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Effect of Xinjikang on left ventricular hypertrophy remodeling in hypertensive rats

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ABSTRACT

Objective: To investigate the effects of Xinjikang on the left ventricular hypertrophy remodeling and myocardial activity in hypertension. **Methods:** Sixty Wistar rats were randomly divided into four groups. The pressure-loaded left ventricular hypertrophy model was established with abdominal aorta ligation method. Rats in A and B groups were intragastrically administered with physiological saline, while C and D groups were administered with Xinjikang and metoprolol, respectively. The changes in blood pressure, E/A ratio, myocardial pathological morphology, myocardial lipoperoxides and superoxide dismustase activity in four groups were observed and compared before and after treatment. **Results:** There were statistically significant differences in E/A ratio between C group after treatment and model group ($P<0.05$), while no difference was observed between A and D groups ($P>0.05$); after treatment the myocardial lipoperoxides and superoxide dismustase contents in C and D groups were improved significantly compared with model group ($P<0.05$). **Conclusions:** Xinjikang can improve myocardial injury, restore myocardial parenchyma and myocardial interstitial remodeling functions in hypertensive rats with the left ventricular hypertrophy.

1. Introduction

Myocardial hypertrophy is a compensatory response of the heart to the pressure or volume load, and 1/3 of patients with hypertension may develop the left ventricular hypertrophy, which is the major risk factor for sudden death and coronary heart disease[1]. The pathological mechanism underlying the left ventricular hypertrophy includes myocardial hypertrophy, fibrosis and collagen deposition, and the local manifestations are similar to symptoms of amassment disease in traditional Chinese medicine, which suggest that the left ventricular hypertrophy is the results of qi, blood, phlegm stagnation and stasis[2]. In this study, the pressure-overload left ventricular hypertrophy models established in Wistar rats were treated with Xinjikang, in a broader attempt to investigate the effects of Xinjikang on the left ventricular hypertrophy remodeling and myocardial activity in hypertensive rats.

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2. Materials and methods

2.1. Experimental animals

Sixty Wistar rats aged 6 months, weighing (220±10) g, males or females, were provided by the Experimental Animal Center, Yuhuangding Hospital of Yantai, Yantai, Shandong Province, China. Rats were housed at (23±3) ℃, allowing free access to food and water. Experimental disposals were in strict accordance with the Guideline on Administration of Laboratory Animals.

2.2. Drugs and instruments

Metoprolol tablet containing 50 mg metoprolol tartrate was provided by Wuxi Huarui Pharmaceutical Co., Ltd., Wuxi, Jiangsu Province, China. Xinjikang decoction was consisted of Radix Salviae miltiorrhiae 15 g, Radix Astragali 20 g,

Rhizoma Pinelliae Preparata 10 g, Radix Notoginseng 5 g, Concha Haliotidis 20 g, Fructrs Trichosanthis 12 g, Radix Acanthopanacis Bidentatae and chrysanthemum 12 g. Each mL decoction contained crude drug 1 g. Lipoperoxides (LPO) and superoxide dismustase (SOD) detection kits were provided by Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, China.

2.3. Establishment of models

The pressure-overload left ventricular hypertrophy models were established with the abdominal aortic coarctation method. In brief, rats were anesthetized with intraperitoneal chloral hydrate, after the skin was disinfected, an incision was made along the xiphoid process for laparotomy, peritoneum and other soft tissue were separated, exposing the abdominal aorta. Subsequently the abdominal aorta was clamped and occluded using a silver clip above renal artery. We reset abdominal organs and closed the abdominal cavity after no bleeding was observed. Rats were intramuscularly injected with penicillin to prevent infection at 3 d postoperatively and restored the feeding 6 h later. The models can be defined success when the cardiac mass index and the left ventricular mass index are significantly increased.

2.4. Methods

Sixty rats were randomly divided into four groups: A, B, C, D, with 15 rats in each group. A is control group (receiving no ligation or occlusion) and B is model group, rats in A and B groups were treated with physiological saline (1 mL/100 g) at 4 weeks after modeling; C is Xinjikang group (rats were intragastrically given Xinjikang 3 mL/kg per day, twice a day); D is metoprolol group (rats were intragastrically given metoprolol 80 mg/mL/kg per day, twice a day).

