1/2 inhibitor PD98059 for 1 h, followed by treatment with Ang II for 48 h. (f) AP-1 transcriptional activity of cardiac fibroblasts evaluated by luciferase reporter assay. Cardiac fibroblasts pretreated with the ROS scavenger NAC, the ERK1/2 inhibitor PD98059, the p38 inhibitor SB205580, the JNK inhibitor SP600125, or the AP-1 inhibitor SR11302 for 1 h were incubated with Ang II for 48 h. (g) Level of leptin protein expression and mRNA transcription in cardiac fibroblasts, as determined by Western blotting and quantitative RT-PCR, respectively. Cardiac fibroblasts were incubated in the presence or absence of the AP-1 inhibitor SR11302 for 1 h before treatment with Ang II for 48 h. Ang II: angiotensin II; AP-1: activation protein 1. N = 4–6. Data are reported as mean ± SEM; *$P < 0.05$ vs. control group; #$P < 0.05$ vs. Ang II, Dp44mT or C6 ceramide group.

also increased AP-1 transcriptional activity in cardiac fibroblasts, an increase inhibited by NAC, PD98059, or the AP-1 inhibitor SR11302, but not by SB205580 or SP600125 (Figure 2(f)). SR11302 significantly inhibited leptin synthesis and expression induced by Ang II in cardiac fibroblasts ($P < 0.05$) (Figure 2(g)). These results show that Ang II can stimulate leptin synthesis and secretion in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway, revealing the molecular crosstalk between local myocardial RAAS and leptin.

Leptin induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 pathway

TGF-β1 is a classic factor that promotes myocardial fibrosis. Treatment of cardiac fibroblasts with leptin stimulated TGF-β1 expression and secretion in a concentration- and time-dependent manner (Supplementary Figure 3). Leptin treatment also enhanced the expression of type 1 collagen (COL 1A1) and TIMP-1, reduced the expression of MMP-1, and activated the JAK2/STAT3-TGF-1/Smad3 signaling

