ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Feb; 23 (2): 124 - 128
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Effects of simvastatin on activities of endogenous antioxidant enzymes and angiotensin-converting enzyme in rat myocardium with pressure-overload cardiac hypertrophy[1]

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KEY WORDS angiotensin Ⅱ; antioxidants; cardiomegaly; peptidyl-dipeptidase A; simvastatin

ABSTRACT

AIM: To investigate effects of simvastatin (Sim) on the activities of antioxidant enzymes and angiotensin-converting enzyme in rat myocardium with pressure-overload cardiac hypertrophy. **METHODS**: Left ventricular hypertrophy (LVH) was induced by partly constricting rat abdominal aorta between the left and right renal artery. Rats were given ig Sim 1.8 and 3.6 mg·kg^{-1}·d^{-1} for 8 weeks following 6 weeks aortic constriction. Activities of antioxidant enzymes and angiotensin-converting enzyme, and lipid peroxidation of left ventricular (LV) tissue were determined. **RESULTS**: Contents of angiotensin Ⅱ and thiobarbituric acid reactive substances (TBARS), and activity of ACE in LVH group ($n = 8$) were increased by 163 %, 90 %, and 130 %, respectively ($P < 0.01$) compared with sham-operated group ($n = 7$), and were decreased by 30 %, 37 %, and 51 %, respectively ($P < 0.01$) in high dosage Sim treatment group ($n = 9$) compared with LVH group. Activities of catalase and glutathione peroxidase of LV tissue in LVH group were decreased by 29 % and 23 % ($P < 0.01$) compared with sham-operated group, and were increased by 32 % and 22 % ($P < 0.01$) in high dosage Sim treatment group compared with LVH group. Activity of Cu, Zn-superoxide dismutase (SOD) of LV tissue was increased by 33 % in LVH group compared with sham-operated group. Sim treatment did not significantly affect activity of Cu, Zn-SOD. **CONCLUSION**: Alteration of redox status in myocardium is associated with cardiac hypertrophy and inhibitory effects of Sim on cardiac hypertrophy in rats model might be linked to its antioxidant effects.

INTRODUCTION

Recent studies showed that norepinephrine[1], angiotensin Ⅱ, tumor necrosis factor-α[2], and ouabain[3] stimulated the intracelluar reactive oxygen species (ROS) production in cultured neonatal rat cardiomyocytes. These stimuli induced ROS-dependent cardiomyocyte hypertrophy. These results suggest that ROS plays an important role in signal transduction of the neonatal rat cardiomyocyte hypertrophy. Thus, abnormal ROS accumulation caused by the hypertrophic factors might lead to cardiomyocyte hypertrophy. Therefore, we investigated the status of antioxidant enzymes and lipid peroxidation in the rat heart with aortic stenosis. We also determined effects of simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on endogenous antioxidant enzymes and angiotensin converting enzyme (ACE) activity in hypertrophic heart, since HMG-CoA inhibitors significantly preserved endogenous antioxidant enzyme activity[4] in the aorta of rabbit fed high cholesterol diet and showed antioxidant action[1,5].

MATERIALS AND METHODS

Materials All chemicals were of analytical grade. Simvastatin, xanthine, and xanthine oxidase were products of Merck, Sharp & Dohme Inc. Cytochrome C was purchased from Serva Chemical Co. Bovine SOD and beef liver catalase were from Sigma. Sprague-Dawley rats (Grade Ⅱ, Certificate No 99A033), ♂, weighing 77 g ± 6 g, were purchased from Guangdong Medical Laboratory Animal Center.