2.5. Main outcome measures

Changes in caudal artery blood pressure were observed before treatment, and at 2, 4, 6 weeks after treatment. E/A ratio (the ratio of the early to late ventricular filling velocities) was measured using color Doppler ultrasound examination before modeling, before and after treatment. After rats in each group were killed at 6 weeks after treatment, cardiac muscle cells were stained by hematoxylin-eosin and morphological changes of myocardial tissue were observed under electron microscopy. Myocardial tissue was grinded and the supernatant was collected, LPO levels were determined by thiobarbituric acid reaction[3] and SOD activity was assayed with pyrogallol autoxidation method[4].

2.6. Statistical analysis

Data were analyzed using SPSS 12.0 software (SPSS, Chicago, IL, USA) and measurement data were expressed as mean±SD using the *t*-test. A $P<0.05$ value was considered statistically significant differences.

3. Results

3.1. Comparison of blood pressure of rat tail artery in four groups before and after treatment

The tail arterial pressure in B, C, D groups of rats was significantly increased compared with that in A group at 4 weeks after modeling ($P<0.05$), and there was no significant difference among B, C, D groups ($P>0.05$). The tail arterial pressure in C and D groups of rats began to decreased at 2, 4, 6 weeks after treatment, which was significantly lower than B group ($P<0.05$); at 6 weeks, no difference was observed in the tail arterial pressure of C and D groups of rats compared with A group ($P>0.05$; Table 1).

Table 1
Comparison of tail arterial pressure before and after treatment (mmHg).

Group	4 weeks after modeling	After treatment		
		2 weeks	4 weeks	6 weeks
A	110.82±3.11	109.82±3.264	113.80±3.92	112.41±3.25
B	142.73±6.60*	153.13±4.74	158.95±4.55	165.09±4.21
C	142.31±4.70*	130.92±4.11△	122.50±4.45△	116.32±4.45△
D	140.20±5.63*	127.13±3.89△	119.23±3.64△	113.91±3.32△

*$P<0.01$, *vs.* A group; △$P<0.05$, *vs.* B group. A: control group; B: model group; C: Xinjikang group; D: metoprolol group.

3.2. Comparison of E/A ratio in four groups before and after treatment

The E/A ratio showed no significant differences among four groups before modeling ($P>0.05$). At 4 weeks after modeling, the E/A ratio in B, C, D groups of rats was significantly lower in that in A group ($P<0.05$); at 6 weeks after treatment, the E/A ratio in C and D groups of rats was significantly increased compared with B group ($P<0.05$; Table 2).

Table 2
Comparison of E/A ratio in each group at different time points.

Group	Before modeling	4 weeks after modeling	6 weeks after treatment
A	1.12±0.06	1.12±0.08	1.12±0.07
B	1.14±0.06	0.84±0.07*	0.75±0.03*
C	1.13±0.04	0.86±0.05*	1.06±0.06△
D	1.12±0.05	0.87±0.04*	0.97±0.05△

*$P<0.05$, *vs.* A group; △$P<0.05$, *vs.* B group. A: control group; B: model group; C: Xinjikang group; D: metoprolol group.

3.3. Changes in morphology of myocardial tissue

In B group, myocardial fibers were thick and ruptured, myocardial cells showed focal or patch degeneration and became necrotic. In C group, myocardial cells presented mild degeneration and hypertrophy, nuclear morphology was slightly irregular, myocardial microvessels were normal, interstitial edema was evident, and myocardial fibers were not ruptured. In D group, myocardial fibers partially fractured, myocardial cells presented mild degeneration, occasional spotty necrosis, interstitial edema, and unclear boundaries with surrounding cells (Figure 1).

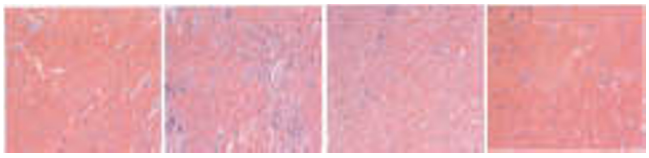

Figure 1. Morphological changes of myocardial tissue in 4 groups (Hematoxylin-eosin staining, ×200).
A: control group; B: model group; C: Xinjikang group; D: metoprolol group.

3.4. Comparison of LPO level and SOD activity in four groups

The SOD activity in myocardial tissue was significantly increased, while LPO levels were significantly reduced in C group after treatment, compared with B group ($P<0.05$). There was no significant difference in the SOD and LPO levels between C group and D group after treatment ($P>0.05$; Table 3).

Table 3
Comparison of LPO levels and SOD activity in four group.

