Effects and potential mechanism of atorvastatin treatment on Lp-PLA2 in rats with dyslipidemia

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Abstract

Introduction: The effects of statins on lipoprotein-associated phospholipase A2 (Lp-PLA2) are controversial, and the present study aimed to investigate whether atorvastatin could reduce Lp-PLA2 in rats with dyslipidemia.

Material and methods: A high-fat and high-cholesterol diet was prescribed to produce a dyslipidemia model. Thereafter, low-dose atorvastatin (5 mg/kg/day), high-dose atorvastatin (20 mg/kg/day) or saline (without-treatment group) was prescribed for 14 days. At 6 weeks after dyslipidemia model establishment and 14 days of atorvastatin treatment, fasting venous blood was drawn for biochemical analysis. Between-group differences and Pearson correlation analysis were conducted.

Results: Compared to the normal-control group, fasting plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels were significantly increased in dyslipidemia groups, while plasma nitric oxide (NO) levels were significantly decreased with attendant elevation of plasma C-reactive protein (CRP) and rho-associated kinase 1 (ROCK1) levels (p < 0.05). At 14 days of atorvastatin treatment, compared to the without-treatment group, plasma levels of TC and LDL-C in the high-dose group were significantly reduced (p < 0.05); and compared to low-dose and without-treatment groups, NO up-regulation (1.8 ±1.1 μmol/l), and CRP (–0.8 ±0.4 ng/ml), ROCK1 (–124 ±65 mmol/l) and Lp-PLA2 (–3.8 ±1.2 ng/ml) reduction were more significant (p < 0.05). Pearson correlation analysis showed that TC (r = 0.365), LDL-C (r = 0.472), CRP (r = 0.501) and ROCK1 (r = 0.675) were positively correlated with Lp-PLA2, while NO (r = –0.378) and atorvastatin (r = –0.511) were negatively correlated with Lp-PLA2.

Conclusions: Atorvastatin treatment is beneficial for reducing the Lp-PLA2 level in rats with dyslipidemia, which may be related to reduced ROCK1 expression in a dose-dependent manner.

Key words: dyslipidemia, endothelial dysfunction, statins, lipoprotein-associated phospholipase A2.
**Introduction**

Dyslipidemia, mainly characterized by an increased plasma level of low-density lipoprotein cholesterol (LDL-C), is a major risk factor for atherosclerotic cardiovascular diseases (ASCVD) [1–4]. Previously, numerous studies have shown that endothelial dysfunction was the underlying mechanism contributing to dyslipidemia-mediated atherogenesis and ASCVD development [5–7]. In recent decades, some studies have revealed that an increased plasma level of lipoprotein-associated phospholipase A2 (Lp-PLA2) was also independently associated with atherosclerosis progress and ASCVD [8–11]. Briefly, Lp-PLA2 is an enzyme excreted by inflammatory cells such as macrophages which hydrolyze oxidized-LDL into lysophosphatidylcholine (Lyso-PC) and oxidized non-esterified fatty acids (oxNEFAs), both of which subsequently play critical and complex roles in atherosclerotic plaque expansion and rupture [12].

Statins are a HMG-CoA reductase inhibitor and has been broadly applied in clinical practice for dyslipidemia and ASCVD treatment. The underlying mechanisms attributing to statins’ cardiovascular benefits include lowering plasma LDL-C level and anti-inflammation, endothelial protection, anti-oxidation and pro-angiogenesis [13, 14]. However, whether statin therapy is beneficial for decreasing plasma Lp-PLA2 level is controversial and the underlying mechanisms are also not fully explored yet.

We produced a dyslipidemia rat model and used different doses of atorvastatin to evaluate the change of plasma Lp-PLA2 level. The results from our current research might help to broaden our knowledge about the effects of statins on Lp-PLA2 regulation and its associated potential mechanisms.

**Material and methods**

**Dyslipidemia rat model production**

A total of 40 male Sprague-Dawley (SD) rats weighing 210–220 g were used in the present research (rats were obtained from the Experimental Animal Center of Sun Yat-sen University, Guangzhou, Guangdong, China). All rats received humane care in compliance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Among the 40 rats, 10 were randomly selected as the normal-control group with prescription of normal rat chow, and the other 30 rats were used to produce a dyslipidemia model. The protocol for dyslipidemia model production was in accordance with a previously described study with prescription of a high-fat and high-cholesterol diet including cholesterol 4.0%, propylthiouracil 0.3%, cholic acid 0.4%, and lard 10.0% for a total of 6 weeks [15]. Thereafter, fasting lipid profiles were evaluated to confirm successful establishment of the dyslipidemia model. Fasting lipid profiles in the normal-control group were also measured using an auto-analyzer (Hitachi 7100, Japan) with the corresponding kits from Wako Inc (Enid, OK, USA). The plasma Lp-PLA2 level measurement kit was brought from CUSABIO BIOTECH CO., Ltd. USA (Catalog No. CSB-E08320r) and the performances were conducted in accordance to the manual instructions. In brief, the enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 ±2 nm. The concentration of Lp-PLA2 in the samples was then determined by comparing the optical density of the samples to the standard curve, and the minimum detectable dose of Lp-PLA2 was typically less than 1.95 ng/ml. Each measurement was performed 3 times and the values were averaged.