[1] Project supported by Education Ministry of China (No 0110-B042) and Education Committee of Guangzhou.
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Received 2001-07-23 Accepted 2001-10-25

Preparation of animals Rats were anesthetized with pentobarbital sodium (45 mg/kg, ip). The abdominal aorta between the left and right renal artery was constricted to a diameter of 0.60 mm, equivalent to the external diameter of a # 5 needle, which was included in and then withdrawn from a ligature. In the sham-operated group, as surgical and age-matched controls, the same surgical operations were performed as in the above-mentioned rats except the aorta was constricted ($n = 7$). After surgical operation, each rat was given im benzylpenicillin 50 kU to prevent infection. Six weeks after aortic partial coarctation, rats were allocated randomly into 3 groups: (A) Low dosage Sim treated group (LVH/LSim), receiving Sim 1.8 mg·kg^{-1}·d^{-1} (ig, $n = 8$); (B) High dosage Sim treated group (LVH/HSim), receiving Sim 3.6 mg·kg^{-1}·d^{-1} (ig, $n = 9$); (C) LVH control group, receiving normal saline ($n = 8$). Drug treatments continued for 8 weeks following 6 weeks aortic constriction.

Homogenization of LV tissue The LV tissues were sliced into small pieces and thoroughly washed with potassium phosphate buffer 50 mmol/L, to avoid contamination from blood. Homogenization of the LV tissue was performed following the method described by Lee *et al*[6]. The homogenates were used for determination of lipid peroxidation and antioxidant enzyme activity. Protein concentration was determined by Lowry method[7] using bovine serum albumin as a standard.

Assay of ACE activity in LV tissue The ACE activity in the LV tissue was determined by the production rate of hippuric acid from the synthetic tripeptide substrate, hippuryl-*L*-histidyl-*L*-leucine (HHL), by previously described method[8].

Determination of angiotensin Ⅱ concentration in LV tissue The LV tissues were thawed on ice and chilled in 10 mL ice-cold Tris·HCl buffer (pH 7.4). After centrifugation (at 480 × *g* for 15 min), the supernatant was diluted with 2 mL of ice-cold KCl 1 mol/L and centrifuged at 100 000 × *g* for 30 min. The resulting supernatant was lyophilied and the dry residues dissolved in 1.2 mL of Tris·HCl reconstitution buffer (pH 7.4). Angiotensin Ⅱ was determined with a RIA kit (Beijing Biological Technology Institute) according to the instructions of the manufacturer.

Superoxide dismutase assay Superoxide dismutase (SOD) activity was determined spectrophotometrically by the ability of the supernatants to inhibit the reduction of cytochrome C by $O_2^{\cdot-}$ generated by the addition of xanthine 118 μmol/L and xanthine oxidase 10 U/L[9]. One unit of SOD is defined as that amount of enzyme which inhibits the rate of cytochrome C reduction by 50 %.

Catalase (CAT) assay The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. Activity of CAT in LV tissue homogenates was assayed by monitoring decomposition of H_2O_2 10 mmol/L by the rate of decrease in absorbance at 240 nm, as previously described by Aebi[10].

Glutathione peroxidase (GSH-Px) assay GSH-Px activity was assessed spectrophotometrically using the method of Flohe and Gunzler[11]. The tert-butyl hydroperoxide was used as substrate instead of H_2O_2 and sodium azide 1 mmol/L to inhibit CAT. Reaction rate was determined at 340 nm and 37 ℃ for 5 min.

Lipid peroxidation assay Lipid peroxidation formation in the homogenates of LV tissues was determined spectrophotometrically by examining the content of thiobarbituric acid reactive substances (TBARS) as described[12]. A standard curve of malondialdehyde (MDA) was generated as a reference and TBARS are reported as mol equivalents.

Statistic analysis The statistical analysis of differences between LVH, LVH/LSim, LVH/HSim, and sham groups was compared by ANOVA, followed by Newman-Keuls test. Statistical significance was accepted at the level of $P < 0.05$.

RESULTS

ACE activity and content of angiotensin Ⅱ in LV tissue In LVH group, ACE activity and content of angiotensin Ⅱ in LV tissue were increased by 130 % and 163 %. Low and high dosage simvastatin could reduce the ACE activity in LV tissue by 33 % and 51 %, and decrease angiotensin Ⅱ concentration in LV tissue by 17 % and 30 % respectively compared with LVH group (Fig 1).

