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Perindopril improves cardiac fibrosis through targeting the AngII/AT1R pathway

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ABSTRACT

To uncover the potential effect of Perindopril on cardiac fibrosis caused by pressure overload and the underlying mechanism. The cardiac fibrosis model in mice was established by the TAC method. Mice were assigned into sham group, TAC group, 2 mg/kg Perindopril group (Per (2 mg/kg)) and 8 mg/kg Perindopril group (Per (8 mg/kg)). Cardiac structure changes were assessed by measuring HW/BW, HW/TBL, LW/BW and LW/TBL in each group. Echocardiography was performed to assess mouse cardiac function by recording EF, LVIDd, IVSd and LVPWd. Relative levels of fibrosis markers were determined. AngII content was examined by ELISA. Besides, mRNA levels of key genes in the AngII/AT1R pathway were finally detected. TAC-induced cardiac insufficiency, left ventricular dilatation, cardiac hypertrophy and myocardial collagen deposition in mice. In addition, fibrosis markers were upregulated in mice of the TAC group. Perindopril markedly reversed TAC-induced pathological changes in the cardiac structure and function of mice. Meanwhile, Perindopril dose-dependently reversed the upregulated genes in the AngII/AT1R pathway. Perindopril improves cardiac fibrosis induced by pressure overload by activating the AngII/AT1R pathway.

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Introduction

The incidence of heart failure increases sharply, with a total amount of 22.5 million affected patients. Seriously, there are 2 million new onset of heart failure cases globally, posing a great health problem owing to its high morbidity and mortality (1). Cardiac fibrosis is a typical feature of progressive coronary heart disease, which is closely linked to the pathogenesis of hypertension, myocardial infarction, myocardiopathy and heart failure. It is demonstrated that cardiac fibrosis is of significance during ventricular remodeling (2, 3). Effective approaches to prevent and treat cardiac fibrosis are urgently required.

Angiotensin II (AngII) is the major bioactive peptide product of the renin-angiotensin-aldosterone system. It exerts a vital biological role in the cardiovascular system alongside angiotensin type 1 receptor (AT1R), including regulation of blood pressure, systemic inflammatory response, interstitial collagen deposition and tissue fibrosis (4, 5). AngII-induced pressure overload is commonly applied for constructing experimental models of progressive cardiac fibrosis (6, 7). Extensive clinical and experimental evidences supported the protective effects of angiotensin-converting enzyme inhibitors (ACEI) and AT1R blockers on cardiac fibrosis (8-10). It is confirmed that AngII promotes fibrogenesis through AT1R-stimulated synthesis and release of TGF-β1 (11, 12).

Perindopril is a typical type of ACEI capable of lowering systemic vascular resistance, dilating arteries and veins, relieving cardiac load by reducing aldosterone release and protecting cardiac remodeling through enhancing vascular compliance (13, 14). In addition, Perindopril also exhibits higher selectivity and potency in angiotensin-converting enzyme (ACE) blockade and is more tolerant. It is found that Perindopril protects myocardial fibrosis by downregulating Gal-3 to improve ventricular remodeling in ischemic heart failure (15). However, its role in cardiac fibrosis caused by pressure overload is unclear.

In this analysis, we first constructed a ventricular pressure overload model in mice by performing transverse aortic constriction (TAC) (16). We further determined the protective role of Perindopril in cardiac fibrosis and the involvement of the AngII/AT1R pathway.

Materials and Methods

Animals

Male 8-week-old C57BL/6 mice weighing 22.3±1.2 g were provided by Chengde Medical University Affiliated Hospital Animal Center. Mice were habituated in a standard environment at the temperature of 22±2°C, relative humidity of 50-60%, and light/dark cycle for 12 h/12 h. Humanitarian care was given to experimental animals. Perindopril (C18H17NO5) was purchased from (MedChem Express, Monmouth Junction, NJ, USA). Animal procedures were approved by the Animal Ethic Committee of Chengde Medical University Affiliated Hospital.

