



# Effects of atorvastatin on ADP-, arachidonic acid-, collagen-, and epinephrine-induced platelet aggregation

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## Abstract

**Objective:** Atorvastatin reduces the incidence of cardiovascular events. However, the effects of atorvastatin on platelet aggregation are unknown.

**Methods:** Blood samples were obtained from 126 healthy volunteers. Prepared isolated platelet suspensions were adjusted with saline to three different concentrations of  $100 \times 10^9$ ,  $300 \times 10^9$ , and  $600 \times 10^9$  platelets/L. Platelet samples were incubated with atorvastatin ( $10^{-7}$  mol/L,  $10^{-6}$  mol/L or  $10^{-5}$  mol/L), and stimulated with ADP ( $10 \mu\text{mol/L}$ ), arachidonic acid ( $0.5 \text{ mmol/L}$ ), collagen ( $2 \mu\text{g/mL}$ ), and epinephrine ( $1 \text{ mg/mL}$ ). The maximal amplitude of aggregation and the curve slope were measured by electric impedance aggregometry.

**Results:** Atorvastatin inhibited platelet aggregation at moderate ( $300 \times 10^9/\text{L}$ ) and high ( $600 \times 10^9/\text{L}$ ) concentrations. However, an inhibitory effect of atorvastatin at low concentrations ( $100 \times 10^9/\text{L}$ ) was not observed.

**Conclusions:** The study shows that atorvastatin inhibits platelet aggregation *in vitro*, and this inhibitory effect is related to platelet concentrations.

## Keywords

Atorvastatin, ADP, arachidonic acid, collagen, epinephrine, platelet aggregation

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## Introduction

Cardiovascular disease impairs health and is the leading cause of death worldwide. Activation of platelets plays a major role in the occurrence and development of cardiovascular disease. At present, aspirin combined with clopidogrel or tirofiban is

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an integral part of therapy for patients with cardiovascular disease, especially for those with acute coronary syndrome and those undergoing percutaneous coronary intervention.

As other antiplatelet drugs, statins are strongly recommended in acute coronary syndrome therapy. Statins are effective lipid-lowering drugs, which inhibit cholesterol biosynthesis by inhibition of 3-hydroxy-3-methylglutaryl CoA reductase. Many studies have shown that statins can reduce the incidence of cardiovascular events, such as myocardial infarction, stroke, and cardiovascular death.<sup>1,2</sup> Moreover, evidence has shown that statins have other effects that are independent of their cholesterol-lowering function, including inhibition of formation of foam cells, stabilizing plaques, anti-inflammatory effects, and inhibition of platelet activation.<sup>3-5</sup> These characteristics of statins may be not related to their cholesterol-lowering effects, which are called pleiotropic effects of statins. Several clinical trials have demonstrated that statins significantly reduce the risk of cardiovascular events in patients with hyperlipidaemia, as well as normocholesterolemic patients.<sup>5,6</sup> Results from these trials indicate that the beneficial effects of statins on cardiovascular events cannot be only explained by their cholesterol-lowering effects. Therefore, further research is needed to clarify the exact mechanism of antiplatelet effects of statins.

This study aimed to investigate the effects of atorvastatin on platelet aggregation and whether the effects are related to platelet concentrations. Our findings may provide evidence of clinical use of atorvastatin in patients with or without hyperlipidaemia.

## Materials and methods

### Materials

Atorvastatin was obtained from the National Institute for the Control of Pharmaceutical and Biological Products.

ADP, arachidonic acid (AA), collagen, and epinephrine (EP) were purchased from Chrono-Log Corp. (USA).

### Blood samples

Blood samples were obtained from 126 healthy volunteers. The donors were healthy adults aged 18 to 55 years (female patients did not have their menstrual period). None of the volunteers took any drugs that could have an interaction with platelets at least 10 days before collection of blood. All of the subjects provided informed consent. Blood biochemical indicators and haematological parameters of all of the subjects were tested and were all within the normal range.