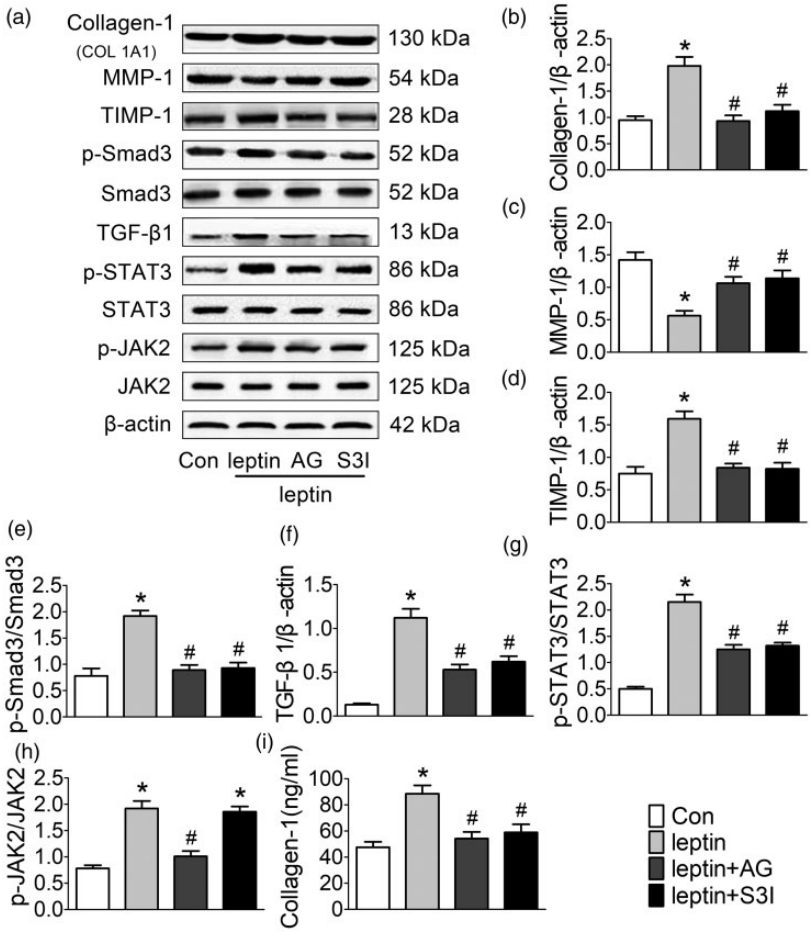

Figure 3. Leptin induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 pathway. Cardiac fibroblasts were pretreated with the JAK2 inhibitor AG-490 or the STAT3 inhibitor S3I-201 for 1 h before treatment with leptin (100 ng/ml) for 48 h. (a) Expression of collagen-1 (COL 1A1), MMP-1, TIMP-1, JAK2/STAT3, and TGF-β1/Smad3 signaling pathway proteins by Western blotting. (b–h) Graphs showing Western blotting-determined expression of collagen-1 (COL 1A1), MMP-1, TIMP-1, JAK2/STAT3, and TGF-β1/Smad3 signaling pathway proteins normalized to β-actin, JAK2, STAT3, or Smad3 expression in the same samples. (i) Collagen-1 concentrations in the supernatants of cardiac fibroblasts determined by ELISA. N = 4–6. Data are reported as mean ± SEM; *$P < 0.05$ vs. control group; #$P < 0.05$ vs. leptin group.

pathway in cardiac fibroblasts (Figure 3(a) to (h)). Leptin also increased the secretion of type 1 collagen by cardiac fibroblasts (Figure 3(i)). The JAK2 inhibitor AG-490 and the STAT3 inhibitor S3I-201 improved leptin-induced collagen metabolism disorder (Figure 3), indicating that leptin induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 signaling pathway.

Telmisartan improves collagen metabolism disorder in cardiac fibroblasts by inhibiting leptin autocrine activity induced by local Ang II

Ang II increased type 1 collagen (COL 1A1) expression in cardiac fibroblasts in a concentration- and time-dependent manner (Supplementary Figure 4(a)), an increase significantly inhibited by the leptin antagonist Leptin tA ($P < 0.05$) (Supplementary Figure 4(b)), indicating that Ang II enhances collagen expression in cardiac fibroblasts via a leptin pathway. As expected, telmisartan was as effective as Leptin tA in reducing Ang II-induced leptin synthesis and secretion (Figure 4(a) to (c)). Telmisartan and Leptin tA inhibited the Ang II-induced increases in type 1 collagen (COL 1A1) and TIMP-1 expression and improved the Ang II-induced decreases in MMP-1 expression in cardiac fibroblasts (Figure 4(d) to (e)). Telmisartan and Leptin tA also inhibited the Ang II-stimulated secretion of type 1 collagen by cardiac fibroblasts (Figure 4(f)). Taken together, these findings indicate that telmisartan can improve collagen metabolism disorder in cardiac fibroblasts by inhibiting the leptin autocrine activity induced by local Ang II.

We also found that telmisartan was superior to the leptin antagonist in improving collagen metabolism disorder induced by Ang II in cardiac fibroblasts (Figure 4(d) to (f)). These findings suggest that telmisartan may improve collagen metabolism disorder through mechanisms other than inhibition of the leptin autocrine activity.

Telmisartan ameliorates collagen metabolism disorder induced by Ang II in cardiac fibroblasts by activating PPAR-γ

Because telmisartan is a PPAR-γ partial agonist, we assessed the effects of telmisartan and the PPAR-γ complete agonist pioglitazone on PPAR-γ expression in cardiac fibroblasts. Telmisartan enhanced PPAR-γ expression by almost three-fold compared with control cells, an increase similar