SOD activity in LV tissue At the end of the 8 weeks treatment period, the Cu, Zn-SOD activity in LV tissue of LVH group was significantly higher than sham-operated group (increased by 33 %). There was no significant difference in Mn-SOD activity between LVH and sham-operated group. Simvastatin had no effects on the Cu, Zn-SOD and Mn-SOD activities of LV tissue (Fig 2A, 2B).

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Feb; 23 (2)
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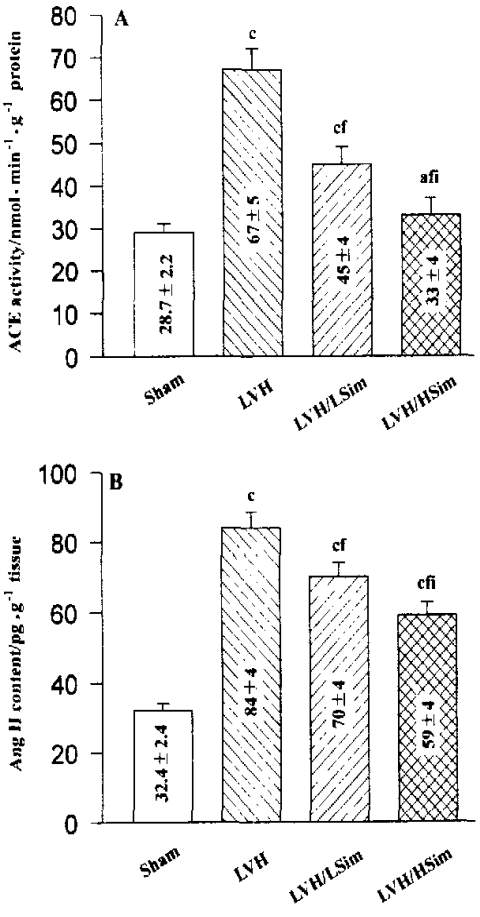

Fig 1. Effects of simvastatin on ACE activity (A) and content of angiotensin Ⅱ (B) in LV tissue. Sham: sham-operated control group ($n = 7$); LVH: untreated aortic stenosis group ($n = 8$); LVH/LSim: simvastatin-treated (1.8 mg·kg^{-1}·d^{-1}) LVH group ($n = 8$); LVH/HSim: simvastatin-treated (3.6 mg·kg^{-1}·d^{-1}) LVH group ($n = 9$). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* Sham. $^fP < 0.01$ *vs* LVH. $^iP < 0.01$ *vs* LVH/LSim.

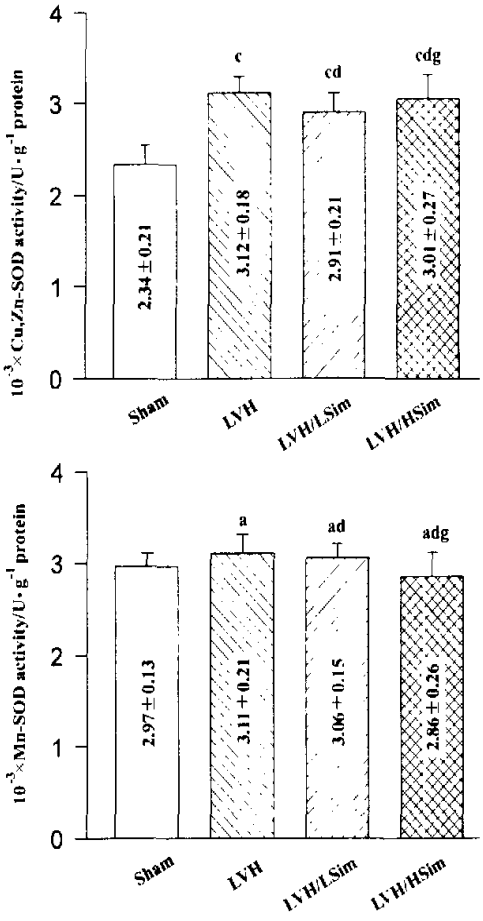

Fig 2. Effects of simvastatin on SOD activitiy in LV tissue. Sham: sham-operated control group ($n = 7$); LVH: untreated aortic stenosis group ($n = 8$); LVH/LSim: simvastatin-treated (1.8 mg·kg^{-1}·d^{-1}) LVH group ($n = 8$); LVH/HSim: simvastatin-treated (3.6 mg·kg^{-1}·d^{-1}) LVH group ($n = 9$). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* Sham. $^dP > 0.05$ *vs* LVH. $^gP > 0.05$ *vs* LVH/LSim.

