Effects of tetrandrine on hydroxyproline content and myosin ATPase activity of hypertrophied myocardium in renovascular hypertensive rats

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Abstract: To study the effects of tetrandrine (Tet) on hypertrophied myocardial hydroxyproline content and myosin ATPase activity, left ventricular hypertrophy (LVH) was induced by renovascular hypertension (two-kidney, one-clip) in rats. Eight weeks after operation Tet 50 mg·kg^{-1}·d^{-1} and enalapril (Ena) 6 mg·kg^{-1}·d^{-1} were given by gavage for 8 weeks. The results showed that hydroxyproline content in LVH group was much higher than that of sham-operated one [(5.9 ± 0.3) *vs* (3.6 ± 0.4) mg·g^{-1} dry weight], and was decreased by 28.2% and 39.0% in Tet and Ena groups, respectively. Myosin ATPase activity in LVH group was much lower than that of sham-operated group [(0.43 ± 0.09) *vs* (0.97 ± 0.06) mmol Pi·min^{-1}·g^{-1} protein, $P < 0.01$]. In Tet and Ena groups they were 60.5% and 118.6% higher than that of LVH group, respectively. The results suggest that Tet or Ena partially reduce the hydroxyproline content and elevate myosin ATPase activity of hypertrophied myocardium in renovascular hypertensive rats.

Key words: tetrandrine; enalapril; collagen; myosin ATPase; hypertension, renovascular; heart hypertrophy

CLC number: R972.4
Document code: A
Article ID: 1000-3002(2001)02-0121-04

In hypertensive left ventricular hypertrophy (LVH) myocardial collagen content increased due to myocardial fibrosis which is the determinant factor of increase in myocardial diastolic stiffness[1]. At the same time α-myosin heavy chain (adult type, α-MHC) was converted into β-type isoenzyme (fetal type, β-MHC) due to the action of many hormones, neurotransmitters and growth factors. The ATPase activity of β-MHC is lower than that of α-MHC. This shift is related to the decrease in active tension and velocity of shortening of myocardium[2]. Tetrandrine (Tet) is a bis-benzylisoquinoline alkaloid isolated from the roots of *Stephania tetrandra* S. Moore, which has been verified as a calcium channel blocker[3] and has long been used for treatment of hypertension and silicosis. Tet reversed LVH induced in deoxycorticosterone-salt hypertensive rats and spontaneously hypertensive rats[4,5]. Our previous works indicated that Tet improved the cardial function of renovascular hypertensive rats[6]. This study was to investigate the changes in myosin ATPase activity and hydroxyproline content in the hypertrophied myocardium of rats resulted from renovascular hypertension (two-kidney, one-clip model), a hyperreninemia hypertension animal model. Enalapril (Ena), an angiotensin converting enzyme inhibitor generally recognized as a LVH reversing agent, was used as a positive drug.

Received date: 2000-03-09 **Accepted date**: 2000-10-17

Foundation item: The project supported by Natural Science Foundation of Guangdong Province (960560)

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1 MATERIALS AND METHODS

1.1 Reagents

Tet (> 98%), purchased from Chengdu Gaoxing Phytogenic Raw Pharmaceutical Factory. Ena was a product of Changzhou Pharmaceutical

Factory. Hydroxyproline, bovine serum albumin and adenosine 5′-triphosphate (disodium salt, ATP) were obtained from Sigma and Shanghai Boao Biologic Science and Technique Co, respectively. Other reagents were all of AR grade.

1.2 Animal model

Male Sprague-Dawley rats (clean animal, certificate №97001, Experimental Center of Nanjing Medical University), weighing (210 ± 15) g ($\bar{x} \pm s$), were used. Hypertension was produced by placing a silver clip (internal diameter 0.2 mm) around the left renal artery under chloral hydrate anesthesia. Sham-operated group was performed by sham operation. All the rats were maintained on normal rat chow and tap water *ad lib*. The systolic blood pressure (SBP) of the conscious rats was monitored regularly by the tail-cuff method using a multichannel physiological polygraph (RM-6200C, Chengdu Instrument Factory, China). After 8 weeks, rats that developed sustaining hypertension were randomly divided into 3 groups: LVH group, Tet group (50 mg·kg^{-1}) and Ena group (6 mg·kg^{-1}). Eight weeks after operation LVH should have significantly developed[2] and therefore the drugs were administrated daily by gavage for 8 weeks from the 9th week after operation for observation of the reversing effects of drugs on LVH. The LVH group and the sham-operated rats received solvent of the same volume.