Group	LPO (nmol/mg)	SOD (U/mg)
A	0.63±0.05	135.61±14.59
B	1.19±0.16*	101.19±11.22*
C	0.81±0.12△	128.74±13.53△
D	0.84±0.20△	126.42±12.59△

*$P<0.05$, *vs.* A group; △$P<0.05$, *vs.* B group. A: control group; B: model group; C: Xinjikang group; D: metoprolol group.

4. Discussion

Myocardial hypertrophy is the common pathological process of a series of cardiovascular diseases, and the adaptive pathological change caused by myocardial overload[5-7]. Early myocardial hypertrophy is a beneficial compensatory response, but long-term sustained hypertrophy leads to dilated cardiomyopathy. Therefore improvement of left ventricular hypertrophy is regarded a key target in the treatment of hypertension[6]. In this study, we established the left ventricular pressure overload model with abdominal aortic coarctation method to produce the left ventricular hypertrophy, which was similar to clinical hypertension-caused left ventricular hypertrophy.

In the Xinjikang's prescription, Radix Salviae Miltiorrhiae can dilate peripheral vessels, improve microcirculation and inhibit fibrosis, its component tanshinone Ⅱ-A has anti-myocardial ischemia/hypoxia, anti-thrombosis and myocardial calcium channel blocking effects in modern pharmacology studies[8-12]. Additionally protective effects against pituitrin-induced ischemic electrocardiography changes are definitely displayed[8]. Radix Astragali can dilute peripheral vessels, lower blood pressure, and reduce collagen content, thus preventing myocardial fibrosis. It is highly involved in the protection of cardiac myocardial cells under oxygen/glucose deprivation conditions, increment of intracellular mitochondria and glycogen granules, and enhancement of myocardial cell metabolism and compensatory ability[9]. Radix Acanthopanacis Bidentatae has diuretic, antihypertensive, and blood promoting effects, it also reduces blood viscosity, hematocrit and aggregation index[13-15]. Radix Notoginseng is capable of dilating blood vessels, lowering blood pressure and improving myocardial ischemia. The negative autorhythmicity and negative conduction of Radix Notoginseng are the pharmacological basis for antiarrhythmic effects[16,17]. Chrysanthemum contains glycosides, which functions to reduce blood pressure, increase coronary blood flow and raise hypoxia tolerance. Fructrs Trichosanthis can expand coronary artery, lower blood fat and improve myocardial ischemia. Concha Haliotidis has hepatoprotective effect. The combination of the aforementioned herbs can improve cardiovascular diastolic functions, lower blood pressure, reduce serum type III procollagen terminal peptide and hyaluronic acid levels, and improve blood rheology conditions, thus the heart may develop from a chronic stress state to a harmony state with abundant qi, blood, nutrition and energy[18-20].

In this study, the blood pressure of rats showed no significant difference between C group and D group, where rats were treated by Xinjikang or metoprolol ($P>0.05$), indicating that Xinjikang can significantly reduce blood pressure levels. At 4 weeks after modeling, the E/A ratio in B, C, D groups of rats was significantly lower than that in A group. This evidence implies that pressure overload left ventricular diastolic functions have changed. The E/A ratio significantly increased in the C group after treatment,

suggesting that Xinjikang, similar to western medicine metoprolol, can improve ventricular diastolic functions. The hematoxylin-eosin staining results displayed that, after rats were treated with Xinjikang, cardiac muscle cell hypertrophy and mild degeneration were observed, cardiac muscle fibers were not ruptured, and nucleus morphology was slightly irregular under light microscope. These evidence supported that, Xinjikang can significantly attenuate myocardial injury, thus reversing myocardial parenchyma remodeling. Additionally the sharp decline of LPO level and significant increased SOD activity implicate the role of Xinjikang on improving metabolic disorder of oxygen free radicals, thus reversing the left ventricular hypertrophy .

In summary, our findings show that Xinjikang can improve myocardial injury, restore myocardial parenchyma and myocardial interstitial remodeling functions, thus it has a potential positive effect for the treatment of the left ventricular hypertrophy.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Ma YH, Li YD, Zhao JX, Wang T. Protection of ultra-filtration extract mixture from *Angelica sinensis* and *Hedysarum polybotrys* on oxidative damage in cardiomyocytes of neonatal rats and its mechanism. *Zhongcaoyao Zazhi* 2010; **41**(4): 602-607.