TAC procedures

Mice were assigned to the sham group, TAC group, Per (2 mg/kg) and Per (8 mg/kg), with 10 in each group. The mouse was anesthetized by intraperitoneal injection of 4% sodium pentobarbital, and placed in a supine position. A median sternal incision was cut to expose the mediastinal cavity in layers. After removing the thymus, the aortic arch was bluntly separated at the base of the heart. A blunt 27-G

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Table 1. Primer sequences.

Gene		Primer sequences
collagen type I	F	5′-TGAGCCAGCAGATTGAGAACAT-3′
	R	5′-TGTCGCAGAAG ACCTTGATGG-3′
collagen type III	F	5′-GTACAACTAGCATTCCTCCGACTG-3′
	R	5′-TTAGAGCAGCCATC CTCCAGAAC-3′
α-SMA	F	5′-GCGTGGCTATTCCTTCGTGACTAC-3′
	R	5′-CCATCAGGCAGTTCGTAGCTCTTC-3′
periostin	F	5′-ACTTCCACGAGGTGTCCTAG-3′
	R	5′-CCTCCCATAATAGACTCAGAACA-3′
AGT	F	5′-GACAGCACCCTACTTTTCAACAC-3′
	R	5′-TCTATCCAAGTCAGGAGGTCGTT-3′
ACE	F	5′-CAGACAACAACTCACCAAGCAAC-3′
	R	5′-TCTGCGTACTCGTTCAACAACAC-3′
AT1R	F	5′-CTCTGCCACATTCCCTGAGTT-3′
	R	5′-CTTGGGGCAGTCATCTTGGA-3′
TGFβ1	F	5′-AACAACGCCATCTATGAGAAAAC-3′
	R	5′-GTAACGCCAGGAATTGTTGCTAT-3′
GAPDH	F	5′-TGGTGAAGGTCGGTGTGAAC-3′
	R	5′-GCTCCTGGAAGATGGTGATGG-3′

needle (0.4 mm in external diameter) was placed parallel to the outer wall of the aorta. Between the brachiocephalic trunk and the left common carotid artery, the needle and the aortic arch were constricted together with a 7-0 surgical thread, and subsequently, the needle was removed. The incision was sutured layer by layer. Mice in the sham group received the same procedures except for constriction of the aortic arch. Cardiac function in mice was performed at postoperative 2nd, 4th and 8th week. Mice were then sacrificed for collecting the heart.

Echocardiography

Echocardiography was performed by a high-resolution ultrasound imaging system (VINNO 6, Vinno Corporation, Suzhou, China) with a 23MHz probe. M-mode echocardiograms were obtained on the long-axis and short-axis of the peristaltic left ventricle. Left ventricular ejection fraction (EF), left ventricular internal-diastolic diameter (LVIDd), end-diastolic interventricular septal thickness (IVSd) and left ventricular posterior wall diameter (LVPWd) in each mouse were recorded.

Assessment of cardiac remodeling

After weighing the body weight (BW) of each mouse, all mice were sacrificed. Heart weight (HW/mg), HB-to-BW ratio (HB/BW, mg/g), lung weight (LW/mg) and LW-to-BW ratio (LW/BW, mg/g) were recorded. In addition, the heart weight-to-tibial length ratio (HW/TBL, mg/mm) and lung weight-to-tibial length ratio (LW/TBL, mg/mm) were calculated.

Enzyme-linked immunosorbent assay (ELISA)

The serum sample of each mouse was collected for determining the serum content of AngII using the ELISA kit (BlueGene Biotech, Shanghai, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Myocardial tissues were homeogenated for isolating RNAs. After reverse transcription into complementary deoxyribose nucleic acid (cDNA), quantitative real-time polymerase chain reaction (qRT-PCR) was performed at 96°C for 4 min, followed by 45 cycles at 95°C for 15 s, 60°C for 60 s, and finally 60°C for 5 min. Primer sequences were as follows (Table 1).