Citrate anti-coagulated venous blood (final citrate/blood ratio 1:9 vol/vol) was placed in tubes for laboratory analyses. Platelets were isolated from blood by centrifugation, as previously described.<sup>7</sup> To simulate the human body platelet concentration, prepared isolated platelet suspensions were adjusted with saline to three different concentrations of  $100 \times 10^9$ ,  $300 \times 10^9$ , and  $600 \times 10^9$  platelets/L.

The study was approved by the ethical committee of Hebei Medical University, China (approval no.201600131). All of the participants provided written informed consent and received compensation for their participation.

### Platelet treatment with atorvastatin

Atorvastatin was dissolved in Dimethylsulfoxide (DMSO) into stock solution and prepared as three different doses immediately before use. Different concentrations of platelet suspensions were incubated (10 min, 37°C) with atorvastatin at final concentrations of  $10^{-7}$  mol/L,  $10^{-6}$  mol/L, or  $10^{-5}$  mol/L in the experimental groups.<sup>8,9</sup> The effects of atorvastatin in the experiments were compared with the same concentration of DMSO as a control.

**Table 1.** Effects of atorvastatin on platelet aggregation with a concentration of  $100 \times 10^9$  platelets/L induced by ADP, AA, collagen, and EP.

Group	ADP (10 $\mu$ mol/L) (n = 90)		AA (0.5 mmol/L) (n = 80)		Collagen (2 $\mu$ g/mL) (n = 130)		EP (1 mg/mL) (n = 100)	
	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope
Control	3.00 (1.00)	2.00 (2.00)	3.50 (1.00)	1.50 (1.00)	8.50 $\pm$ 1.78	2.00 (1.00)	4.00 (1.00)	2.00 (1.00)
$10^{-7}$ mol/L	3.00 (1.00)	2.00 (1.00)	3.00 (1.00)	1.00 (1.00)	8.00 (3.00)	2.00 (1.00)	4.00 (1.00)	2.00 (1.00)
$10^{-6}$ mol/L	3.00 (2.00)	2.00 (1.00)	3.00 (2.00)	1.00 (1.00)	8.00 (2.00)	2.00 (0.00)	4.00 (3.00)	2.00 (1.00)
$10^{-5}$ mol/L	3.14 $\pm$ 1.06	2.00 (1.00)	3.00 (2.00)	1.00 (1.00)	8.00 (3.00)	2.00 (0.00)	4.00 (2.00)	1.50 (1.00)

Data are mean  $\pm$  SD or M (QR).

ADP: adenosine diphosphate; AA: arachidonic acid; EP: epinephrine.

The concentration of DMSO was  $\leq 0.1\%$ , at which concentration platelet function is not affected.<sup>10,11</sup>

### Platelet aggregation test

Platelet aggregation was tested with induction by ADP (10  $\mu$ mol/L), AA (0.5 mmol/L), collagen (2  $\mu$ g/mL), and epinephrine (1 mg/mL). The maximal amplitude of aggregation and the curve slope were monitored for up to 10 min in response to agonists. The changes were measured by electric impedance aggregometry (Model 590-4D; Chrono-Log Corporation) according to the manufacturer's protocol.

### Statistical analysis

The Shapiro–Wilk test was used to determine whether the data were normally distributed. For data with a normal distribution, continuous variables are expressed as mean  $\pm$  SD. Overall comparison in each group was evaluated by single-factor ANOVA analysis. For non-normally distributed data, the variables are expressed as median and quartile (QR). Overall comparison in each group was evaluated by Friedman's M test. A value of  $P < 0.05$  was considered to be statistically significant.

## Results

### Effects of atorvastatin on low-concentration platelet aggregation

As shown in Table 1, atorvastatin ( $10^{-7}$  mol/L,  $10^{-6}$  mol/L, or  $10^{-5}$  mol/L) did not affect platelet aggregation and the curve slope with a concentration of  $100 \times 10^9$  platelets/L induced by ADP (10  $\mu$ mol/L), AA (0.5 mmol/L), collagen (2  $\mu$ g/mL), and EP (1 mg/mL).