1.3 Heart weight

At the end of the treatment period, the body weight was measured and the rats were killed by neck pulling, and the heart was rapidly excised. The atria, great vessels and right ventricle free walls were carefully dissected from the left ventricle. The left ventricle was opened, rinsed in cold saline, blotted dry, weighed. The right ventricle free wall was also weighed. It was taken to calculate the ratio of left ventricle wet weight to body weight (LVWW/BW) and right ventricle wet weight to body weight (RVWW/BW).

1.4 Hydroxyproline content assay

Myocardium of about 100 mg wet weight was placed into an oven with 120℃ for 15 h. The dried specimen was used to determine the content of hydroxyproline with colorimetric method[7] using hydroxyproline (5 mg·L^{-1}) as standard.

1.5 Myosin ATPase activity assay

According to the method[8], myocardium of about 100 mg was used to determine the activity of myosin ATPase. The reaction system included: extracting solution (0.05 mol·L^{-1} borate buffer, 0.5 mol·L^{-1} KCl, pH 7.4) 0.4 mL, 0.1 mol·L^{-1} $CaCl_2$ 0.1 mL, myosin supernant 0.4 mL, 50 mmol·L^{-1} ATP 0.1 mL. One mL of 1.22 mol·L^{-1} trichloroacetic acid was added in to stop the reaction 10 min after incubation in 30℃ water bath. The inorganic phosphorus (Pi) was measured with ammonium molybdate method. The protein was determined with Bradford method using bovine serum albumin as the standard.

1.6 All data were expressed as $\bar{x} \pm s$. Statistical analysis was performed using t test.

2 RESULTS

2.1 Effects of tetrandrine and enalapril on systolic blood pressure and heart weight

SBP was significantly elevated after clipping the renal artery (Tab 1). With drugs for 8 weeks, SBP was reduced by 43.9% in Tet group and 47.3% in Ena group, respectively, compared with that before administration. RVWW/BW in all groups was not different from each other. LVWW/BW was significantly elevated in LVH group, whereas LVWW/BW in Tet and Ena groups were only 77.5% and 71.5% of that in LVH group, which meant that the myocardial hypertrophy was reversed by drugs (Tab 1).

2.2 Effects of tetrandrine and enalapril on hydroxyproline content

Hydroxyproline content of LVH group increased compared with that of sham-operated one. They were 28.2% and 39.0% lower than that of LVH in Tet and Ena groups, respectively. The results suggest that Tet and Ena inhibit the synthesis of collagen from myocardium (Tab 2).

2.3 Effects of tetrandrine and enalapril on myosin ATPase activity

In LVH group, myosin ATPase activity was

Tab 1. Effects of tetrandrine(Tet) on systolic blood pressure(SBP) and ventricle weight in renovascular hypertensive rats

Group	SBP/kPa		(LVWW/BW) /mg·g^{-1}	(RVWW/BW) /mg·g^{-1}
	Before	After		
Sham-operated	15.0 ± 0.8	14.9 ± 0.7	1.80 ± 0.15	0.53 ± 0.15
LVH	25.5 ± 2.4**	26.2 ± 3.6**	2.84 ± 0.33*	0.58 ± 0.19
LVH + Tet	24.9 ± 1.6**	14.7 ± 1.6##	2.20 ± 0.26*#	0.59 ± 0.07
LVH + Ena	25.8 ± 2.2**	13.8 ± 1.8##	2.03 ± 0.50#	0.52 ± 0.05

Left ventricular hypertrophy(LVH) was induced by 2K1C renovascular hypertension for 8 weeks. Tet 50 mg·kg^{-1}·d^{-1} and enalapril(Ena) 6 mg·kg^{-1}·d^{-1} were administered from 9th weeks after operation by gavage, once daily for 8 weeks. LVWW: left ventricle wet weight; BW: body weight; RVWW: right ventricle wet weight. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with sham-operated; # $P < 0.05$, ## $P < 0.01$, compared with LVH.

(0.43 ± 0.09) mmol Pi·min^{-1}·g^{-1} protein and lower than that of sham-operated one significantly. However, the myosin ATPase activity in Tet and Ena groups were restored to different levels and 60.5%, 118.6% higher than that in LVH group, respectively(Tab 2).

