ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9): 855 - 860
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Effects of fluvastatin on structure and function of resistant vessels in spontaneously hypertensive rats

LIN Zhi-Hong, XIE Liang-Di[1], WU Ke-Gui, WANG Hua-Jun, XU Chang-Sheng
(*Hypertension Division, First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, China*)

KEY WORDS fluvastatin; hydroxymethylglutaryl-CoA reductase inhibitors; inbred SHR rats; thoracic aorta; mesenteric arteries; vasodilation; vasoconstriction; hypertension

ABSTRACT

AIM: To evaluate the effects of fluvastatin, a hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor, on the alterations of structure and function of resistant vessels in spontaneously hypertensive rats (SHR). **METHODS**: Eight-week-old male SHR were given fluvastatin 20 $mg \cdot kg^{-1} \cdot d^{-1}$ by gavage. Rats were decapitated at 16 wk. Wall-to-lumen area ratios (W/L) of thoracic aorta and mesenteric arteries (3rd grade branch) were assessed by morphometric assay. The effects of fluvastatin on vascular reactivity to sodium nitroprusside (SNP) and norepinephrine (NE), were studied with rings of thoracic aorta and mesenteric arteries isolated from rats. **RESULTS**: After 8 wk of treatment, histological examination showed that the wall-to-lumen area ratio was lower in SHR_{flu} than that in SHR (0.44 ± 0.09 *vs* 0.79 ± 0.09, $P < 0.05$). EC_{50} of vasodilation response was much lower in SHR_{flu} than that in SHR [(4.9 *vs* 190) $pmol \cdot L^{-1}$, $P < 0.05$], while EC_{50} of mesenteric artery rings from SHR_{flu} was somewhat lower than that of SHR [(0.02 *vs* 0.04) $nmol \cdot L^{-1}$, $P > 0.05$]. In both aortic and mesenteric artery rings, EC_{50} of vasoconstriction in response to NE from SHR_{flu} was higher than that of SHR [thoracic aorta: (0.20 *vs* 0.02) $nmol \cdot L^{-1}$, $P < 0.05$; mesentric arteries: (1.46 *vs* 0.72) $nmol \cdot L^{-1}$, $P < 0.05$]. **CONCLUSION**: Short-term treatment with fluvastatin ameliorated the vasomotoricity of resistant vessels, enhanced the sensitivity to vasodilator and depressed the sensitivity to vasoconstrictor; fluvastatin also attenuated the resistant vascular hypertrophy during the development of *hypertension in SHR*.

[1] Correspondence to Prof XIE Liang-Di.
Phn 86-591-335-7199, ext 756/269. Fax 86-591-331-8716.
E-mail LdXie@163.net
Accepted 1998-03-04

INTRODUCTION

As a potent inhibitor of 3-hydroxy-3-methyl-gluyraryl coenzyme A (HMG-CoA) reductase (the rate limiting enzyme in cholesterol biosynthesis), fluvastatin was effective in not only reducing serum lipids, but also inhibiting cultured vascular smooth muscle cells (VSMC) proliferation and migration[1]. It exhibited the ability to reverse endothelial dysfunction and to prevent restenosis after successful percutaneous transluminal coronary balloon angioplasty (PTCA) in high cholesterol-fed rabbits[2-4].

The "amplifier" role of resistant vessels hypertrophy was demonstrated in the development of hypertensive "vicious cycle", which might be partially accounted for the overproliferation of VSMC[5].

In hyperlipidemia, the beneficial effects of HMG-CoA reductase inhibitors have been demonstrated. However, The effects of statins on resistance vessels in spontaneously hypertensive rats (SHR) have not been studied previously. The purpose of this study was to test the effects and underlying mechanism of fluvastatin treatment on resistant vessels during the development of hypertension in SHR.

