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Effects of simvastatin on left ventricular hypertrophy and function in rats with aortic stenosis

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KEY WORDS left ventricular hypertrophy; heart function tests; simvastatin; abdominal aorta; vascular stenosis; hydroxyproline

ABSTRACT

AIM: To investigate the effects of simvastatin (Sim) on left ventricular hypertrophy in rats with pressure-overload cardiac hypertrophy. **METHODS**: The left ventricular hypertrophy (LVH) of rats was induced by partly occluding abdominal aorta below right renal artery. The rats were given ig Sim (1.8 and 3.6 mg·kg^{-1}·d^{-1}) for 8 wk. Three days after operation, left ventricular function was measured. Then the left ventricle (LV) + septum and the right ventricle (RV) were weighed. Hydroxyproline content of LV was measured. **RESULTS**: Eight weeks later, in the LVH group, LV weight (LVW), LVW/body weight (BW), LVW/RV weight (RVW), LV ending diastolic pressure (LVEDP), and hydroxyproline content increased by 36 %, 51 %, 28 %, 92 %, and 23 %, respectively (all $P < 0.01$) compared with the sham group. LV + dp/dt_{max} and $-dp/dt_{max}$ decreased by 39.2 % and 39.4 % (all $P < 0.01$). After the rats were given ig Sim 3.6 mg·kg^{-1}·d^{-1}, LVW, LVW/BW, LVW/RVW, left ventricle ending diastolic pressure (LVEDP), and hydroxyproline content decreased by 22 %, 21 %, 23 %, 24 %, and 11 % compared with LVH group (all $P < 0.01$), LV + dp/dt_{max} and $-dp/dt_{max}$ increased by 60 % and 32 % (all $P < 0.01$). **CONCLUSION**: Sim inhibited development of LV hypertrophy and improved LV function in rats with aortic stenosis.

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INTRODUCTION

Mevalonate (Mev) pathway plays a role in cell growth. Mev is intracellularly synthesized from 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), and this process is catalyzed by HMG-CoA reductase, the rate-limiting enzyme in this pathway[1]. Mev metabolism yields a series of isoprenoids that are vital for diverse cellular function[2]. Several proteins post-translationally modified by the covalent attachment of Mev-derived isoprene groups (prenylation), farnesyl-PP or geranylgeranyl-PP, have been identified. These proteins must be prenylated as a prerequisite for membrane association, which, in turn, is required for their function[3-5]. Among the prenylated proteins, much attention has been focused on Ras for its key role in the pathophysiology of cell proliferation. Ras, a guanine nucleotide-binding protein, is activated by signal transduction pathways involved in growth and differentiation. Activated Ras has been linked to cardiac hypertrophy[6,7]. Simvastatin (Sim), an inhibitor of HMG-CoA reductase, inhibits the production of Mev. The aim of the present study was to investigate the effects of Sim on hypertrophic remodeling in rats with pressure-overload cardiac hypertrophy.

MATERIALS AND METHODS

Materials Sim was purchased from Merck, Sharp & Dohme Inc (Woodbridge NJ). It was dissolved in distilled water. Sprague-Dawley rats (n = 31, Grade Ⅱ, Certificate № 98A033), ♂, body weight (BW) 76 g ± 5 g, were purchased from Guangdong Medical Laboratory Animal Center.

Aortic stenosis The rats were anesthetized with pentobarbital sodium (45 mg·kg^{-1}, ip). The

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abdominal aorta just below right renal artery was only separated but not ligated in the rats of sham operation group (Sham). The abdominal aorta was occluded with No 5 needle in the other rats, then the needle was drawn out. After operation, each rat was given im benzylpenicillin 50 kU. The rats were divided into 4 groups at random. 1) Sham: 3 d after operation, the rats were given ig distilled water 10 mL·kg^{-1} as Sim groups; 2) left ventricle hypertrophy (LVH): the same as sham except that abdominal aorta was occluded; 3) low dosage of Sim: 3 d after operation, the rats were given Sim (1.8 mg·kg^{-1}·d^{-1}, ig for 8 wk); 4) high dosage of Sim: the same as 3), except for Sim 3.6 mg·kg^{-1}·d^{-1}.

Heart function and LVH evaluation At the end of the 8-wk treatment, rats were selected for measurement of LV pressure. The left ventricular apex was punctured by a 22 G needle filled with heparinized saline[9]. Pressure in the LV was measured with a Statham pressure transducer with its diaphragm located at the heart level, and recorded on LMS-2B (Chengdu Medical Equipment Factory) physical recording apparatus. Immediately after the functional study, the right ventricle (RV) was separated from the left ventricle and septum (LV).