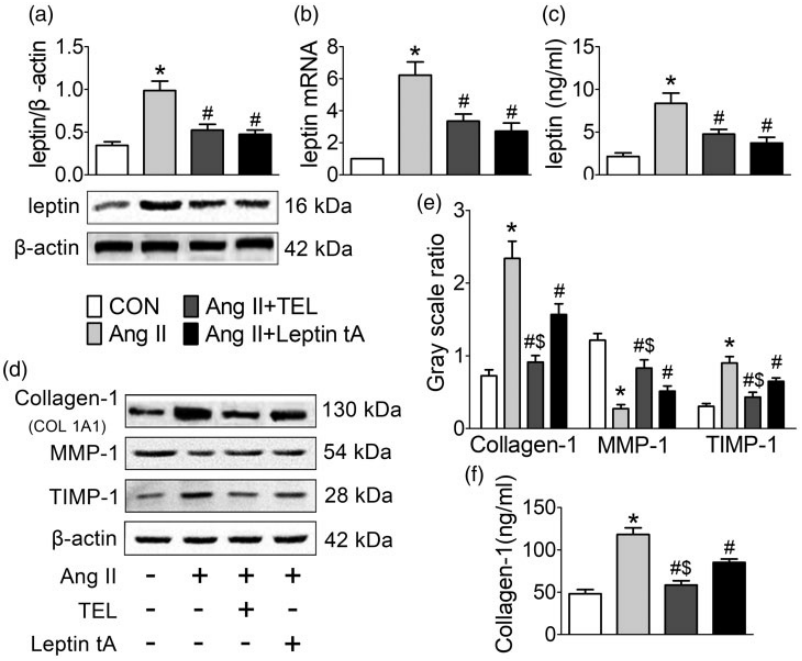

Figure 4. Telmisartan improves collagen metabolism disorder in cardiac fibroblasts by inhibiting leptin autocrine activity induced by Ang II. The cardiac fibroblasts were pretreated with telmisartan or leptin tA for 1 h before treatment with Ang II (10^{-5} mol/l) for 48 h. (a) Level of leptin expression in cardiac fibroblasts detected by Western blotting. (b) Level of leptin transcription mRNA in cardiac fibroblasts detected by quantitative RT-PCR. (c) Leptin concentrations in supernatants of cardiac fibroblasts detected by ELISA. (d) Collagen-1 (COL 1A1), MMP-1, and TIMP-1 protein expression in cardiac fibroblasts detected by Western blotting. (e) Graphs showing Western blotting-determined expression of collagen-1 (COL 1A1), MMP-1, and TIMP-1 proteins normalized to β-actin expression in the same samples. (f) Collagen-1 concentrations in the supernatants of cardiac fibroblasts determined by ELISA. N = 4–6. Data reported as mean ± SEM; $^{*}P < 0.05$ vs. Control group; $^{\#}P < 0.05$ vs. Ang II group; $^{\$}P < 0.05$ vs. Ang II+ Leptin tA group.
Ang II: angiotensin II; TEL: telmisartan; Leptin tA: leptin antagonist.

to that of pioglitazone. The PPAR-γ inhibitor GW9662 significantly inhibited the telmisartan-induced increase in PPAR-γ expression ($P < 0.05$) (Figure 5(a)). Telmisartan treatment also increased myocardium PPAR-γ expression in hypertensive LVH rats (Supplementary Figure 5), suggesting that the PPAR-γ agonist activity of telmisartan may play an important role in its improvement of myocardial remodeling in hypertensive LVH rats. The Ang II-induced collagen metabolism disorder (increased COL 1A1 and TIMP-1 expression and type 1 collagen secretion, and reduced MMP-1 expression) ameliorated by telmisartan in cardiac fibroblasts could be partly inhibited by pretreatment with the PPAR-γ inhibitor GW9662 (Figure 5(b) to (d)). These results suggest that telmisartan could improve collagen metabolism disorder by activating PPAR-γ as well as by inhibiting leptin autocrine activity induced by Ang II. Because leptin acts downstream of Ang II-AT1R and induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 pathway (Figure 3), we assessed the influence of PPAR-γ agonist effects of telmisartan on leptin signaling to determine its specific mechanism in improving collagen metabolism disorder. Telmisartan improved collagen metabolism disorder and reduced STAT3 phosphorylation induced by leptin in cardiac fibroblasts (Figure 5(e) to (h)). These effects of telmisartan did not occur when the cells were pretreated with the PPAR-γ inhibitor GW9662 (Figure 5(e) to (h)), indicating that telmisartan can improve Ang II-induced collagen metabolism disorder in cardiac fibroblasts by inhibiting leptin downstream signaling of STAT3 phosphorylation through the activation of PPAR-γ, thus revealing a new molecular mechanism by which telmisartan improves myocardial remodeling.

Discussion

The present study had three main findings: (1) Ang II stimulates leptin autocrine activity in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway; (2) leptin induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3-TGFβ1/smad3 signaling pathway; and (3) telmisartan improves myocardial remodeling by inhibiting Ang II-induced leptin autocrine activity and by reducing leptin downstream signaling of STAT3 phosphorylation through the activation of PPAR-γ (Figure 6).

