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Effects of atorvastatin on vascular remodeling in spontaneously hypertensive rats

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Abstract: Objective: To investigate the structural changes of aorta, and evaluate the effects of atorvastatin on the remodeling of thoracic aorta in spontaneously hypertensive rats(SHR). Methods: Twelve eight-week-old SHR were randomized into atorvastatin treated group(ATV group, $n=6$) and distilled water group(DW group, $n=6$); Wistar-Kyoto rats(WKY) were used as normal controls. Atorvastatin was administered to ATV group for 10 weeks by gavage in mixture with distilled water(1 ml); the latter two groups were given the same amount of distilled water by gavage for 10 weeks. Systolic blood pressure of caudal artery was examined before and after treatment, and serum concentrations of total cholesterol, triglycerides and HDL-C were measured. Wall thickness, media thickness, medial cross-sectional area and lumen diameter of thoracic aorta were assessed with computed video processing. Results: Systolic blood pressure in ATV group was markedly lower than that in DW group($P<0.01$). Compared with DW group and WKY group, serum concentrations of total cholesterol, triglycerides and HDL-C in ATV group were significantly lower($P<0.01$, $P<0.05$). Wall thickness, media thickness, and medial cross-sectional area to lumen ratio in DW group were significantly higher than those in WKY group and ATV group($P<0.01$, $P<0.05$), but no such difference was found between WKY group and ATV group($P>0.05$). Conclusion: Vascular structural changes of aorta are due to the alteration of the vessel wall in early stage of SHR. Atorvastatin can markedly improve vascular remodeling.

Key words: Atorvastatin, Hypertension, Vascular remodeling
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INTRODUCTION

Hypertension is always accompanied by increases in artery wall thickness, mainly caused by proliferation, hypertrophy, migration and apoptosis of vascular smooth muscle cells(VSMC), and elevated content of connective tissue. These structural changes in blood vessels are known as vascular remodeling(Dzau *et al.*, 1994). Recent study showed that statins such as atorvastatin had pleiotropic effects: inhibiting VSMC proliferation and migration, facilitating VSMC apoptosis, and ameliorating endothelial function(Bellosta *et al.*, 2000). Past researches in this field mainly focused on small arteries, less on big ones. Moreover, there are still several unsolved problems: during the development of hypertension, what are the characters of thoracic aorta remodeling? And what are the effects of atorvastatin on aortic remodeling? With the model of spontaneously hypertensive rats, we investigated the structural changes of aorta, and evaluated the effects of atorvastatin on aortic remodeling.

MATERIALS AND METHODS

Drugs and chemicals

Atorvastatin was donated by Pfizer Pharmaceuticals, Ltd.(Dalian, China). Lipids test kits: cholesterol, triglycerides and HDL were products of No.1 Chemical Company, Japan.

Rats and treatment

Eighteen eight-week-old spontaneously hypertensive rats(SHR) and Wistar-Kyoto rats(WKY)(SHR 12, WKY 6, Grade II), male, body weight 118g ± 3g respectively, were purchased from the Department of Pharmacology, the Second Military University. After measurement of systolic blood pressure(SBP), SHR

were randomized into atorvastatin treated group (ATV group, $n = 6$) and distilled water group (DW group, $n = 6$), with WKY as normal controls. Atorvastatin mixed with 1 ml distilled water was administered to ATV group for 10 weeks by gavage. While, the latter two groups were given 1 ml distilled water by gavage.

Systolic blood pressure (SBP) measurement and materials taken from the rats

SBP was measured in conscious rats using tail-cuff technique (HX-II computer control sphygmomanometer for rats, Laboratory of Cardiovascular Physiology, Hunan Medical University) before and at the end of the treatment. At the end of the ten-week treatment, the rats were anesthesiaed by 2% soluble pentobarbitone; 2 ml arterial blood was drawn for determination of serum concentrations of total cholesterol, triglycerides and HDL-C. The thoracic aorta was removed and treated carefully by Hanks' balance solutions, then immersed in 10% formaldehyde. After desiccation, and being hyaline and paraffin embedded, was cut into 4μm sections for hematoxylin and eosin (HE) staining.