Activities of CAT, GSH-Px, and lipid peroxides in LV tissue In LVH group, activities of CAT and GSH-Px in LV tissue were decreased by 29 % and 23 %. Low and high dosage simvastatin could increase the CAT activity by 19 % and 32 %, and increase the GSH-Px activity by 11 % and 22 % respectively compared with LVH group (Fig 3). LV tissue lipid peroxides levels were significantly increased in LVH group compared with sham-operated group (increased by 90 %). Low and high dosage simvastatin decreased lipid peroxides levels in LV tissue (reduced by 27 % and 37 %).

DISCUSSION

The major new finding of this work is that imbalance of ROS-producing materials and antioxidant enzymes plays an important role in cardiac hypertrophy of rats with aortic stenosis. Firstly, the ACE activity and content of Ang Ⅱ, a stimulator of H_2O_2 production, in LV tissue of LVH group are higher than those of sham rats. Secondly, the activity of SOD that converts $O_2^{\cdot-}$ into H_2O_2 in LV tissue of LVH group is significantly increased than that of sham rats. Thirdly, the activities of CAT and GSH-Px that can specifically decompose H_2O_2 in LV tissue of LVH group are significantly decreased than those

of sham rats. Fourthly, HMG-CoA reductase inhibitor simvastatin can reduce content of Ang Ⅱ and increase the activities of CAT and GSH-Px in LV tissue of LVH group.

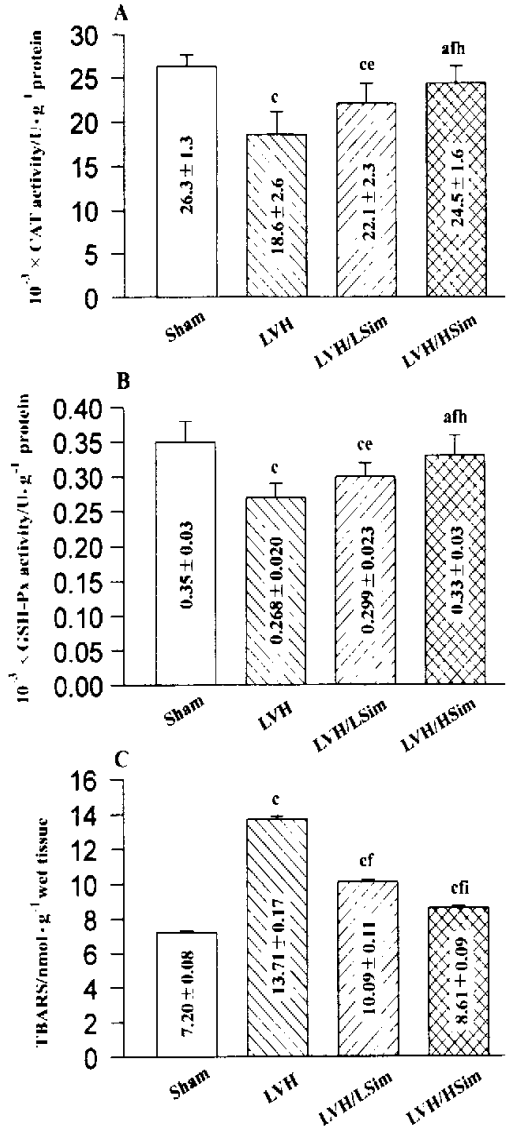

Fig 3. Effects of simvastatin on activities of CAT (A), GSH-Px (B), and lipid peroxides (C) in LV tissue. Sham: sham-operated control group (n = 7); LVH: untreated aortic stenosis group (n = 8); LVH/LSim: simvastatin-treated (1.8 mg·kg^{-1}·d^{-1}) LVH group (n = 8); LVH/HSim: simvastatin-treated (3.6 mg·kg^{-1}·d^{-1}) LVH group (n = 9). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* Sham. $^eP < 0.05$, $^fP < 0.01$ *vs* LVH. $^bP < 0.05$, $^iP < 0.01$ *vs* LVH/LSim.