The pathogenesis of hypertensive LVH is complex, with myocardial compensatory hypertrophy caused by pressure overload[1] regarded as the classic pathogenic mechanism underlying hypertensive LVH. Therefore, antihypertensive therapy is the most common treatment for patients with hypertensive LVH. Reducing blood pressure can partially reverse hypertensive LVH,[21] with early initiation of antihypertensive treatment being critical to avoid irreversible LVH.[22] Other factors involved in the formation of LVH in hypertension include, in addition to pressure overload, the sympathetic catecholamine system,[23] the RAAS system,[2] inflammation,[24] insulin-like growth factor,[25] and even adipokines (adiponectin, leptin).[5,26] The complex crosstalk among various neuroendocrine factors is also involved in hypertensive LVH.

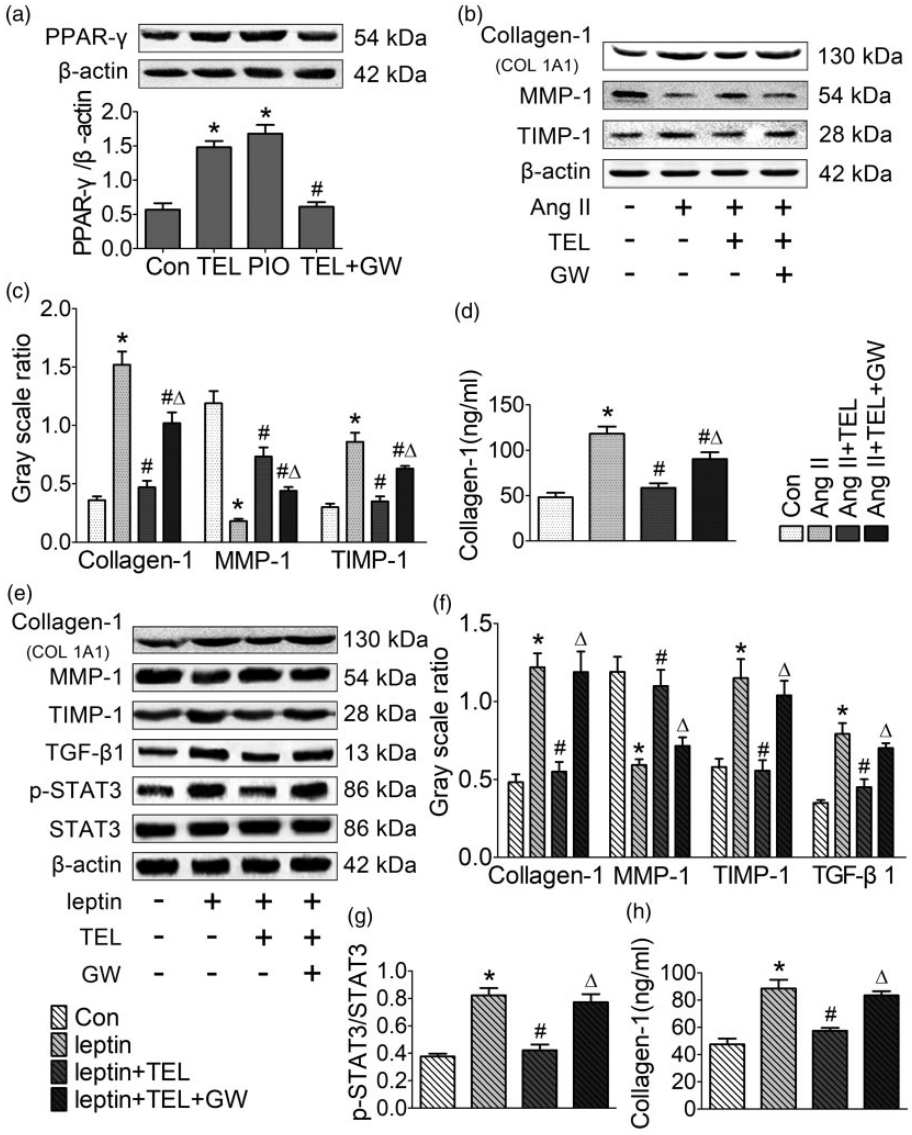

Figure 5. Telmisartan ameliorates Ang II-induced collagen metabolism disorder in cardiac fibroblasts by activating PPAR-γ. (a) PPAR-γ expression in cardiac fibroblasts determined by Western blotting and normalized to β-actin expression in the same samples. Cardiac fibroblasts were incubated with the PPAR-γ complete agonist pioglitazone and telmisartan in the presence or absence of the PPAR-γ inhibitor GW9662.
TEL: telmisartan; PIO: pioglitazone. N = 4–6. Data reported as mean ± SEM; $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ vs. TEL group. (b) Collagen-1 (COL 1A1), MMP-1, and TIMP-1 expression in cardiac fibroblasts determined by Western blotting. (c) Graphs showing Western blotting-determined expression of collagen-1 (COL 1A1), MMP-1, and TIMP-1 proteins normalized to β-actin expression in the same samples. (d) Collagen-1 concentrations in the supernatants of cardiac fibroblasts determined by ELISA. Cardiac fibroblasts were pretreated with telmisartan with or without the PPAR-γ inhibitor GW9662 for 1 h before stimulation with Ang II (10^{-5} mol/l) for 48 h. (e) Collagen-1 (COL 