Lipids measurement

Arterial blood (2 ml) was centrifugated (2000 r/min × 5 min) to have the serum isolated. Total cholesterol (TC), triglycerides (TG) and HDL-C were measured in a routine diagnostic analyzer (Hitachi, 7600) using enzymatic colorimetric assays (TC, CHOD-PAP assay; TG, GPO-PAP assay; and HDLs, PEG-cholesterolesterase-cholesteroloxidase assay).

Morphometric analysis of vascular wall thickening

Four points in every vascular circle was perpendicularly chosen as measurement areas in the sections stained with HE. Wall thickness, media thickness, medial cross-sectional area and lumen diameter of thoracic aorta were assessed with computed video processing (Institute of Biochemical Engineering and Apparatus Science, Zhejiang University).

Statistical analysis

Data were expressed as $\bar{x} \pm s$; comparison between two groups was done by t test and analysis of variance. All above were processed by software SPSS 10.0. $P < 0.05$ indicated statistical significance.

RESULTS

Effect of atorvastatin on SBP

SBP in ATV group and DW group were equal before the treatment began, and were higher than that in WKY group ($P < 0.01$). After the ten-week treatment, SBP in ATV group was markedly decreased ($P < 0.01$) compared with that before treatment or in DW group, while SBP in WKY group stayed at a nearly stable level (Table 1).

Table 1 Comparison of SBP in three groups

Groups	8 weeks	18 weeks
WKY	108.33 ± 4.18	119.67 ± 1.63[a]
DW	153.83 ± 4.40[b]	173.33 ± 3.78
ATV	155.69 ± 4.96[b]	134.17 ± 3.60[a]

$n = 6$ rats. $\bar{x} \pm s$. [a] $P < 0.01$, *VS* DW group; [b] $P < 0.01$, *VS* WKY group

Comparison of TC, TG and HDL-C in three groups

After the ten-week treatment, serum concentrations of TC, TG and HDL-C in ATV group were significantly lower compared with DW group and WKY group ($P < 0.01$, $P < 0.05$) (Table 2).

Table 2 Serum concentrations of TC, TG and HDL-C in three groups

Groups	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)
WKY	1.49 ± 0.38[b]	1.09 ± 1.46[b]	0.82 ± 0.23[a]
DW	1.53 ± 0.22[b]	1.18 ± 1.55[b]	0.79 ± 0.13[a]
ATV	1.03 ± 0.16	0.71 ± 0.19	0.62 ± 0.14

$n = 6$ rats. $\bar{x} \pm s$. [a] $P < 0.05$; [b] $P < 0.01$, *VS* ATV group

Morphometric parameters of the thoracic aorta remodeling in every group

Wall thickness, media thickness, and medial cross-sectional area, to lumen ratio in various groups, the parameters in DW group were significantly higher than those in WKY group and ATV group ($P < 0.01$, $P < 0.05$), but no such difference was found between WKY group and

ATV group ($P > 0.05$) (Table 3).

Table 3 Effects of atorvastatin on the parameters of thoracic aorta remodeling

Groups	Wall thickness (mm)	Media thickness (mm)	Media area (mm^2)	Media area/Lumen diameter (mm)
WKY	0.114 ± 0.004^{a}	0.086 ± 0.001^{a}	0.366 ± 0.008^{a}	0.518 ± 0.031^{b}
DW	0.138 ± 0.015	0.115 ± 0.014	0.397 ± 0.013	0.599 ± 0.056
ATV	0.115 ± 0.008^{ac}	0.087 ± 0.010^{ac}	0.366 ± 0.014^{ac}	0.521 ± 0.045^{ac}

$n = 6$ rats. $\bar{x} \pm s$. [a] $P < 0.01$; [b] $P < 0.05$, *VS* DW group; [c] $P > 0.05$, *VS* WKY group

DISCUSSION

Vascular remodeling is the adaptive process of blood vessel to the changes of hemodynamics or body fluid factors. It is not only an important pathological change, but also a structural approach for dealing with hypertension. Previous researches in this field mainly focused on small arteries and arterioles, less on big arteries because of their being regarded as a low resistance system in the regulation of blood pressure (Bouthier *et al*., 1985) showed that decreased aortic adaptation to the effects of hypertension and elevated stress on aorta, was an important factor leading to myocardial hypertrophy and heart failure. Knowledge gained in research on the structural characters of aortic remodeling during the development of hypertension, will contribute to better understanding of aortic functional changes.

