Asia Pacific
Academy of Science Pte. Ltd.

Original Research Article

Effect of short-term high-dose atorvastatin on apoE^{-/-} mouse atherosclerosis model

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ABSTRACT

Objective: To explore the intervention mechanism of short-term high-dose atorvastatin on atherosclerotic (as) plaque. **Methods:** 4-week-old male apoE^{-/-} mouse were fed with high fat for 6 weeks to establish as model. They were randomly divided into control group, model group, atorvastatin conventional dose group, medium dose group and high dose group (load dose group and non load dose group). After 2 weeks, the blood lipid level, inflammatory factor concentration, platelet number, platelet membrane protein expression level and plaque area of each group were compared. **Results:** Compared with the model group, HDL-C increased significantly, LDL-C decreased significantly, the levels of inflammatory factors in serum and plaque decreased significantly, PLT decreased significantly, and the fluorescence intensity of CD31 and CD62p decreased significantly (all $P < 0.05$); The level of ox LDL in the load dose group was significantly lower than that in the model group ($P < 0.05$). The plaque area of atorvastatin intervention groups was significantly lower than that of model group (all $P < 0.05$). **Conclusion:** Short-term high-dose atorvastatin may intervene as by reducing blood lipid level, inhibiting the release of related inflammatory factors and regulating the number of platelets and the expression of platelet membrane protein.

Keywords: atherosclerosis; atorvastatin; inflammatory factor

1. Introduction

Atherosclerosis (as) is a common and frequently occurring disease, which is the pathological basis of cardiovascular and cerebrovascular diseases. Clinical epidemiological investigation found that patients with as will eventually develop cardiovascular and cerebrovascular diseases, and the death caused by cardiovascular and cerebrovascular diseases accounts for about the fourth place of disease death[1]. Among them, acute coronary syndrome caused by as is the most common as heart disease[2]. Percutaneous coronary intervention (PCI) is an effective method for the treatment of acute coronary syndrome. The study found that giving high-dose atorvastatin 12 hours before PCI can significantly reduce cardiovascular events such as perioperative myocardial infarction[3]. From the perspective of treatment benefit time, its mechanism is obviously impossible to be obtained by reducing blood lipid and improving atherosclerotic plaque. Relevant animal studies have found that atorvastatin has anti-inflammatory, antioxidant and antithrombotic effects[4,5]. However, there are

few studies on the effect of short-term high-dose atorvastatin on atherosclerotic plaquein this study, as model animal mice were used to explore the mechanism of short course and high-dose atorvastatin on as plaque.

2. Materials and methods

2.1. Experimental materials

Experimental animals:

Seventy 4-week-old male apolipoprotein E gene knockout (apolipoprotein E$^{-/-}$, apoE$^{-/-}$) mice (provided by Beijing weitonglihua Experimental Animal Technology Co., Ltd., production certificate No.: scxk (Beijing) 2016–0006, without fertility history), weighing 17~22G, were randomly divided into blank group ($n = 5$) and model group ($n = 65$) after 1 week of adaptive feeding.

Animal modeling method and grouping:

The model group was fed with high-fat diet (high-fat mixed feed: 1.5% cholesterol, 10% egg yolk powder, 5% lard and ordinary feed) for 6 weeks according to the classical as modeling method[6], and then five random mice in the blank group and the model group were dissected to confirm the success of as modeling After that, 60 model mice were randomly divided into ordinary feed feeding group (control group), high-fat group (model group), atorvastatin conventional dose + high-fat group (conventional dose group), medium dose + high-fat group (medium dose group), with 10 mice in each group; 20 rats in high dose + high fat group (high dose group).

Experimental drugs and administration methods:

Atorvastatin (Pfizer Pharmaceutical Co., Ltd., gyzz h20051407, specification: 10 mg × 7 tablets, 7 tablets/box) is 10 mg/d for adults According to pharmacology of experimental animals[7], the conversion coefficient of conventional drugs between mouse (20 g) and human (70 kg) is 9.1, so the dosage of each mouse is 2.5 mg/(kg·d) That is, 2.5 mg/(kg·d) in the conventional dose group, 5.0 mg/(kg·d) in the medium dose group and 10 mg/(kg·d) in the high dose group Dissolve the drug with 0.9% normal saline and then gavage. The gavage volume is 0.1 mL/animal, once a day, for 2 weeks. The animals in the high-dose group were further divided into load dose group (10 animals) and non load dose group (10 animals). Atorvastatin 2.5 mg/kg was added in the load dose group 2 h before the end of the intervention, but not in the non load dose group. The control group and the model group were fed with ordinary feed and high-fat feed respectively for 2 weeks.