Western blot

Tissues were homeogenated for extracting proteins. After concentration determination, protein samples were loaded on polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, non-specific antigens were blocked in 5% skim milk for 2 h. Membranes were then incubated with primary and secondary antibodies. Band exposure and grey value analysis were finally conducted.

Statistical analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± SD (standard deviation). Comparison between multiple groups was done using a one-way ANOVA test followed by Post Hoc Test (Least Significant Difference). $P<0.05$ indicated a significant difference.

Results

Perindopril relieved cardiac remodeling and pulmonary congestion following TAC

Cardiac remodeling was assessed by weighing heart weight. It is shown that HW/BW (Figure 1A), HW/TBL (Figure 1B), LW/BW (Figure 1C) and LW/TBL (Figure 1D) were markedly elevated in the TAC group than those in the sham group. Nevertheless, their elevations were dose-dependently reduced by Perindopril treatment. It is suggested that Perindopril effectively alleviated myocardial hypertrophy and the subsequent pulmonary congestion following TAC.

Perindopril relieved cardiac function following TAC

Echocardiography was performed for assessing changes in mouse cardiac functions. EF was remarkably reduced in

the TAC group than the sham group, and it was elevated by Perindopril (Figure 2A). Besides, increased LVIDd (Figure 2B), IVSd (Figure 2C) and LVPWd (Figure 2D) were partially reversed by Perindopril. As a result, Perindopril protected cardiac function against cardiac fibrosis.

Perindopril relieved cardiac fibrosis following TAC

Chronic ventricular pressure overload leads to cardiac hypertrophy, which is manifested as cardiac fibrosis (17, 18). There are many fibrosis markers used for evaluating the fibrotic level (19, 20). Here, mRNA levels of Col I and Col III (Figure 3A), α-SMA (Figure 3B) and periostin (Figure 3C) were remarkably higher in the TAC group than those of the sham group, and their levels were reduced by Perindopril treatment. Identically, upregulated protein levels of Col I, Col III, α-SMA and periostin were also abolished by Perindopril (Figure 3D).

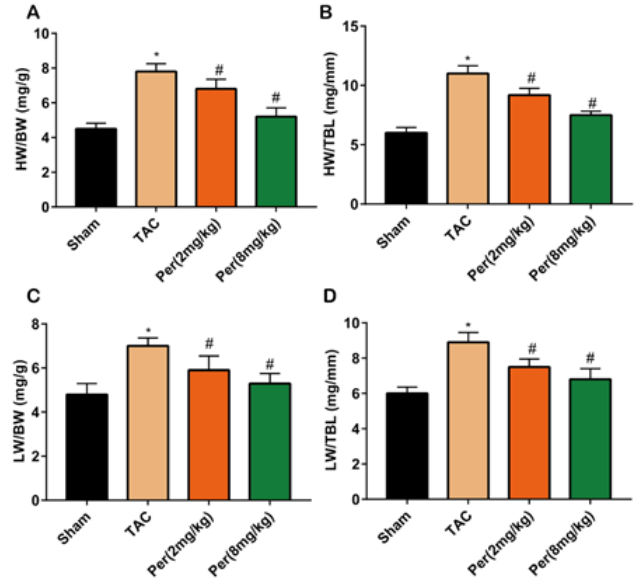

Figure 1. Perindopril relieved cardiac remodeling and pulmonary congestion following TAC. Mice were assigned to the sham group, TAC group, Per (2 mg/kg) and Per (8 mg/kg), with 10 in each group. HW/BW (A), HW/TBL (B), LW/BW (C) and LW/TBL (D) in each group.