Physiological level of ROS is necessary to maintain normal cellular activity. There are many enzymatic sources for ROS in all cell types. The activity or expression of these enzymes can be regulated by cytokines and growth factors. Recent studies showed that norepinephrine, angiotensin Ⅱ, tumor necrosis factor-α, and ouabain stimulated the intracelluar reactive oxygen species (ROS) production in cultured neonatal cardiomyocyte of rat and antioxidant could inhibit enlargement of cardiac myocytes induced by norepinephrine, angiotensin Ⅱ, tumor necrosis factor-α[1-3]. Ang Ⅱ increases H_2O_2 production by activating an NADH/NADPH oxidase[9]. H_2O_2 can cause myocyte hypertrophy through activating MAPK and p^{38} MAPK signal transduction pathways[13]. These results suggested that alteration of redox status were involved in cardiac hypertrophy.

Our results previously showed that simvastatin inhibited cardiac hypertrophy in rats with aortic stenosis and myocyte hypertrophy in neonatal rat cardiomyocytes[1,14,15]. In this study, our results indicated that anti-hypertrophic effect of simvastatin might be related to its antioxidant effect since simvastatin lowered Ang Ⅱ level and increased CAT and GSH-Px activities in hypertrophic myocardium. Simvastatin also reduced TBARS content in the heart of rats with aortic stenosis.

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ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Feb; 23 (2)
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辛伐他汀对压力超负荷心肌肥厚大鼠心肌内源性抗氧化酶及血管紧张素转化酶活性的影响[1]

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关键词 血管紧张素Ⅱ; 抗氧化剂; 心肥大; 肽基二肽酶 A; 辛伐他汀

目的: 研究辛伐他汀对压力超负荷心肌肥厚大鼠心肌内源性抗氧化酶及血管紧张素转化酶活性的影响. **方法**: 在左右肾动脉之间部分结扎腹主动脉诱导左心室肥厚(LVH). 6 周后, 大鼠 ig 辛伐他汀 1.8 和 3.6 $mg \cdot kg^{-1} \cdot d^{-1}$ 8 周, 然后测定左心室组织内源性抗氧化酶和血管紧张素转化酶(ACE)活性以及脂质过氧化物(TBARS)含量. **结果**: LVH 组($n = 8$)大鼠左心室组织血管紧张素Ⅱ (Ang Ⅱ)和 TBARS 含量、ACE 及 Cu,Zn-超氧化物歧化酶(SOD)活性分别比假手术组($n = 7$)大鼠增加 163 %、90 %、130 %和33 % ($P < 0.01$), 而过氧化氢酶(CAT)和谷胱甘肽过氧化物酶(GSH-Px)的活性则分别比假手术组大鼠减少 29 %和 23 % ($P < 0.01$). 3.6 $mg \cdot kg^{-1} \cdot d^{-1}$ 辛伐他汀处理的大鼠($n = 9$) Ang Ⅱ、TBARS 含量及 ACE 活性比 LVH 组大鼠分别减少 30 %, 37 % 和 51 %, 而 CAT 和 GSH-Px 的活性比 LVH 组大鼠分别增加 32 %和 22 % ($P < 0.01$). 辛伐他汀对 SOD 活性没有明显的影响. **结论**: 心肌内抗氧化酶活性的变化与压力超负荷性心肌肥厚的形成和发展有关. 辛伐他汀对大鼠心肌肥厚的抑制作用可能与其抗氧化作用有关.

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