1A1), MMP-1, TIMP-1, TGF-β1, p-STAT3, and STAT3 expression in cardiac fibroblasts determined by Western blotting. (f–g) Graphs showing Western blotting-determined expression of collagen-1 (COL 1A1), MMP-1, TIMP-1, TGF-β1, and p-STAT3 proteins normalized to β-actin or STAT3 expression in the same samples. (h) Collagen-1 concentrations in the supernatants of cardiac fibroblasts determined by ELISA. Cardiac fibroblasts were pretreated with telmisartan with or without the PPAR-γ inhibitor GW9662 for 1 h before stimulation with leptin (100 ng/ml) for 48 h. N = 4–6. Data reported as mean ± SEM; $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ vs. Ang II or Leptin group; $^{\triangle}P < 0.05$ vs. Ang II+ TEL or Leptin+ TEL group.

Ang II, an important component of the RAAS system, is involved in the development of hypertensive LVH. Circulatory and local Ang II, including Ang II in the blood vessels and heart, are closely associated with LVH pathogenesis.[2,18] Ang II binding to its receptors activates various downstream signaling pathways, including the ERK, MAPK, JNK, and mTOR pathways, to induce protein synthesis and cardiac hypertrophy.[11,13] Ang II has been shown to directly promote cardiac hypertrophy throughout the whole body, as well as in individual organs and cultured cells.[27,28] The present study showed that the AT1R antagonist telmisartan could reduce local cardiac Ang II concentrations and improve myocardial fibrosis in hypertensive LVH rats. However, telmisartan treatment itself increased plasma Ang II levels, suggesting that telmisartan inhibition of peripheral tissue AT1R stimulates Ang II expression in serum by a feedback mechanism. The inconsistent effects of telmisartan on Ang II levels in myocardium and plasma indicate that myocardial RAAS is regulated independently of systemic RAAS, suggesting the need to explore these regulatory mechanisms.

We also found that telmisartan reduced local leptin levels and in situ leptin expression of myocardium, as