**Atorvastatin treatment**

In the normal-control group, 3 ml saline was administered by gavage in the morning around 8–10 am, and the 30 dyslipidemia rats were randomly divided into low-dose atorvastatin (5 mg/kg/day), high-dose atorvastatin (20 mg/kg/day) and without-treatment groups (3 ml saline). The doses of atorvastatin we used in the current study were based on our previous study in which we found that 5 mg/kg/day and 20 mg/kg/day were safe and efficacious for rats with dyslipidemia [16]. In brief, atorvastatin was dissolved in dimethyl-sulfoxide (DMSO, working concentration 10 mmol/l) and was administered by gavage which was similar to our previous study [17]. The duration of treatment was 14 days and then fasting plasma was used for evaluation of lipid profiles and plasma Lp-PLA2 level.

**Measurement of C-reactive protein, nitric oxide and rho-associated kinase 1 levels**

At 6 weeks of high-fat and high-cholesterol diet treatment and at 14 days of atorvastatin treatment, fasting plasma was used for C-reactive protein (CRP) assessment with an enzyme linking immune-absorbent assay (ELISA) kit (Biomatik, USA, Cat. No. EKE51580); all performances were in accordance with the manual instructions, and the detection range was 0.156–10 ng/ml. Nitric oxide level was evaluated by the nitrite reductase method using the Total Nitric Oxide Kit (Beyotime, Haimen, China, Cat. No. S0023). Rho-associated kinase 1 (ROCK1) level was also measured using an ELISA kit (CUSABIO, USA, Cat. No. CSB-EL020058RA) with a detection range of 125–8000 pg/ml. Three independent measurements were performed and the values were averaged.
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Alanine aminotransferase and creatine kinase measurement

Plasma alanine aminotransferase (ALT) and creatine kinase (CK) levels were also measured using the Automatic Biochemistry Analyzer (Hitachi 7150, Tokyo, Japan) to evaluate whether atorvastatin treatment would cause potential side effects.

Statistical analysis

All continuous variables were expressed as mean ± SD, and analyses were performed with SPSS software, version 17.0 (SPSS Science, Chicago, IL, USA). Statistical significance of differences between groups was evaluated using one-way ANOVA, and changes of pre- and post-atorvastatin treatment within each group were also compared using one-way ANOVA. Pearson correlation analysis was performed to evaluate the association of parameters of interest and Lp-PLA2; and a value of \( p < 0.05 \) was considered statistically significant.

Results

Assessment of dyslipidemia model establishment

At 6 weeks of high-fat and high-cholesterol diet treatment, parameters of interest were evaluated and compared. As shown in Table I, compared with the normal-control group, fasting plasma total cholesterol (TC) and LDL-C levels were significantly increased in the dyslipidemia groups, indicating that the dyslipidemia model was successfully established. In addition, compared with the normal-control group, plasma NO levels were significantly decreased in the dyslipidemia groups with attendant elevation of plasma CRP and ROCK1 levels \( (p < 0.05) \).

Assessment of the effects of atorvastatin treatment

At 14 days of atorvastatin treatment, parameters of interests were evaluated and compared between different groups. As shown in Table II, compared with the without-treatment group, plasma levels of TC and LDL-C in the high-dose atorvastatin group were significantly reduced \( (p < 0.05) \), and there were no significant differences in TC or LDL-C between the high-dose and low-dose groups or the low-dose and without-treatment groups. Notably, compared with the low-dose and without-treatment groups, NO up-regulation and CRP and ROCK1 reduction were more significant in the high-dose group \( (p < 0.05) \). Compared with without-treatment group, significant differences in NO, CRP and ROCK1 levels were also observed in the low-dose group \( (p < 0.05) \). No significant elevations of ALT or CK were observed in either atorvastatin treatment group.

Changes of plasma Lp-PLA2 levels

Plasma Lp-PLA2 levels were evaluated at 6 weeks of high-fat and high-cholesterol diet treatment and at 14 days of atorvastatin treatment. As shown in Figure 1, compared with the normal-control group, plasma Lp-PLA2 levels were significantly elevated in the dyslipidemia group at 6 weeks of high-fat and high-cholesterol diet treatment \( (p < 0.05) \). At 14 days of atorvastatin treatment, compared with the without-treatment group, the plasma Lp-PLA2 level was significantly reduced in the high-dose group \( (p < 0.05) \); although plasma Lp-PLA2 level was lower in the low-dose group when compared with the without-treatment group, no statistical significance was observed \( (p = 0.96) \).