2.2. Animal treatment

After intragastric administration of drugs for 2 weeks, the mice were killed by cutting off their necks, blood was taken from their eyeballs, centrifuged at 3500 r/min by high-speed centrifuge (thermo, USA) for 10 min, and the serum was taken and stored at −80 °C for standby.

2.3. Index detection

The level of lipoprotein and inflammatory factors were detected by ELISA kit:

Take about 0.5 cm of thoracic aorta tissue from each group, add 0.5 mL normal saline to homogenize for 2 min, centrifuge at 500 r/min for 5 min, and take the supernatant and store it at −80 °C. Follow the steps in the ELISA Kit instructions, Serum high density lipoprotein cholesterol (HDL-C) (Nanjing Jiancheng bioengineering company, batch No.: 20201118847), low density lipoprotein cholesterol (LDL-C) (Nanjing Jiancheng bioengineering company, batch No.: 20201118786), oxidized low density lipoprotein cholesterol (ox-LDL) were measured (Nanjing Jiancheng bioengineering company, batch No.: 20201118684) level; Tumor necrosis factor (TNF-α) in serum and plaque of mice in each group (Tiangen Biochemical Technology

Co., Ltd., batch No.: 20201017879), interleukin-6 (IL-6) (Tiangen Biochemical Technology Co., Ltd., batch No.: 20201016679), monocyte chemoattractant protein-1 (MCP-1) (Tiangen Biochemical Technology Co., Ltd., batch No.: 20201015379).

Platelet count and platelet membrane protein detection:

The number of platelets (PLT) in peripheral blood was detected by blood cell counter Calculate the number of cells according to the formula: Number of cells = (Total number of 4 large cells/4) × Dilution ratio (10) × 10^6. The average fluorescence intensity of platelet membrane proteins CD62P and CD31 was detected by flow cytometry and analyzed by flowjo software.

HE staining of thoracic aorta:

Take about 0.5 cm thoracic aorta tissue, wash it with PBS solution for about 3 min, fix it with 4% formaldehyde for 24~48 h, dehydrate it with 10%, 20% and 30% sucrose gradient for 1 day and 3 days respectively, and then embed it in paraffin and slice it After paraffin section, routine HE staining, neutral resin sealing, and the area of intravascular plaque are analyzed by automatic imaging analysis software system (Olympus). Five sections are taken from each animal to calculate the average value of plaque area.

2.4. Statistical methods

Spss19 0 statistical software to process the data. The measurement data are expressed by means ± standard deviation ($x \pm$ SD). The data conform to the normal distribution, and the analysis of variance is used for the comparison between groups. If not, the rank sum test is used. The counting data were expressed by frequency (%), and the difference between groups was expressed by frequency (%) χ^2 inspection. The difference was statistically significant when $p < 0.05$.

3. Results

3.1. Comparison of serum HDL-C, LDL-C and ox LDL levels in each group

During intragastric administration, one mouse in the model group died, and no mice in the other groups died Compared with the model group, HDL-C in the control group, routine dose group, medium dose group, load dose group and non load dose group increased significantly (all $P < 0.05$), and LDL-C decreased significantly (all $P < 0.05$) Compared with the model group, ox LDL in the load dose group decreased ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of serum HDL-C, LDL-C and Ox-LDL levels in each group ($\bar{x} \pm s$).

Group	HDL-C (mmol/L)	LDL-C (mmol/L)	Ox-LDL (µg/mL)
Control group ($n = 10$)	3.52 ± 0.71[a]	3.14 ± 0.52[a]	1.14 ± 0.31
Model group ($n = 9$)	2.25 ± 0.62	4.62 ± 0.72	1.29 ± 0.32
Routine dose group ($n = 10$)	3.60 ± 0.75[a]	3.16 ± 0.74[a]	1.00 ± 0.33
Medium dose group ($n = 10$)	3.71 ± 0.52[a]	3.09 ± 0.61[a]	0.94 ± 0.41
Loading dose group ($n = 10$)	3.92 ± 0.82[a]	2.94 ± 0.73[a]	0.71 ± 0.24[a]
Non load dose group ($n = 10$)	3.88 ± 0.72[a]	3.01 ± 0.67[a]	0.94 ± 0.36

Note: HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; Ox LDL: oxidized low density lipoprotein cholesterol Compared with the model group, [a]$P < 0.05$.