MATERIALS AND METHODS

Rats and treatment SHR (Certificate № 30-25) and WKY (Certificate № 30-27) rats were bred in our laboratory, 4 to 6 per cage at (22 ± 2) ℃, humidity of 55 % ± 5 %, a 12-h light/dark cycle

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

(07:00 – 19:00), and fed with standard rat chow and tap water *ad lib*.

Eight-week-old male SHR rats (n = 14) were given fluvastatin (Lescol, 20 $mg \cdot kg^{-1} \cdot d^{-1}$, NOVARTIS Pharmaceutical, Ltd) by gavage in mixture with water. Treatment was maintained until 16 wk of age. Sex and age matched untreated SHR (n = 12) and WKY (n = 12) were given only some water. Experiments were performed at 16 wk old.

Measurement of systolic blood pressure (SBP) and serum lipids SBP was measured using tail-cuff technique (MRB-Ⅲ A computer control sphygmomanometer for rats, Shanghai Institute of Hypertension Research) at the beginning and the end of treatment under consciousness.

Rats were decapitated at the end of experiment. Three mL of blood sample was collected and centrifugated immediately, total cholesterol (TC) and triglyceride (TG) concentrations were determined by CHOD-PAP and GPO-PAP methods respectively within 3 h. The concentration of low density lipid protein (LDL)-cholesterol was calculated by the formula of Friedwald: LDL = TC – HDL – TG/2.2 (TG < 4.52 $mmol \cdot L^{-1}$).

Morphometric determination After rats were decapitated, abdominal aorta was cannulated immediately, sodium nitroprusside (0.1 $mg \cdot L^{-1}$) and heparin (0.1 $mg \cdot L^{-1}$) were perfused for 5 min to assure maximal vasodilation and anticoagulation. Thereafter, 10 % formaldehyde in Hanks' balanced solution was used for *in situ* fixation under a pressure of 2.93 kPa for 24 h. Thoracic aorta (elastic conducting vessels) and the 3rd grade branch vessels of mesenteric arteries (arteriolar resistance arteries) were processed and embeded in paraffin for transverse sections (6 – 10 μm) and stained with haematoxylin/esoin (HE) method. Wall/lumen (W/L) ratio of the two arteries denoted the ratio of the media cross sectional area/lumen area.

The areas of various component of vessels were measured using 2 methods: visual point counting and video image planimeter (CMIAS-B multifunctional colored pathology image analysis system, the Imaging Center of Beijing University of Aeronautics and Astronautics, and Air Force General Hospital). Linear regression showed a close correlation between the two methods (n = 59, r = 0.997).

Vascular responsiveness After rats were decapitated, thoracic aorta and mesenteric arteries (1st grade branch) were isolated and placed into ice-cold modified Tyrod's buffer solution (NaCl 136.8; KCl 5.4; $CaCl_2$ 1.8; $NaHCO_3$ 1.2; $MgCl_2$ 1.05; Tris 5.0; glucose 11.0 $mmol \cdot L^{-1}$) immediately. The vessels were cleaned of excess fat and connective tissue, and cut into rings 3 – 5 mm in longitudinal length. Responses of the rings to sodium nitroprusside (SNP) and NE in various concentration were conducted as described by Mulvany[6].

The ring preparations were suspended between stainless steel triangular holders and transferred to muscle baths containing bathing medium at 37 ℃ ± 0.5 ℃ and bubbled with 95 % O_2 and 5 % CO_2 mixture gas. The changes in isometric force under a resting force of 1 g for mesenteric arteries and 2 g for thoracic aorta were monitored through a force displacement transducer (Da Hua Machine Co, Shanghai). Before the measurement, the rings were equilibrated for 60 min in the bathing medium and the medium was replaced every 15 min. Preparations were pre-contracted with NE 31.6 $nmol \cdot L^{-1}$, then SNP at concentrations of 3.16 $pmol \cdot L^{-1}$ to 31.6 $nmol \cdot L^{-1}$ was added to examine the relaxation effects. After the relaxation experiment, the samples were washed with Tyrod's solution and equilibrated again for 30 – 45 min as described. Then various concentrations of NE from 3.16 $pmol \cdot L^{-1}$ to 31.6 $nmol \cdot L^{-1}$ were added to examine the contraction effects.