Determination of myocardial hydroxyproline concentrations Quantification of LV myocardial hydroxyproline concentrations was determined[8]. LV specimens of approximately 90 mg were hydrolyzed in HCl 6 mol·L^{-1} at 100 ℃ for 22 h. The hydrolyzed material was dried and reconstituted in 5 mL H_2O. Hydrolysate 200 μL was mixed with chloramine T 200 μL (1.4 % in citrate uffer) and allowed to oxidize at 25 ℃ for 20 min. Ehrlich's reagent (400 μL), containing *p*-dimethylaminobenzaldehyde 12 g in ethanol 40 mL + H_2SO_4 2.7 mL, was added. After 3 h of incubation at 37 ℃, extinction at 573 nm was measured.

Statistics Data were compared with *t* test.

RESULTS

Effect of Sim on LVH In the LVH group, LVW, the ratio of LVW/BW, and LVW/RVW increased by 36 % ($P<0.01$), 51 % ($P<0.01$) and 28 % ($P<0.01$) compared with the sham group.

Sim partly prevented the changes mentioned above (Tab 1).

Effects of Sim on LV function LVSP, LV Dev P/g, and LVEDP in hypertrophy group increased by 48 % ($P<0.01$), 8 % ($P>0.05$), and 92 % ($P<0.01$) compared with the sham group. But LV

Tab 1. Effects of SIM on left ventricular hypertrophy with aortic stenosis and on left ventricular function in the rats. $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* sham. $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* LVH. $^{g}P>0.05$, $^{h}P<0.05$, $^{i}P<0.01$ *vs* SIM 1.8 mg·kg^{-1}·d^{-1}.

	Sham	LVH	SIM 1.8 mg·kg^{-1}·d^{-1}	SIM 3.6 mg·kg^{-1}·d^{-1}
n	9	7	8	7
Left ventricular hypertrophy				
BW, g	245 ± 12	231 ± 9^{a}	239 ± 11^{ad}	228 ± 12^{adg}
LVW, g	0.661 ± 0.017	0.904 ± 0.012^{c}	0.81 ± 0.03^{cf}	0.70 ± 0.04^{bfi}
LVW/BW, g·kg^{-1}	2.59 ± 0.24	3.91 ± 0.16^{c}	3.30 ± 0.17^{cf}	3.08 ± 0.06^{cfi}
RVW, g	0.144 ± 0.013	0.168 ± 0.018^{c}	0.151 ± 0.012^{bd}	0.156 ± 0.011^{cdg}
LVW/RVW, g·g^{-1}	4.56 ± 0.34	5.87 ± 0.22^{c}	5.40 ± 0.37^{cf}	4.53 ± 0.19^{dfi}
Left ventricle function				
LVSP, kPa	15.6 ± 1.0	23.1 ± 2.2^{c}	22.6 ± 2.3^{cd}	23.3 ± 1.9^{cdg}
LV Dev P, kPa	23.7 ± 2.1	25.6 ± 2.7^{a}	27.9 ± 2.2^{cd}	33.2 ± 2.1^{cfi}
LV + d*p*/d*t*max, kPa·s^{-1}	550 ± 82	334 ± 46^{a}	474 ± 55^{bf}	536 ± 57^{adg}
LV − d*p*/d*t*max, kPa·s^{-1}	424 ± 85	257 ± 50^{c}	292 ± 63^{cd}	341 ± 79^{aeg}
LVEDP, kPa	0.54 ± 0.05	1.04 ± 0.09^{c}	0.86 ± 0.05^{cf}	0.79 ± 0.10^{cfg}
Heart rate, beat·min^{-1}	249 ± 8	253 ± 6^{a}	249 ± 10^{ad}	254 ± 9^{adg}

$+dp/dt_{max}$ and $-dp/dt_{max}$ decreased by 39.2 % ($P<0.01$) and 39.4 % ($P<0.01$). Sim reversed the changes of LV $+dp/dt_{max}$ and $-dp/dt_{max}$, and LVEDP.