3.2. TNF in serum and plaque of each group-α, comparison of IL-6 and MCP-1 levels

Compared with the model group, serum TNF in the control group and drug intervention group TNF-α, IL-6 and MCP-1 decreased significantly (all $P < 0.05$). Serum in load dose group TNF-α, IL-6 and MCP-1

were significantly lower than those in the control group and the other three drug groups (all $P < 0.05$). Serum in medium dose group, load dose group and non load dose group TNF-α, IL-6 and MCP-1 were lower than those in the control group (all $P < 0.05$). TNF-α in plaque of each group, the expression trend of IL-6 and MCP-1 is consistent with that of inflammatory factors in serum, as shown in **Table 2**.

Table 2. Comparison of serum and plaque TNF-α, IL-6 and MCP-1 levels in each group ($\bar{x} \pm s$).

Group	TNF-α (ng/L)		IL-6 (pg/L)		MCP-1 (ng/L)	
	Serum	In plaque	Serum	In plaque	Serum	In plaque
Control group ($n = 10$)	1.25 ± 0.13[ab]	1.15 ± 0.25[ab]	20.65 ± 4.23[ab]	17.89 ± 3.34[ab]	17.02 ± 4.15[ab]	17.14 ± 4.41[ab]
Model group ($n = 9$)	1.68 ± 0.26	2.13 ± 0.33	31.13 ± 4.36	21.24 ± 4.35	21.54 ± 5.27	21.92 ± 5.57
Routine dose group ($n = 10$)	1.07 ± 0.17[ab]	0.92 ± 0.35[ab]	17.92 ± 4.60[ab]	14.14 ± 4.40[ab]	15.05 ± 4.73[ab]	13.05 ± 4.57[ab]
Medium dose group ($n = 10$)	0.95 ± 0.22[abc]	0.89 ± 0.32[abc]	14.81 ± 5.25[abc]	11.02 ± 3.37[abc]	13.15 ± 3.38[abc]	10.29 ± 4.40[abc]
Loading dose group ($n = 10$)	0.77 ± 0.23[ac]	0.69 ± 0.30[ac]	12.67 ± 3.21[ac]	9.04 ± 3.32[ac]	7.28 ± 3.30[ac]	7.04 ± 3.92[ac]
Non load dose group ($n = 10$)	0.88 ± 0.19[abc]	0.72 ± 0.38[abc]	14.78 ± 4.24[abc]	10.98 ± 3.74[abc]	10.28 ± 3.02[abc]	8.07 ± 4.57[abc]

Note: TNF-α: Tumor necrosis factor; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1 Compared with the model group, [a]$P < 0.05$; Compared with the load dose group, [b]$P < 0.05$; Compared with the control group, [c]$P < 0.05$.

3.3. Comparison of PLT, CD62P and CD31 levels in each group

Compared with the model group, the PLT in the control group and the drug intervention group were significantly decreased, and the fluorescence intensity of CD31 and CD62P were significantly decreased (all $P < 0.05$). Compared with the control group, the CD31 fluorescence intensity of the drug intervention group was significantly decreased (both $P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of PLT, CD31 and CD62P levels in each group ($\bar{x} \pm s$).

Group	PLT(×109/L)	CD31 (fluorescence intensity)	CD62P (fluorescence intensity)
Control group ($n = 10$)	607.3 ± 217.1[a]	22.6 ± 2.1[a]	3.3 ± 0.9[a]
Model group ($n = 9$)	872.4 ± 302.9	26.3 ± 2.8	4.5 ± 1.0
Routine dose group ($n = 10$)	541.8 ± 254.8[a]	17.4 ± 1.9[ab]	2.8 ± 0.9[a]
Medium dose group ($n = 10$)	520.3 ± 270.2[a]	17.1 ± 2.0[ab]	2.6 ± 0.8[a]
Loading dose group ($n = 10$)	482.7 ± 241.0[a]	16.5 ± 2.2[ab]	2.5 ± 0.7[a]
Non load dose group ($n = 10$)	533.0 ± 254.3[a]	16.7 ± 1.8[ab]	2.5 ± 0.8[a]

Note: compared with the model group, [a]$P < 0.05$; Compared with the control group, [b]$P < 0.05$.

3.4. Comparison of plaque area in aorta of each group

Compared with the model group, the plaque area of conventional dose group, medium dose group, load dose group and non load dose group decreased significantly (all $P < 0.05$), as shown in **Table 4**.

Table 4. Corrected plaque area of aorta in each group ($\bar{x} \pm s$).