Statistical analysis Data were expressed as $\bar{x} \pm s$, and tested by Student-Newman-Keul's test (ANOVA), EC_{50} of relaxation and contraction were calculated using Probit analysis (Regression).

RESULTS

Effects of short-term fluvastatin treatment on SBP and body weight (BW) At 8 wk of age, there was no difference of SBP in SHR and SHR_{flu} [(22.2 ± 0.9 *vs* 22.5 ± 1.0) kPa, $P > 0.05$]. After 8-wk treatment of fluvastatin, SBP was lower in SHR_{flu} than that in SHR [(25.5 ± 1.7 *vs* 28.4 ± 1.1) kPa, $P < 0.05$]. BW of SHR_{flu} was similar to that of untreated SHR [(271.6 ± 14.1 *vs* 278.5 ± 16.0) g, $P > 0.05$].

Effects of fluvastatin on serum lipids Serum lipids were decreased in SHR_{flu} as compared with

SHR. TC, TG, and LDL concentration was 25 %, 25 %, and 11 %, lower in SHR_{flu} than in SHR, respectively. Of note, only the decrease in serum TC concentration reached statistical significance [(1.84 ± 0.34) *vs* (2.31 ± 0.39) mmol · L^{-1}, $P < 0.05$] (Tab 1).

Tab 1. Serum lipids in 16-wk-old WKY, SHR, and SHR_{flu}. $^bP < 0.05$ *vs* SHR.

Rats		Concentration/mmol·L^{-1} TC	TG	LDL
WKY	8	2.73 ± 0.37^b	1.02 ± 0.41	1.28 ± 0.41^b
SHR	8	2.31 ± 0.39	0.94 ± 0.27	0.81 ± 0.43
SHR_{flu}	14	1.84 ± 0.34^b	0.75 ± 0.24	0.73 ± 0.39

Effects of short-term fluvastatin treatment on vascular morphometrics W/L of SHR was greater than that of WKY at 16 wk of age in both aorta and mesenteric arteries (3rd grade branch) [thoracic aorta: (0.33 ± 0.03 *vs* 0.24 ± 0.05), $P < 0.05$; mesenteric arteries: (0.79 ± 0.09 *vs* 0.26 ± 0.02), $P < 0.05$], suggesting vascular hypertrophy in SHR. Short-term fluvastatin treatment markedly reduced the ratio of mesenteric vessel (0.44 ± 0.09 *vs* 0.79 ± 0.09, $P < 0.05$). Whereas, there was no significant difference of W/L in thoracic aorta between SHR_{flu} and SHR ($P > 0.05$) (Fig 1).

Effects of short-term fluvastatin treatment on vascular function The relaxation sensitivity to SNP was suppressed in SHR as compared with WKY. 50 % of effective concentration (EC_{50}) for dilation was much higher in SHR than that in WKY [thoracic aorta: (190 *vs* 2.5) pmol · L^{-1}, $P < 0.05$; mesenteric arteries: (40 *vs* 3.97) pmol·L^{-1}, $P < 0.05$]. Short-term fluvastatin treatment markedly increased relaxation sensitivity in thoracic aorta to the level of WKY[(4.9 *vs* 2.5) pmol · L^{-1}, $P > 0.05$]. In mesenteric arteries, EC_{50} for dilation in SHR_{flu} was only 50 % of that in SHR, but this difference did not reach statistical significance (Fig 2).