### Effects of atorvastatin on moderate-concentration platelet aggregation

Effects of atorvastatin on platelet aggregation with a concentration of  $300 \times 10^9$  platelets/L are shown in Table 2. There were no significant differences in aggregation and the curve slope among the four groups after incubation with atorvastatin and induction by ADP (10  $\mu$ mol/L) and AA (0.5 mmol/L).

Effects of atorvastatin on platelet aggregation induced by collagen (2  $\mu$ g/mL) and EP (1 mg/mL) among the five groups were observed. As shown in Table 2 and Figure 1(a), atorvastatin suppressed aggregation of platelets that were stimulated with collagen in a dose-dependent manner ( $P < 0.05$ ). Similar effects were observed with curve slopes induced by collagen.

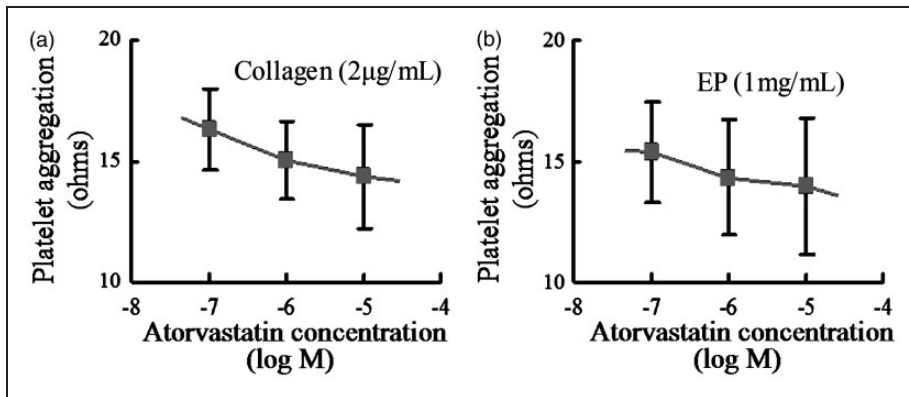
**Table 2.** Effects of atorvastatin on platelet aggregation with a concentration of  $300 \times 10^9$  platelets/L induced by ADP, AA, collagen, and EP.

Group	ADP (10 $\mu$ mol/L) (n = 90)		AA (0.5 mmol/L) (n = 80)		Collagen (2 $\mu$ g/mL) (n = 130)		EP (1 mg/mL) (n = 100)	
	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope
Control	10.97 $\pm$ 1.71	4.00 (2.00)	12.00 (2.00)	10.33 $\pm$ 1.35	17.14 $\pm$ 1.36	15.55 $\pm$ 1.57	15.50 $\pm$ 1.48	17.98 $\pm$ 2.81
$10^{-7}$ mol/L	11.00 (2.00)	4.00 (2.00)	12.00 (2.00)	9.83 $\pm$ 1.27	16.29 $\pm$ 1.68*	15.00 $\pm$ 2.02†	15.37 $\pm$ 2.10†	17.44 $\pm$ 3.61†
$10^{-6}$ mol/L	10.42 $\pm$ 1.91	4.00 (1.00)	12.00 (2.00)	9.72 $\pm$ 1.60	15.03 $\pm$ 1.62*	14.23 $\pm$ 1.75†	14.32 $\pm$ 2.39†	16.33 $\pm$ 3.87†
$10^{-5}$ mol/L	10.00 (3.00)	4.00 (1.00)	11.21 $\pm$ 1.51	9.68 $\pm$ 1.46	14.36 $\pm$ 2.15*	13.86 $\pm$ 1.98†	13.93 $\pm$ 2.82†	15.46 $\pm$ 4.06†

Data are mean  $\pm$  SD or M (QR).

\*P < 0.05, vs. control group; †P < 0.01, vs. control group.

ADP: adenosine diphosphate; AA: arachidonic acid; EP: epinephrine.



**Figure 1.** Effects of atorvastatin on platelet aggregation with a concentration of  $300 \times 10^9$  platelets/L induced by collagen (a) and EP (b).

Moreover, atorvastatin also affected platelet aggregation after EP stimulation in a dose-dependent manner (Figure 1(b)).