Group	Corrected plaque area (μm²)
Control group ($n = 10$)	280.12 ± 1.58[a]
Model group ($n = 9$)	345.73 ± 1.60
Routine dose group ($n = 10$)	268.14 ± 1.86[a]
Medium dose group ($n = 10$)	246.65 ± 1.76[a]
Loading dose group ($n = 10$)	202.83 ± 1.89[a]
Non load dose group ($n = 10$)	218.01 ± 1.68[a]

Note: compared with the model group, [a]$P < 0.05$.

4. Discussion

Preventing plaque rupture, bleeding, dilation and blockage of blood vessels is an effective way to treat and improve the prognosis of as patients Atorvastatin belongs to statin lipid-lowering drugs and is a basic therapeutic drug for as commonly used in clinic[8] According to pharmacology, atorvastatin is a highly selective 3-hydroxy-3-methylglutaric acid Monoacyl coa reductase, which can reduce cholesterol synthesis by reducing enzyme activity. While controlling blood lipid, atorvastatin can also play an anti platelet aggregation role by reducing the expression of platelet aggregation proteins such as CD31[9]. In addition, atorvastatin may also inhibit the inflammatory response and antagonize the occurrence of as by inhibiting the aggregation and infiltration of inflammatory cells in the aorta and down regulating the expression of inflammatory factors, such as vascular cell adhesion molecule-1 (VCAM-1) and MCP-1 in the aortic intima[10]. Administration of high-dose atorvastatin 12 h before PCI can significantly reduce the incidence of perioperative myocardial infarction and other cardiovascular events[3]. How short-term high-dose atorvastatin affects atherosclerotic plaque and then reduces the risk of perioperative myocardial infarction, heart failure and other cardiovascular diseases is unknown.

In this study, as model mice were taken as the research object. It was found that atorvastatin could inhibit TNF by reducing LDL-C and increasing HDL-C-α. The levels of inflammatory factors such as IL-6 and MCP-1 antagonize platelet aggregation and finally reduce the plaque area to achieve the purpose of treating as, which is consistent with previous studies[11]. Although the conventional dose group, medium dose group, loaded dose group and non loaded dose group can improve the above indicators, only the loaded dose group can reduce ox LDL As activates oxidative stress and local vascular inflammatory reaction, produces excessive superoxide and oxygen free radicals, and oxidizes LDL into ox LDL[12]. Macrophages phagocytosis of ox-LDL into foam cells is an important condition for forming AS. By reducing LDL, especially ox LDL level, the incidence of cardiovascular events can be significantly reduced[13].

After the injury of endothelial cells, leukocytes adhered to the endothelial cells and activated the immune cells to release inflammatory mediators such as IL TNF. The latter made macrophages express scavenger receptors, and ox-LDL absorbed large amounts of ox-LDL to form foam cells. Inflammation activation not only accelerated the occurrence and development of AS, but also participated in thrombosis formation, promoting plaque rupture[14]. Abela et al.[15] found that serum TNF was down regulated-α. And IL-6 levels can significantly reduce the incidence of cardiovascular accidents in patients with as No matter how careful the operation is, the "irritation" of PCI on arterial plaque is difficult to avoid. Compared with before operation, the level of inflammatory factors in patients after PCI is significantly increased, which is an important factor leading to adverse cardiovascular events[16]. It has chemotactic activity and can activate monocytes/macrophages Inflammatory factors are the main mediators that activate the above cells to secrete MCP-1[17]. This study found that atorvastatin has a stronger effect on reducing serum inflammatory factors than inflammatory factors in plaque, which may be related to drug metabolism and distribution.

Hyperactivation of blood coagulation is also an important inducement of cardiovascular events. Plaque rupture, cellulose cell necrosis substances, foam cells and other contents spilt, activate platelets to induce disseminated intravascular coagulation, microthrombosis with blood flow obstruction, heart and brain microvessels are the main factors causing myocardial infarction and generalized cerebral ischemia and infarction[18]. Tan et al.[19] found that compared with low dose, high dose atorvastatin has more significant effect on reducing platelet aggregation rate and platelet adhesion rate. Deng et al.[20] believe that high-dose atorvastatin can not only reduce platelet concentration and inhibit platelet activation, but also significantly reduce blood lipid level and inflammatory factor concentration Atorvastatin inhibits platelet activation, which may be related to reducing the expression of platelet surface adhesion protein CD31. Blood lipids,

inflammatory factors, platelets and other as promoting substances are reduced, while the plaque area is significantly reduced[21].

In conclusion, short-term high-dose atorvastatin may intervene in the formation of as plaque by reducing blood lipid level, inhibiting the release of relevant inflammatory factors, regulating the number of platelets and the expression of platelet membrane protein.

Conflict of interest

The authors declare no conflict of interest.

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