Meanwhile, contraction response to NE in SHR was more sensitive than that in WKY [EC_{50} for thoracic aorta: (0.02 *vs* 0.23) nmol · L^{-1}, $P < 0.05$; for mesenteric arteries: (0.72 *vs* 1.67) nmol·L^{-1}, $P < 0.05$]. Both aorta and mesenteric arteries, the

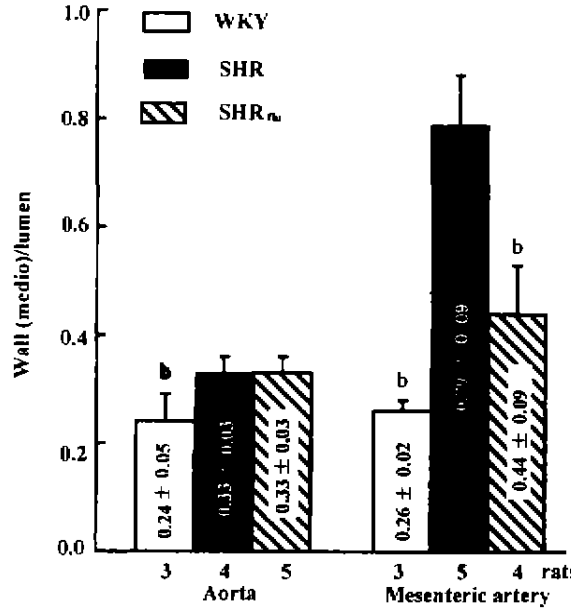

Fig 1. Wall-to-lumen area ratio of thoracic aorta and mesenteric artery from WKY, SHR, and SHR_{flu}. $^bP < 0.05$ *vs* SHR.

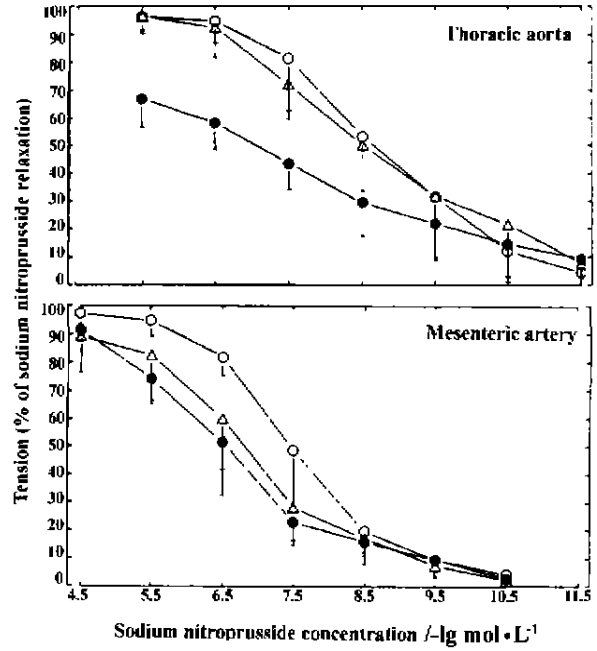

Fig 2. Effects of fluvastatin on sodium nitroprusside (SNP)-induced relaxation in thoracic aorta and mesenteric artery (1st) from WKY (○, $n = 6$ rats), SHR (●, $n = 6$ rats), and SHR_{flu} (△, $n = 7$ rats). Preparations were contracted with norepinephrine 31.6 nmol·L^{-1}.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9)
E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