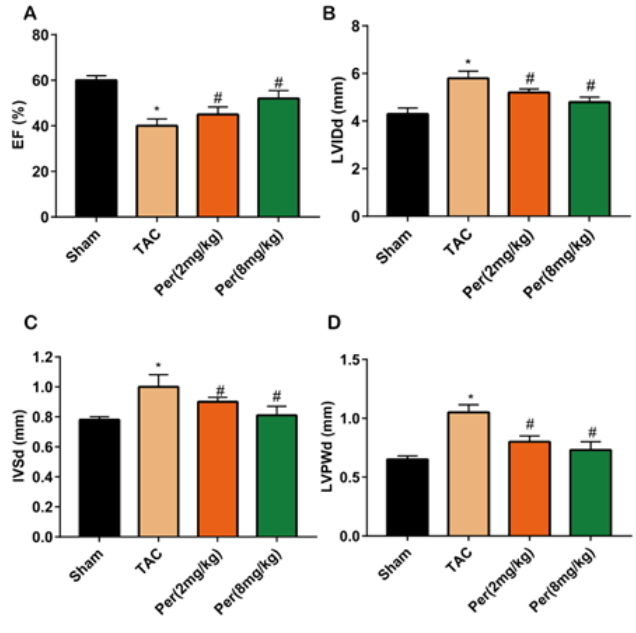

Figure 2. Perindopril relieved cardiac function following TAC. Mice were assigned to the sham group, TAC group, Per (2 mg/kg) and Per (8 mg/kg), with 10 in each group. EF (A), LVIDd (B), IVSd (C) and LVPWd (D) in each group.

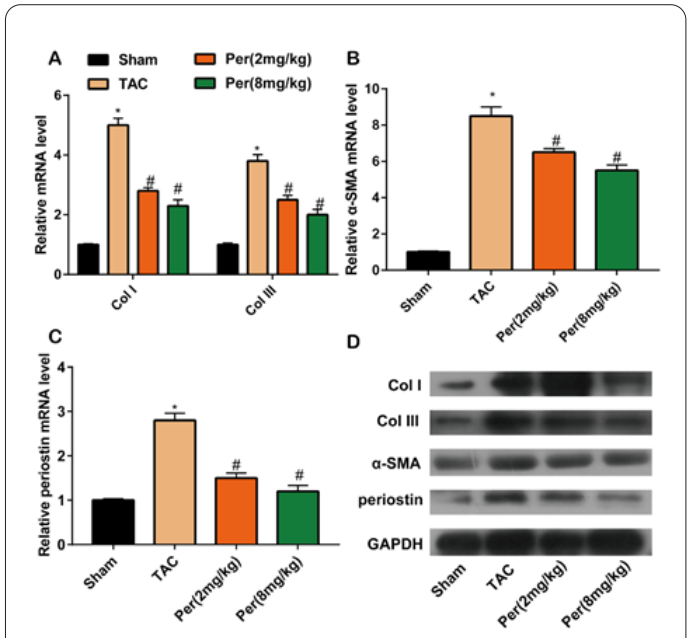

Figure 3. Perindopril relieved cardiac fibrosis following TAC. Mice were assigned to the sham group, TAC group, Per (2 mg/kg) and Per (8 mg/kg), with 10 in each group. (A-C) The mRNA levels of Col I and Col III (A), α-SMA (B) and periostin (C) in each group. (D) Protein levels of Col I, Col III, α-SMA and periostin in each group.

Perindopril inhibited cardiac fibrosis by inactivating the AngII/AT1R pathway

AngII is abundantly secreted under the stimulation of pressure overload, which is a vital pro-fibrosis factor (21, 22). It is previously reported that the ACE/AngII/AT1R-TGFβ1 axis attributes to the mechanism of fibroblast transdifferentiation (23). Our findings uncovered that mRNA levels of AngII (Figure 4A), AGT (Figure 4B), ACE (Figure 4C), AT1R (Figure 4D) and TGFβ1 (Figure 4E) were upregulated in the TAC group than those of the sham group. Notably, their levels were dose-dependently reduced by Perindopril. Similarly, the protein level change of AT1R was identical to its mRNA level (Figure 4F). Collectively, the AngII/AT1R pathway was responsible for the protective effect of Perindopril on cardiac fibrosis.