Spontaneously hypertensive rat (SHR) is a model of chronic essential hypertension, whose blood pressure rises at six weeks old, and in which left ventricular hypertrophy and myocardial fibrosis exist at ten weeks old (Makino *et al*., 1997). However, the structural characters of thoracic aorta remodeling at early stage of SHR are not clear. Our findings demonstrated that systolic blood pressure, wall thickness, media thickness, medial cross-sectional area to lumen diameter ratio of thoracic aorta in DW group were significantly higher than those in WKY group, indicating that vascular structural changes of aorta were the alteration of the vessel wall at early stage of SHR.

Our data showed that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor-atorvastatin treatment led to significant reduction of systolic blood pressure, wall thickness, media thickness, medial cross-sectional area and medial cross-sectional area to lumen diameter ratio of thoracic aorta, in addition to its lowering serum lipid concentrations, indicating that atorvastatin could effectively reverse vascular remodeling. The mechanisms by which atorvastatin affected blood pressure and vascular remodeling could likely be explained as follow. Firstly, atorvastatin could reverse the state of imbalance between growth and apoptosis of VSMC during the development of vascular remodeling, by inhibiting proliferation, hypertrophy and migration of VSMC, and facilitating VSMC apoptosis. The effects of atorvastatin on growth and apoptosis of VSMC might be associated with its ability to inhibit synthesis of mevalonate (Mev). Mev pathway plays a role in cell growth. Mev is intracellularly synthesized from HMG-CoA, and this process is catalyzed by HMG-CoA reductase, the rate-limiting enzyme in this pathway (Corsini *et al*., 1998). The Mev metabolism yields a series of isoprenoids that are vital for the posttranslational isoprenylation of some proteins such as ras and rho (Laufs and Liao, 1998). Ras, a guanine nucleotide-binding protein, is activated by signal transduction pathways involved in growth and differentiation (Yang *et al*., 2001). Inhibited prenylation of p21 Rho B may induce apoptosis of VSMC (Guijarro *et al*., 1999). Secondly, atorvastatin could improve endothelial function by recovering the balance between NO and Ang II. Recent study suggested that atorvastatin enhanced the NO/O_2-concentration ratio after stimulation of NO synthase (NOS), resulting in an increase of NO bioavailability in bovine endothelial cells (Dobrucki *et al*., 2001). Wassmann *et al*. (2001) reported that treatment of SHR with atorvastatin caused profound endothelial dysfunction improvement mediated by a reduction of free

radical release in the vasculature. The underlying mechanism could in part be based on the atorvastatin-induced downregulation of AT1 receptor expression and decreased expression of the NAD(P)H oxidase subunit p22phox, and upregulation of endothelial cell NOS(ecNOS) mRNA expression and enhanced ecNOS activity in the vessel wall. Finally, atorvastatin could keep VSMC responsiveness normal by stabilizing cell membrane, improving Ca^{2+} homeostasis and inhibiting VSMC contraction. Tesfamariam *et al*. (1999) found that isolated aorta from normocholesterolemic rats incubated with atorvastatin, the smooth muscle contractions caused by phenylephrine were inhibited at high concentration of atorvastatin. In Ca^{2+}-free buffer, the transient contraction caused by phenylephrine, which results from intracellular release of Ca^{2+}, was also inhibited by atorvastatin. In cultured rat aortic smooth muscle cells loaded with fura-2, increases in intracellular free-Ca^{2+} concentration induced by angiotensin II were markedly inhibited in cells incubated with atorvastatin.

In summary, the data in the present study demonstrated that vascular structural changes of aorta are the alterations of the vessel wall at early stage of SHR. Atorvastatin can effectively reverse vascular remodeling.

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