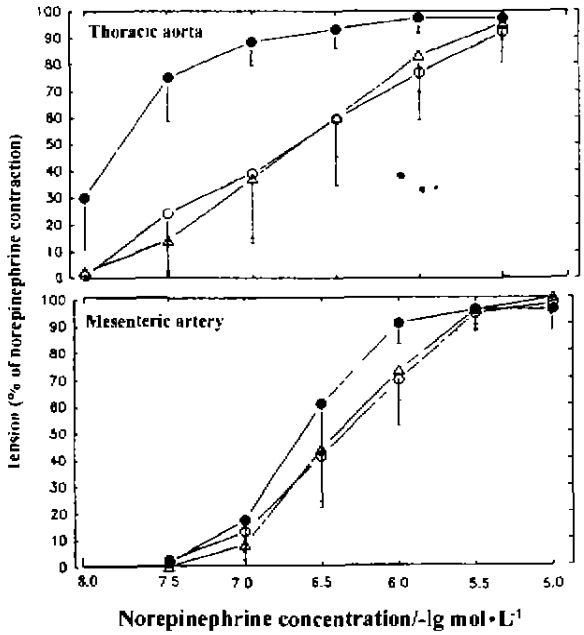

Fig 3. Effects of fluvastatin on the norepinephrine (NE)-induced contraction in thoracic aorta and mesenteric artery (1st) from WKY (○, $n = 4$ rats), SHR (●, $n = 5$ rats) and SHR_{flu} (△, $n = 7$ rats). The contraction response curve to NE was markedly right-wards shifted after 8-wk treatment of fluvastatin in SHR.

constriction sensitivity was significantly depressed to a level close to that of WKY after short-term fluvastatin treatment [EC_{50} for thoracic aorta: (0.20 *vs* 0.23) nmol·L^{-1}, $P > 0.05$; EC_{50} for mesenteric arteries: (1.46 *vs* 1.67) nmol·L^{-1}, $P > 0.05$] (Fig 3).

DISCUSSION

Hypertrophy of resistant vessels played an important role during the development of hypertension, which was related to overproliferation of VSMC stimulated by various factors (vascular stress, local angiotensin Ⅱ, circulating catecholamine etc)[7]. Recent studies indicated that HMG-CoA reductase inhibitors such as fluvastatin, simvastatin, markedly inhibited basic and stimulated proliferation and migration of VSMC at the level of micromolar[1,8]. Furthermore, the inhibitory effects could be completely reversed by adding of mevalonate. This effect was independent of its lipid-lowering action[9]. Another investigator demonstrated the prevention effects of fluvastatin on restenosis after successful PTCA *in vivo*[10]. However, whether fluvastatin has direct effects on hypertrophy of resistant vessels in SHR remains unknown.

In this study, we examined the effects of short-term fluvastatin treatment on hypertrophy of resistant vessels, and found that fluvastatin reduced the W/L of mesenteric arteries (3rd grade branch) after 8-wk treatment. We found that short-term fluvastatin treatment reduced W/L of mesenteric arteries, indicating that fluvastatin adminstration could attenuate remodelling of resistant vessels in SHR. However, the underlying mechanism by which fluvastatin inhibited proliferation of VSMC is not yet known. Recent study suggested that mevalonate was necessary for the proliferation of VSMC. The effects of fluvastatin on vascular growth might be associated with its ability to inhibit synthesis of mevalonate. The latter was not only an important mediator in the synthesis of cholesterol, but the precursor for the synthesis of isoprenoids, which were important for the posttranslational isoprenylation of many oncogene proteins involved in signal transduction pathways such as the low-molecular-weight GTP binding proteins (P^{21}ras and P^{21}rho)[11]. The effects of fluvastatin on VSMC might be secondary to the inhibitory action on these proteins.

Folkow *et al* first suggested the important role of hypertrophic resistance vessels during the development and maintenance of primary hypertension. An elevation of blood pressure caused excessive proliferation of VSMC and led to compensatory vascular wall hypertrophy, which resulted in hypertrophic medial wall and reduction in vessel lumen. Meanwhile, hypertension potentiated pressor responses of vasoconstrictors, eg NE, Ang Ⅱ etc, causing further caliber reduction of vessel lumen. Thus, a "vicious cycle" formed and higher blood pressure perpetuated[5]. It was reported that statins had direct inhibitory effects on DNA synthesis in culture SMC, the antiproliferation effects of statins might play an role in the reduction of W/L of resistance vessels.