EP (1 mg/mL) in a dose-dependent manner. Similar effects were also observed with curve slopes induced by these stimulations.

**Effects of atorvastatin on high-concentration platelet aggregation**

Furthermore, the effects of atorvastatin on platelet aggregation with a concentration of  $600 \times 10^9$  platelets/L were analysed. As shown in Table 3 and Figure 2, atorvastatin suppressed aggregation of platelets that were stimulated with ADP (10  $\mu$ mol/L), AA (0.5 mmol/L), collagen (2  $\mu$ g/mL), and

**Discussion**

Platelet aggregation is a complex process involving multiple signalling processes and other factors. Our study showed that atorvastatin inhibited platelet aggregation at moderate ( $300 \times 10^9$ /L) and high ( $600 \times 10^9$ /L) concentrations. However, an inhibitory effect of atorvastatin at a low concentration ( $100 \times 10^9$ /L) of platelet

**Table 3.** Effects of atorvastatin on platelet aggregation with a concentration of  $600 \times 10^9$  platelets/L induced by ADP, AA, collagen, and EP.

Group	ADP (10 $\mu$ mol/L) (n = 90)		AA (0.5 mmol/L) (n = 80)		Collagen (2 $\mu$ g/mL) (n = 130)		EP (1 mg/mL) (n = 100)	
	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope	Platelet aggregation (ohms)	Curve slope
Control	22.58 $\pm$ 1.79	20.77 $\pm$ 2.36	16.33 $\pm$ 2.66	21.07 $\pm$ 2.28	22.43 $\pm$ 1.75	26.00 $\pm$ 1.55	20.63 $\pm$ 1.86	24.63 $\pm$ 1.77
10 <sup>-7</sup> mol/L	22.35 $\pm$ 2.70*	20.09 $\pm$ 2.53 <sup>†</sup>	15.50 (4) <sup>†</sup>	20.71 $\pm$ 2.37 <sup>†</sup>	22.06 $\pm$ 2.19*	25.00 (3) <sup>†</sup>	19.60 $\pm$ 2.16*	22.93 $\pm$ 2.68*
10 <sup>-6</sup> mol/L	21.21 $\pm$ 2.30*	19.03 $\pm$ 1.94 <sup>†</sup>	15.06 $\pm$ 2.84 <sup>†</sup>	19.41 $\pm$ 1.47 <sup>†</sup>	21.39 $\pm$ 2.57*	24.45 $\pm$ 1.95 <sup>†</sup>	18.97 $\pm$ 1.83*	21.94 $\pm$ 1.92*
10 <sup>-5</sup> mol/L	19.59 $\pm$ 1.85*	18.72 $\pm$ 2.20 <sup>†</sup>	14.00 (3) <sup>†</sup>	18.00 (2) <sup>†</sup>	20.30 $\pm$ 1.94*	22.00 (4) <sup>†</sup>	17.76 $\pm$ 1.66*	20.90 $\pm$ 3.08*

Data are mean  $\pm$  SD or M (QR).