[2] Cuspidi C, Vaccarella A, Negri F, Sala C.. Resistant hypertension and left ventricular hypertrophy: an overview. *J Am Soc Hypertens* 2010; **4**(6): 319-324.

[3] Deng J, Deng XS, Luo J. Protection of Angelica sinensis extract against left ventricular hypertrophy of rats. *Zhongcaoyao Zazhi* 2013; **44**(6): 721-722.

[4] Matsui Y, Sasaki S. Left ventricular reconstruction for severely dilated heart. *Ann Thorac Cardiovasc Surg* 2008;**14**(2): 66-74.

[5] Zhang YM, Li L. Characteristics of left heart remodeling and function in essential hypertensive patients with atrial fibrillation. *Zhonghua Gaoxueya Zazhi* 2009; **17**(2): 141-143.

[6] Lapu-Bula R, Oi li E. From hypertension to heart failure: role of nitric oxide-mediated endothelial dysfunction and emerging insights from myocardial contrast echocardiography. *Am J Cardiol* 2007; **99**(6B): 7D-14D.

[7] Chen YQ, Hu GX, Fu Q, Jin XJ. Effects of stellate ganglion block on blood pressure in spontaneously hypertensive rats. *J Zhejiang Univ Sci B* 2012; **41**(1): 59-63.

[8] Guo P, Wu C, Masaki T, Mori H, Nishiyama A. Subdose of fasudil suppresses myocardial fibrosis in aldosterone-salt-treated uninephrectomized rats. *Pharmazie* 2011; **66**(9): 716-719.

[9] Wang P, Chen SR, Wang XW. Tanshinone ⅡA attenuated oxidative stress and left ventricular hypertrophy induced by renovascular hypertension. *Zhonghua Gaoxueya Zazhi* 2010; **18**(3): 229-234.

[10]Satoh K, Fukumoto Y, Shimokawa H. Rho-kinase: important new therapeutic target in cardiovascular diseases. *Am J Physiol Heart Circ Physiol* 2011; **301**(2): H287-H296.

[11]Surma M, Wei L, Shi J. Rho kinase as a therapeutic target in cardiovascular disease. *Future Cardiol* 2011; **7**(5): 657-671.

[12]Wang P, Chen SR, Wang XW. Tanshinone ⅡA attenuated oxidative stress and left ventricular hypertrophy induced by renovascular hypertension. *Zhonghua Gaoxueya Zazhi* 2010; **18**(3): 229-234.

[13]Bishu K, Hamdani N, Mohammed SF, Kruger M, Ohtani T, Ogut O, et al. Sildenafil and B-type natriuretic peptide acutely phosphorylate titin and improve diastolic distensibility *in vivo*. *Circulation* 2011; **124**(25): 2882-2891.

[14]Piera-Velazquez S, Li Z, Jimenez SA. Role of endothelial-mesenchymal transition (EndoMT) in the pathogenesis of fibrotic disorders. *Am J Pathol* 2011; **179**(3): 1074-1080.

[15]Lee K, Nelson CM. New insights into the regulation of ep-ithelial-mesenchymal transition and tissue fibrosis. *Int Rev Cell Mol Bio* 2012; **294**: 171-221.

[16]Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-beta signaling in cardiac remodeling. *J Mol Cell Cardiol* 2011; **51**(4): 600-606.

[17]Deng J, Wang YW, Chen WM, Wu Q, Huang XN. Role of nitric oxide inginsenoside Rg(1)-induced protection against left ventric-ular hypertrophy produced by abdominal aorta coarctationin rats. *Biol Pharm Bull* 2010; **33**(4): 631-635.

[18]Deng J, Lv XT, Wu Q, Huang XN. Ginsenoside Rg(1) inhibitsrat left ventricular hypertrophy induced by abdominalaorta coarctation: involvement of calcineurin and mitogen-activated protein kinase signalings. *Eur J Pharmacol* 2009; **608**(1-3): 42-47.

[19]Xiang YZ, Shang HC, Gao XM, Zhang BL. A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. *Phytother Res* 2008; **22**(7): 851-858.

[20]Yin J, Kukucka M, Hoffmann J, Sterner-Kock A, Burhenne J, Haefeli WE, et al. Sildenafil preserves lung endothelial function and prevents pulmonary vascu-lar remodeling in a rat model of diastolic heart failure. *Circ Heart Fail* 2011; **4**(2): 198-206.