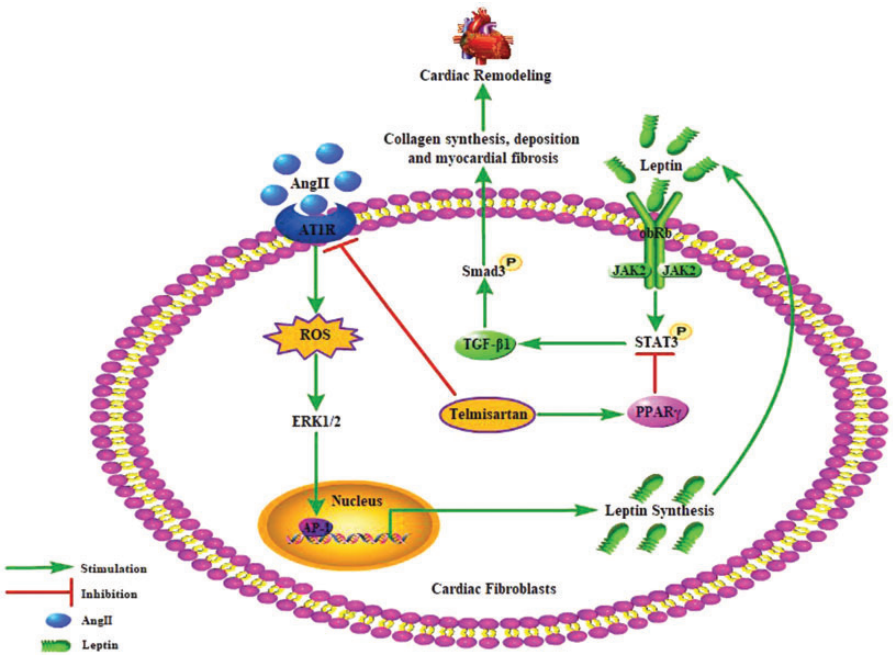

Figure 6. Schematic illustration of the mechanism. Ang II stimulates leptin autocrine activity in cardiac fibroblasts by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway. Leptin induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3-TGFβ1/smad3 signaling pathway. Telmisartan improves myocardial remodeling by inhibiting leptin autocrine activity induced by Ang II, and by inhibiting phosphorylation of the leptin downstream signal STAT3 by activating PPAR-γ. Ang II: angiotensin II; AT1R: angiotensin II type 1 receptor; AP-1: activation protein 1. (A color version of this figure is available in the online journal.)

well as inhibited leptin signaling pathways in rats with hypertensive LVH. The results indicate that local myocardial RAAS and leptin may interact closely, with these interactions playing an important role in the development of myocardial remodeling in hypertensive LVH rats. Studies have shown interactions between Ang II and leptin. For example, mechanical cyclic stretching was found to promote cardiomyocyte hypertrophy through increased leptin expression mediated by the induction of the Ang II-ROS-ERK pathway.[14] In addition, local application of a leptin antagonist attenuated Ang II-induced cardiac remodeling.[16]

Interactions between local myocardial Ang II and leptin, and the effects of these interactions on the development of myocardial remodeling in hypertensive LVH were further explored by in vitro experiments, thus excluding the effects of pressure load and of circulating Ang II and leptin. The pathophysiological processes of myocardial remodeling are complex, involving cardiomyocyte necrosis and apoptosis, cardiomyocyte hypertrophy, and myocardial interstitial fibrosis. Because the myocardial interstitium is an important component of myocardial tissue, the ability of cardiac fibroblasts to secrete collagen, the main component of the myocardial interstitium, was assessed in vitro. Ang II stimulates leptin synthesis and secretion in cardiac fibroblasts in an autocrine manner by promoting AP-1 nuclear translocation via the AT1R-ROS-ERK1/2 pathway, revealing the mechanism of interaction between Ang II and leptin in cardiac fibroblasts.

The adipokine leptin is mainly synthesized by white adipose tissue and released into the circulation. Leptin can also be synthesized and secreted by skeletal muscle,[29] brain,[30] and heart,[31] indicating that leptin is associated with complex effects on various tissues and organs. Leptin was shown to promote myocardial remodeling through several signaling pathways, primarily through the JAK and MAPK pathways.[6,7] Other pathways, including the PPAR-α, mTOR, and RhoA signaling pathways, also participate in leptin-induced myocardial remodeling.[32,33] Additional studies are needed to explore the potential mechanisms underlying leptin-induced myocardial remodeling. Our results indicate that leptin can promote collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3-TGF-β1/Smad3 signaling pathway.

Although we did not evaluate the effects of leptin signaling on cardiac remodeling in our animal model, we found that the expression of leptin/JAK2/STAT3-TGF-β1/Smad3 signaling molecules in myocardial tissue increased in a manner consistent with myocardial collagen metabolism disorder and myocardial remodeling in hypertensive LVH rats. We also confirmed that leptin can induce collagen metabolism disorder via the JAK2/STAT3 pathway in cardiac fibroblasts, by testing the in vitro effects of leptin with various signaling pathway inhibitors. Leptin/JAK/STAT signaling plays an important role in cardiac remodeling,[7,8] and clinical studies have shown that serum leptin concentrations are significantly higher in hypertensive patients with than without LVH.[5] Thus, leptin signaling plays an important role in cardiac remodeling in hypertensive LVH. Future experiments using local myocardial silencing and overexpression of leptin signaling in hypertensive LVH rats will further evaluate the effects of myocardial leptin signaling on cardiac remodeling in vivo.

Taken together, the results of our in vivo and in vitro experiments demonstrate that Ang II-induced leptin promotes myocardial remodeling in hypertensive LVH rats in an autocrine manner by activating the JAK2/STAT3

pathway. These results are consistent with previous findings, showing that leptin acts as an autocrine mediator of Ang II-induced cardiomyocyte hypertrophic effects in vitro.[34] To our knowledge, however, no study to date has focused on the negative regulation of leptin autocrine that mediates Ang II-induced myocardial remodeling.