Tab 2. Effects of tetrandrine on hydroxyproline content and myosin ATPase activity of hypertrophied myocardium in renovascular hypertensive rats

Group	Hydroxyproline /mg·g^{-1} dry weight	Myosin ATPase /mmol Pi·min^{-1}·g^{-1} protein
Sham-operated	3.56 ± 0.41	0.97 ± 0.06
LVH	5.92 ± 0.31**	0.43 ± 0.09**
LVH + Tet	4.25 ± 0.19*#	0.69 ± 0.03*#
LVH + Ena	3.61 ± 0.55#	0.94 ± 0.12##

See legend of Tab 1 for drug treatments. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with sham-operated; # $P < 0.05$, ## $P < 0.01$, compared with LVH.

3 DISCUSSION

The results indicated that after 8 weeks of clipping of renal artery SBP and LVH were increased. Both Tet and Ena could reduce the SBP and LVWW/BW of renovascular hypertensive rats.

Hydroxyproline makes up about 13.4% of total amino acid in collagen which may be used to represent the collagen content. Many studies[1,9] have described that the accumulation of collagen within the extracellular matrix and around intramyocardial coronary arteries of the left ventricle is reponsible for LVH. A significant increase of collagen in LVH was seen and it was reduced in Tet and Ena group which was consistent with our conclusion that Tet and Ena improved the cardiac function and hemodynamics[6]. Calcium in fibroblast cells may facilitate the synthesis of procollagen and inhibit the collagenase activity[11]. Tet, which inhibited both L and T calcium channel current in ventricular cells[3] led to decrease of $[Ca^{2+}]_i$ and inhibiting synthesis of collagen[12] in myocardium. Angiotensin Ⅱ (AngⅡ) and aldosterone also play a crucial role in cardiac fibrosis[11,12]. Ena may decrease the production of AngⅡ and aldosterone, the cardiac fibrosis has been inhibited consequently.

There are two isoforms of myosin heavy chain[13], i.e., α-MHC and β-MHC. Myosin isoenzyme V_1 is the αα homodimer and has the highest ATPase activity; V_3 is the ββ homodimer with the lowest ATPase activity, while V_2 is believed to be the αβ heterodimer. V_1 Is the predominant isoenzyme in adult and V_3 is in fetal animal heart mainly. Myosin isoenzyme may converted from V_1 into V_3 when LVH developed[2]. The decrease of contractility of hypertrophied myocardium was closely associated with the shift of myosin isoform[13]. In this study, myosin ATPase activity in LVH was only 44.3% of that in sham-operated one which suggests that myosin heavy chain in hypertrophied myocardium shift from V_1 to V_2 or V_3 isoform. Tet blocked calcium channel, whereas Ena inhibited angiotensin converting enzyme from

which many neurohormones and growth factors did not play their roles on facilitating emboric-type protein synthesis in myocardial cells[14].

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粉防己碱对高血压心肌肥厚大鼠心肌胶原含量和肌球蛋白 ATP 酶活性的影响

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摘要 观察粉防己碱对高血压心肌肥厚大鼠心肌胶原含量和肌球蛋白 ATP 酶活性的影响. 采用二肾一夹肾血管性高血压造成大鼠左心室肥厚模型，自术后第9周起按粉防己碱50 $mg\cdot kg^{-1}$，依那普利6 $mg\cdot g^{-1}$灌胃给药，每日1次，连续8周. 用分光光度法测定心肌羟脯氨酸含量及肌球蛋白 ATP 酶活性. 结果表明左心室肥厚组心肌羟脯氨酸为(5.9 ± 0.3) $mg\cdot g^{-1}$干重，明显高于假手术对照组[(3.6 ± 0.4) $mg\cdot g^{-1}$干重]，粉防己碱组和依那普利组心肌羟脯氨酸含量分别较左心室肥厚组低28.2%和39.0%. 左心室肥厚组肌球蛋白 ATP 酶活性仅为(0.43 ± 0.09) mmol $Pi\cdot min^{-1}\cdot g^{-1}$蛋白质，较假手术对照组[($0.97\pm0.06$) mmol $Pi\cdot min^{-1}\cdot g^{-1}$蛋白质]明显降低，而粉防己碱组和依那普利组则分别比左心室肥厚组高60.5%和118.6%. 实验结果表明粉防己碱可部分逆转肾血管性高血压所致的大鼠左心室肥厚，降低心肌胶原含量，升高肌球蛋白 ATP 酶活性.

关键词：粉防己碱；依那普利；胶原；肌球蛋白 ATP 酶；高血压，肾血管性；心脏肥厚

基金项目：广东省自然科学基金资助项目(960560)

（本文编辑 董立春）