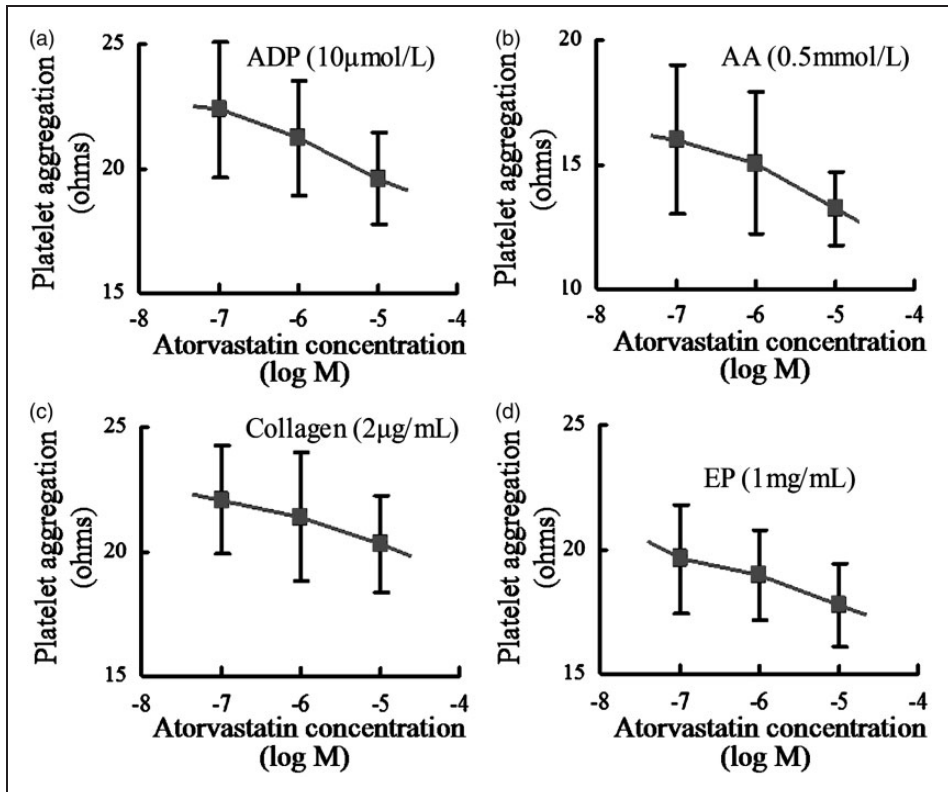
\*P < 0.05, vs. control group; <sup>†</sup>P < 0.01, vs. control group.

ADP: adenosine diphosphate; AA: arachidonic acid; EP: epinephrine.

aggregation was not observed. These findings indicated that atorvastatin could inhibit platelet aggregation *in vitro*, and these inhibitory effects were related to the concentration of platelets.

Recently, statins have been shown to be the most important class of lipid-lowering agents and are recommended as the first-choice treatment for hyperlipidemia.<sup>12</sup> Several studies have demonstrated that hyperlipidaemia is associated with platelet hyperactivity.<sup>13</sup> Platelet activation and aggregation play important roles in the pathogenesis of arterial thrombosis and the occurrence of acute coronary syndrome. Many clinical studies have shown that patients could benefit from statins by reducing myocardial infarction, stroke, and cardiovascular death.<sup>14,15</sup>

The benefit of statin treatment on patients with coronary artery disease cannot solely be attributed to their lipid-lowering effects.<sup>2,16</sup> Atorvastatin has been shown to dose-dependently reduce collagen-induced TXA2 synthesis and platelet aggregation, which are unrelated to its cholesterol lowering effects.<sup>10</sup> Additionally, direct inhibition effects of statins on platelet activation are mediated by peroxisome proliferator-activated receptors and are involved in an interaction with protein kinase C- $\alpha$  (PKC- $\alpha$ ) in platelets.<sup>17</sup> Moreover, some studies have showed that direct antiplatelet effects of statins are related to upregulation of nitric oxide synthetase and suppression of cyclooxygenase-1 activation.<sup>16,18</sup> In our study, we used isolated platelet suspensions to exclude related factors, such as blood cholesterol levels, as well as other blood cells, which might interfere with platelet activity. Atorvastatin is extensively metabolized by cytochrome P450 3A4 to active metabolites, including ortho- and parahydroxy atorvastatin.<sup>19</sup> Our *in vitro* study could not completely provide physiological conditions and represent *in vivo* responses. This is because the effects of atorvastatin on platelet aggregation



**Figure 2.** Effects of atorvastatin on platelet aggregation with a concentration of  $600 \times 10^9$  platelets/L induced by ADP (a), AA (b), collagen (c), and EP (d).

involve absorption by the intestine and metabolism by the liver. Therefore, we speculate that metabolites might also have affected platelet aggregation. The specific mechanism of antiplatelet effects of statins remains to be further investigated.

The present study showed that the effects of atorvastatin on platelet aggregation depended on the concentration of platelets. Further studies are needed to demonstrate the specific mechanism of the inhibitory effects of statins on platelet aggregation.

#### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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