This study also confirmed hypertrophy of resistant vessels in SHR. Furthermore, decreased relaxation sensitivity response to SNP and increased contraction sensitivity response to NE were observed both in resistant vessels and conduct vessels in SHR. Roland and colleagues reported that fluvastatin improved

progressively endothelial function of forearm resistant vessels, at least the flow-mediated vasodilation in patients with hypercholesterolemia. The improvement was ever more pronounced with the prolongation of treatment, although without concomitant reduction in TC and LDL[2]. Short-term fluvastatin treatment improved myocardial perfusion by restoring coronary endothelium function, before anatomic regression of stenosis occurred in hypercholesterolemial patients[12]. To our knowledge, there was no previous study regarding the effects of fluvastatin on vasomotor activity in SHR, a genetic model of hypertension, whose serum lipids were within normal range[13].

Our study indicated that short-term fluvastatin treatment ameliorated abnormal vasoconstrictive and vasodilative sensitivity in SHR, with EC_{50} of contraction response to NE or relaxation response to SNP in both thoracic aorta and mesenteric arteries similar to that of untreated WKY. It was reported that inhibitory effects of HMG-CoA reductase inhibitors on constriction of VSM were related to its effects on the small G proteins (P^{21}ras and P^{21}rho) activity, the latter were confirmed to be involved in the GTP_{γ}-enhanced calcium ion sensitivity of VSM contraction[14]. Recently, it was suggested that relaxation responses to acetylcholine (ACh) were significantly suppressed in cholesterol-fed rabbits, which may be reversed by treatment of fluvastatin and associated with the reduction of vascular ACE activity[3].

Interestingly, in the present study, although the increase of SBP in SHR could not be completely prevented with short-term treatment of fluvastatin, there still had a significant reduction on SBP in fluvastatin-treated SHR, consisting with the demonstration of a reduction on mean arterial pressure with another HMG-CoA reductase inhibitor-lovastatin in SHR[15]. The mechanism under which SBP was decreased by these drugs is not yet known at this stage. It is possible that this may be due to either the prevention of vascular hypertrophy or the amelioration of vasodilation, or the depressed response to vasoconstrictor.

REFERENCES

1 Rogler G, Lacker KJ, Schmitz G. Effect of fluvastatin on growth of porcine and human vascular smooth muscle cells *in vitro*. Am J Cardiol 1995; 76: 114A – 116A.

2 Schmieder RE, Schobel HP. Is endothelial dysfunction reversible? Am J Cardiol 1995; 76: 117A – 121A.

3 Mitani H, Bandoh T, Ishikawa J, Kimura M, Totsuka T, Hayashi S. · Inhibitory effects of fluvastatin, a new HMG-CoA reductase inhibitor, on the increase in vascular ACE activity in cholesterol-fed rabbits. Br J Pharmacol 1996; 119: 1269 – 75.

4 Foley DP, Bonnier H, Jackson G, Macaya C, Shepherd J, Vrolix M, *et al*. Prevention of restenosis after coronary balloon angioplasty: rationale and design of the fluvastatin angioplasty restenosis (FLARE) trail. Am J Cardiol 1994; 73: 50D – 61D.

5 Folkow B. Cardiovascular structural adaption: its role in the initiation and maintenance of primary hypertension. Clin Sci Mol Med 1987; 55 suppl 4: 3.

6 Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1977; 41: 19 – 26.

7 Folkow B. Extrinsic mechanism of vascular growth. J Clin Hypertens 1987; 3: 328 – 36.

8 Hidaka Y, Eda T. Inhibition of cultured smooth muscle cell migration by simvastatin (MK-733). Atherosclerosis 1992; 95: 87.

9 Corsini A, Raiteri M, Soma MR, Bernini F, Fumagalli R, Paoletti R. Pathogenesis of atherosclerosis and the role of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Am J Cardiol 1995; 76: 21A – 28A.

10 Bandoh T, Mitani H, Niihashi M, Kusumi Y, Ishikawa J, Kimura M, *et al*. Inhibitory effect of fluvastatin at doses insufficient to lower serum lipids on the catheter-induced thickening of intima in rabbit femoral artery. Eur J Pharmacol 1996; 315: 37 – 42.