Discussion

Long-term pressure overload results in the decompensation of persistent cardiac hypertrophy, thus leading to ventricular remodeling. Eventually, abnormal ventricular dilation, dysfunction of myocardial energy metabolism, and persistent decrease of cardiac systolic and diastolic function lead to heart failure (24). Hence, delaying ventricular remodeling is the major therapeutic approach to heart failure. Our study established cardiac pressure overload model in mice by performing TAC. It is shown that TAC results in heart weight changes, cardiac hypertrophy and pulmonary congestion. In addition, the cardiac functions of model mice remarkably declined. ACEIs have been identified to improve cardiac remodeling (25). Herein,

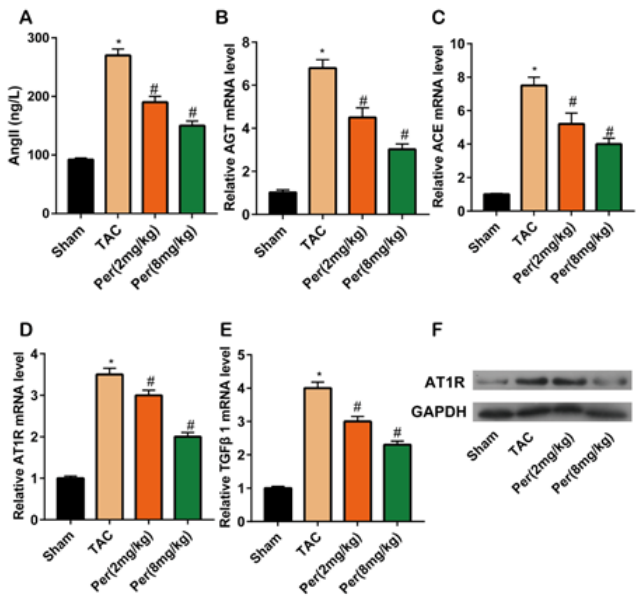

Figure 4. Perindopril inhibited cardiac fibrosis by inactivating the AngII/AT1R pathway. Mice were assigned to the sham group, TAC group, Per (2 mg/kg) and Per (8 mg/kg), with 10 in each group. (A-E) The mRNA levels of AngII (A), AGT (B), ACE (C), AT1R (D) and TGFβ1 (E) in each group. (F) The protein level of AT1R in each group.

Perindopril dose-dependently relieved cardiac remodeling and pulmonary congestion, as well as improved cardiac function in model mice.

Changes in cardiac components and structure following cardiac diseases lead to cardiac fibrosis (26). TAC could result in fibrosis as collagen deposition reveals (27, 28). In our paper, relative levels of Col I, Col III, α-SMA and periostin increased in the TAC group. Their enhanced trends were dose-dependently reversed by Perindopril. Therefore, the protective effects of Perindopril on cardiac fibrosis induced by pressure overload have been confirmed.

AngII, collage deposition, inflammatory response and ROS production are extensively involved in cardiac remodeling (29). Previous studies have proven that AngII enhances blood pressure and stimulates cardiac fibrosis through AT1R (30, 31). AngII-induced upregulation of TGF-β markedly triggers cardiac fibrosis (32). In a mouse model of pressure overload, Telmisartan effectively alleviates cardiac fibrosis by downregulating AT1R and TGFβ1 (33, 34). Cardiac fibrosis would further aggravate myocardial stiffness and TGFβ1 is responsible for this change. Our findings uncovered that expression levels of AngII, AGT, ACE, AT1R and TGFβ1 were markedly upregulated in the TAC group than those of the sham group. Notably, their upregulations were markedly relieved by Perindopril. It is suggested that the ACE/AngII/AT1R-TGFβ1 axis was extensively involved in cardiac fibrosis caused by pressure overload.

Conclusions

Perindopril improves cardiac fibrosis induced by pressure overload by activating the AngII/AT1R pathway.

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Competing interests

The authors declare no competing interests.

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