Effects of Sim on hydroxyproline content in LV tissue In LVH group, hydroxyproline content in LV tissue increased by 23 % [LVH group (2.64 ± 0.22) $mg\cdot g^{-1}$, $n=7$, *versus* sham group (2.15 ± 0.13) $mg\cdot g^{-1}$, $n=9$, $P<0.01$]. Sim reduced the hydroxyproline content by 7.7 % and 11.5 % in low and high dosages of Sim group compared with LVH group respectively [Sim 1.8 $mg\cdot kg^{-1}\cdot d^{-1}$ group (2.39 ± 0.13) $mg\cdot g^{-1}$, $n=8$, $P<0.05$, Sim 3.6 $mg\cdot kg^{-1}\cdot d^{-1}$ group (2.27 ± 0.18) $mg\cdot g^{-1}$, $n=7$ rats, *versus* LVH group (2.64 ± 0.22) $mg\cdot g^{-1}$, $n=7$ rats, $P<0.01$].

DISCUSSION

Sim is an HMG-CoA reductase inhibitor. It is able to inhibit the production of Mev-derivatives and cholesterol. It was used to treat hypercholesterolemia. Now we first proved that Sim was able to inhibit cardiac hypertrophy induced by partly constricted rat abdominal aorta and improved the heart function.

Proliferation of collagen fiber is an important reason of decreased diastolic function in rats with cardiac hypertrophy. Assuming that collagen contains 14 % hydroxyproline and thus that the hydroxyproline content parallels the total collagen content. When the collagen content increased, relax of the left ventricle decreased, and the stiffness of heart increased, these could reduce the diastolic function of the heart. Sim could improve the left ventricular diastolic function through inhibiting proliferation of collagen fiber.

In the present study, we do not answer the mechanism that Sim suppressed the cardiac hypertrophy in rat with pressure-overload. Mev is necessary for cell growth [10]. The activity of Ras protein is dependent on the post-translation of Mev-derivatives. A number of investigators have showed that activated Ras has been linked to cardiac hypertrophy [6,7]. It is possible that Sim inhibits the production of Mev and isoprenyl group in cardiac tissue and makes the Ras protein unable to locate on the membrane and loss its activity. Ras protein is a key component of receptor tyrosine kinase (RTK) signaling pathway. Many peptide hormones bind RTK. Following ligand binding, these receptors autophosphorylate tyrosine residue and lead to formation of Ras·GPT. The Ras·GPT complex initiates the sequential activation of three protein kinases: Raf protein kinase → MEK, a dual-specificity kinase → MAP kinase. Activated MAP kinase phosphorylates numerous substrate protein, including other kinase, cytoskeletal elements, and transcription factors, thereby affecting their activity. So Ras protein plays a central role in controlling cell growth and differentiation [11].

Local renin-angiotensin system plays a key role in the development of cardiac hypertrophy [12]. Many investigators have proved that angiotensin converting enzyme (ACE) inhibitor and angiotensin AT1 receptor blocker show the effect of anti-hypertrophy in rat with pressure-overload [13]. Mitani *et al* [14] recently showed that fluvastatin significantly lowered the tissue ACE in the aorta of rabbits that were fed a 1.5 % cholesterol. It is another possible mechanism of anti-hypertrophy of Sim to inhibit cardiac tissue ACE activity.

The third anti-hypertrophy possible mechanism is that Sim inhibits Na^+/H^+ exchange. Takewaki *et al* [15] have showed that activation of Na^+/H^+ exchange and its gene expression was involved in molecular mechanism of cardiac hypertrophy. There was evidence that Sim was able to inhibit the Na^+/H^+ exchange.

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辛伐他汀对主动脉狭窄大鼠左心室肥大和功能的影响

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关键词 左心室肥大；心脏功能试验；辛伐他汀；腹主动脉；血管狭窄；羟脯氨酸

目的：研究辛伐他汀抗心肌肥厚作用. **方法**：部分狭窄腹主动脉、大鼠 ig 辛伐他汀 8 周后进行各项指标测定. **结果**：8 周后，左心室肥大(LVH)大鼠左心室重量(LVW)，左心室重量/体重(LVW/BW)，左右心室重量比(LVW/RVW)，左心室舒张末期压力 (LVEDP)和羟脯氨酸的含量分别比假手术组大鼠增加 36 %，51 %，28 %，92 %和23 % ($P<0.01$)，$LV+dp/dt_{max}$和$-dp/dt_{max}$分别减少 39.2 %和 39.4 % ($P<0.01$). 辛伐他汀 ig 8 周 (3.6 $mg\cdot kg^{-1}\cdot d^{-1}$)使 LVW，LVW/BW，LVW/RVW，LVEDP 和羟脯氨酸的含量分别比 LVH 大鼠减少 22 %，21 %，23 %，24 %，和 11 % ($P<0.01$). **结论**：辛伐他汀抑制大鼠左心室肥大的形成，改善左心室功能.

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