11 Clarke S. Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. Annu Rev Biochem 1992; 61:355 – 86.

12 Eichstadt HW, Eskotter H, Hoffman I, Arnthauer HW, Weidinger G. Improvement of myocardial perfusion by short-term fluvastatin therapy in coronary artery disease. Am J Cardiol 1995; 76: 122A – 125A.

13 Iritani N, Fukuda E, Nara Y, Yamori Y. Lipid metabolism in spontaneously hypertensive rats (SHR). Atherosclerosis 1977; 28: 217 – 22.

14 Hirata K, Kikuchi A, Sasaki T, Kuroda S, Kaibuchi K, Matsuura Y, *et al*. Involvement of rho P21 in the GTP-enhanced calcium ion sensitivity of smooth muscle contraction. J Biol Chem 1992; 267: 8719 – 22.

15 Jiang J, Roman RJ. Lovastatin prevents development of hypertension in spontaneously hypertensive rats. Hypertension 1997; 30: 968 – 74.

855-860

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9)
E-mail aps@server shcnc.ac.cn Phn/Fax 86-21-6474-2629

氟伐他汀对自发性高血压大鼠阻力血管结构和功能的影响

R972.4
R544.105

林志鸿，谢良地[1]，吴可贵，王华军，许昌声
（福建省高血压研究所、福建医科大学附属第一医院，福州 350005、中国）

关键词 氟伐他汀；羟甲基戊二酰基辅酶 A 还原酶抑制剂；近交系 SHR 大鼠；胸主动脉；肠系膜动脉；血管舒张；血管收缩；高血压

目的：探讨氟伐他汀（HMG-CoA 还原酶抑制剂）对自发性高血压大鼠阻力血管结构和功能的影响。**方法**：8 周龄雄性 SHR 大鼠、氟伐他汀 20 mg·kg^{-1}·d^{-1}灌胃治疗。计算血管壁腔比作为阻力血管结构变化指标；应用离体主动脉和肠系膜动脉环对硝普钠和去甲肾上腺素反应的敏感性，观察治疗后血管的功能变化。**结果**：8 周后，治疗组血管的壁腔比低于对照的 SHR（0.44 ± 0.09 *vs* 0.79 ± 0.09，$P < 0.05$）；氟伐他汀能增加 SHR 主动脉对硝普钠舒张的敏感性，EC_{50}[(4.9 *vs* 190) pmol·L^{-1} $P < 0.05$]；减弱主动脉和肠系膜动脉对去甲肾上腺素收缩的敏感性、EC_{50}[主动脉：(0.20 *vs* 0.02) nmol·L^{-1}，$P < 0.05$；肠系膜动脉：(1.46 *vs* 0.72) nmol·L^{-1}，$P < 0.05$]。**结论**：短期氟伐他汀治疗，改善 SHR 大鼠阻力血管的舒缩功能，同时也抑制了 SHR 大鼠在血压发展过程中伴随的血管壁肥厚现象。

（责任编辑 刘俊娥）

读者、订阅者注意

《中国药理学报》（英文刊名 Acta Pharmacologica Sinica）数年来坚持走国际化道路，发表国内外全英文来稿，受到国内外同行好评。现经上级有关部门批准，2000 年起，将登记为英文刊。刊号（ISSN 0253-9756、CN 31-1347/R）和邮发代号（4-295）不变，内容和现有格式基本不变。但邮发目录中将标注（英文）字样。请读者注意通知有关订购部门继续订购《中国药理学报》（英文）。本刊并无另外中文版本。

国内年定价人民币 300 元，每期定价 25 元。欢迎订阅。

联系地址：200031 上海市太原路 294 号《中国药理学报》编辑部
电话：021-6474-2629、021-6431-1833 转 200，202 分机
传真：021-6474-2629、021-6437-0269
电子邮件（E-mail）：aps@server.shcnc.ac.cn hxiao@mail.shcnc.ac.cn