Telmisartan is a new-generation long-acting non-peptide Ang II receptor antagonist that selectively and irreversibly antagonizes AT1R. Telmisartan could improve hypertensive LVH by reducing pressure load, inhibiting the TGF-β1 signaling pathway, sympathoinhibition, or activating the AMPK signaling pathway.[17,18,35] Our study demonstrates that the AT1R antagonist telmisartan could indirectly improve myocardial remodeling by inhibiting local leptin induced in an autocrine manner by Ang II, and that telmisartan was superior to a leptin antagonist in improving collagen metabolism disorder induced by Ang II in cardiac fibroblasts. These findings suggest that telmisartan may improve collagen metabolism disorder by other mechanisms, in addition to inhibiting leptin autocrine activity.

Telmisartan is a partial PPAR-γ agonist, which acts on the PPAR-γ ligand to induce various metabolic effects.[36] PPAR-γ is a transcription factor belonging to the nuclear receptor superfamily of ligand activators and is therefore a target for the treatment of insulin resistance, diabetes, and metabolic syndrome.[37] Studies have shown, however, that PPAR-γ agonists can not only improve metabolism but also inhibit vascular and cardiac remodeling. For example, PPAR-γ agonists were shown to inhibit cerebral vascular remodeling without affecting blood pressure in hypertensive rats.[38] Moreover, the PPAR-γ agonist chrysin reversed cardiac interstitial fibrosis and improved cardiac function in a rat model of acute myocardial infarction.[39] Our results also showed that telmisartan could improve Ang II-induced collagen metabolism disorder in cardiac fibroblasts, partly by activating PPAR-γ. However, the specific mechanisms by which the PPAR-γ agonist effects of telmisartan improve cardiac remodeling remain unclear. PPAR-γ agonists have been reported to inhibit the phosphorylation of STAT3 signaling molecules, participating in the pathological processes of epithelial mesenchymal transition and cirrhosis.[40,41] Because leptin is downstream of Ang II-AT1R and induces collagen metabolism disorder in cardiac fibroblasts via the JAK2/STAT3 pathway, we assessed the PPAR-γ agonist effects of telmisartan on leptin signaling. We found that telmisartan could directly improve leptin-induced collagen metabolism disorder in cardiac fibroblasts by inhibiting STAT3 phosphorylation via activation of PPAR-γ. These results reveal a novel mechanism by which telmisartan improves cardiac remodeling and may help to identify new therapeutic targets for patients with hypertensive LVH.

This study, however, had several limitations. First, we did not use animal transfection technology or assess the effects of local myocardial silencing, or overexpression of Ang II or leptin. Moreover, we did not include a group of telmisartan-treated sham-operated rats. We intend to perform these experiments in future studies. Second, although leptin expression was assessed immunohistochemically in the myocardial tissue of rats, cardiac fibroblasts were not labeled. Thus, we could not ensure that the in situ expression of leptin in myocardial tissue was entirely due to expression by cardiac fibroblasts. Future experiments will include immunohistochemical staining of rat myocardial tissue for both leptin and cardiac fibroblast markers. Third, we did not directly assess the exact process by which Ang II induces leptin in an autocrine manner in cardiac fibroblast; that is, we did not determine how leptin was secreted by cardiac fibroblasts and subsequently acted on cardiac fibroblasts. This process will be assessed in future studies using an isotope tracing method.

In conclusion, this study shows that telmisartan can improve myocardial remodeling in hypertensive LVH rats by acting as an AT1R antagonist to inhibit leptin autocrine activity induced by local Ang II, and by acting as a PPAR-γ agonist to inhibit phosphorylation of the leptin downstream signaling molecular STAT3 (Figure 6). These findings demonstrate the crosstalk between local myocardial RAAS and leptin and reveal a novel molecular mechanism by which telmisartan improves LVH in hypertension.

Authors' contributions: All authors participated in the design, interpretation of the studies, analysis of the data, and review of the article. Hui Chen and Min Li contributed equally to this article. Hui Chen was responsible for the experimental design, literature review, and drafting of the original article. Min Li and Lei Liu performed the animal and cell experiments. Danjun Zhu analyzed the data. Gang Tian revised the article and approved the final version.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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