Table I. At 6 weeks of high-fat and high-cholesterol diet treatment \( (n = 10 \) each group)

| Variables | Normal – control | High-dose | Low-dose | Without treatment |
|-----------|-----------------|-----------|----------|-------------------|
| TC [mmol/l] | 4.2 ±0.3* | 5.3 ±0.6 | 5.2 ±0.5 | 5.3 ±0.5 |
| LDL-C [mmol/l] | 2.8 ±0.4* | 3.7 ±0.3 | 3.7 ±0.4 | 3.7 ±0.4 |
| HDL-C [mmol/l] | 1.2 ±0.2 | 1.0 ±0.3 | 1.0 ±0.3 | 1.0 ±0.2 |
| TG [mmol/l] | 1.6 ±0.4 | 1.8 ±0.5 | 1.7 ±0.5 | 1.7 ±0.6 |
| CRP [ng/ml] | 2.0 ±0.8* | 5.3 ±1.1 | 5.2 ±1.0 | 5.2 ±1.2 |
| NO [μmol/l] | 10.7 ±1.6* | 6.3 ±1.9 | 6.2 ±1.4 | 6.3 ±1.6 |
| ROCK1 [mmol/l] | 489 ±56* | 844 ±103 | 827 ±124 | 833 ±120 |
| ALT [U/l] | 25 ±8 | 28 ±6 | 26 ±9 | 26 ±7 |
| CK [U/l] | 10 ±2 | 12 ±4 | 10 ±4 | 11 ±3 |

\*\( p < 0.05 \) vs. other 3 groups. TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglyceride, expressed as mean ± SD.
Changes of parameters of interest before and after atorvastatin treatment within each group

As presented in Table III, changes of parameters of interest before and after atorvastatin treatment within each group showed that the magnitude of improvement of dyslipidemia, decreased serum levels of CRP, ROCK1 and Lp-PLA2, and increased serum level of NO were most prominent in the high-dose group when compared to the low-dose and without-treatment groups ($p < 0.05$). Similarly, compared to the without-treatment group, the changes of the above parameters were also significantly greater in the low-dose group when compared to the without-treatment group ($p < 0.05$).

Pearson correlation analysis

Pearson correlation analysis was performed to evaluate the association of plasma Lp-PLA2 level and parameters of interest. As shown in Table IV, TC, LDL-C, CRP and ROCK1 were positively correlated with Lp-PLA2, while NO and statins both were negatively correlated with Lp-PLA2. Notably, the correlation coefficient of ROCK1 was the largest.

Discussion

Our preliminary research shows that in rats with 6 weeks of high-fat and high-cholesterol diet treatment, dyslipidemia establishment is accom-
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Whether statin therapy could reduce Lp-PLA2 level is uncertain. Lp-PLA2 is an enzyme excreted by inflammatory cells such as macrophages which then hydrolyzes ox-LDL into two more potent pro-atherosclerotic metabolites, Lyso-PC and oxNEFAs. It is therefore conceivable that reducing the Lp-PLA2 level would contribute to deceleration of atherosclerosis progression and reduction of cardiovascular events. Statins have pleiotropic effects such as decreasing macrophage infiltration and anti-oxidation, and it is biologically plausible that statin therapy could reduce Lp-PLA2 excretion from macrophages. For example, White et al. [24] reported that Lp-PLA2 attenuation by pravastatin therapy during the first year was significantly associated with reduced ASCVD events, which was independent of LDL-C change. Nevertheless, in another study, Albert et al. [25] found that after adjustment for LDL-C, the effect of pravastatin on Lp-PLA2 reduction was no longer statistically significant. Our current experimental study showed that atorvastatin therapy could reduce Lp-PLA2 in a dose-dependent manner, which was also further supported by the strong correlation coefficient of –0.511 as presented in Table IV. In addition, as evident in the Pearson correlation analysis, the positive relationship was strongest between Lp-PLA2 and ROCK1. In light of the pathophysiological effects of ROCK1 and the effects of statins on ROCK1 expression [26, 27], we speculated that the efficacies of atorvastatin treatment on Lp-PLA2 reduction might be attributed to ROCK1 reduction. Indeed, ROCK1 reduction leads to a macrophage migration decrease which in turn could lead to diminished Lp-PLA2 production. In addition, endothelial function improvement by attenuated ROCK1 could also decrease macrophage infiltration and accumulation, which in turn further attenuate Lp-PLA2 production.

In conclusion, our present research reveals that atorvastatin treatment is beneficial for reducing plasma Lp-PLA2 level in rats with dyslipidemia and the underlying mechanism may be related to attenuation of ROCK1 expression in a dose-dependent manner. The clinical implication of our present research is that even short-term dyslipidemia...
could result in severe impairment of endothelial function and high-dose atorvastatin prescription may result in prompt storage of endothelial function, which in turn may help to prevent atherosclerosis progression.

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Dongdan Zheng and Anping Cai contributed equally to this work.

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**Conflict of interest**

The authors declare no conflict of interest.

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