Effect of simvastatin on endothelium-dependent vasorelaxation and endogenous nitric oxide synthase inhibitor

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ABSTRACT

AIM: To investigate the effect of simvastatin on endothelium-dependent vasorelaxation and endogenous nitric oxide synthesis inhibitor asymmetric dimethylarginine (ADMA) in rats and cultured ECV304 cells. **METHODS:** Endothelial injury was induced by a single injection of low density lipoprotein (LDL) (4 mg/kg, 48 h) in rats or incubation with LDL (300 mg/L) or oxidative-modified LDL (100 mg/L) in cultured ECV304 cells, and vasodilator responses to acetylcholine (ACh) in the aortic rings and the level of ADMA, nitrite/nitrate (NO) and tumor necrosis factor-alpha (TNF-α) in the serum or cultured medium were determined. And the adhesion of the monocytes to endothelial cells and the activity of dimethylarginine dimethylaminohydrolase (DDAH) in the cultured ECV304 cells were measured. **RESULTS:** A single injection of LDL decreased endothelium-dependent relaxation to ACh, markedly increased the serum level of endogenous ADMA and TNF-α, and reduced serum level of NO. Pretreatment with simvastatin (30 or 60 mg/kg) markedly attenuated inhibition of vasodilator responses to ACh, the increased level of TNF-α and the decreased level of NO by LDL, but no effect on serum concentration of endogenous ADMA. In cultured ECV304 cells, LDL or ox-LDL markedly increased the level of ADMA and TNF-α and potentiated the adhesion of monocytes to endothelial cells, concomitantly with a significantly decrease in the activity of DDAH and serum level of NO. Pretreatment with simvastatin (0.1, 0.5, or 2.5 μmol/L) markedly decreased the level of TNF-α and the adhesion of monocytes to endothelial cells, but did not affect the concentration of endogenous ADMA and the activity of DDAH. **CONCLUSION:** Simvastatin protect the vascular endothelium against the damages induced by LDL or ox-LDL in rats or cultured ECV304 cells, and the beneficial effects of simvastatin may be related to the reduction of inflammatory cytokine TNF-α level.

INTRODUCTION

A great deal of information demonstrated that endothelial function is impaired in animal and man with hypercholesterolemia. Oxidative-modified low density lipoprotein (ox-LDL) played a crucial role in the initiation and progression of atherosclerotic plaque. Recently, it was found that asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, was significantly elevated in animals and humans with hypercholesterolemia[1-3]. ADMA, besides inhibiting NO synthesis, also participated in inflammatory reaction in atherosclerosis[4,5]. Preincubation of TNF-α with endothelial cells increased ADMA concentrations and potentiated the adhesiveness of endothelial cells[5], and thus ADMA might be thought as a key factor contributing to endothelial dysfunction.

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Statins, structural analogs of hydroxy-methyl-glutaryl conezyme A (HMG-CoA) reductase, were widely used for treatment of hypercholesterolemia. There was an increasing amount of evidence to suggest that statins decreased the incidence of ischemic strokes and myocardial infarctions in animals and patients with atherosclerosis and hypercholesterolemia. Although the beneficial effects of statins were primarily attributed to their lipid-lowering effects, the results from these researches suggested that there might be other beneficial effects independently of serum cholesterol level. Beyond their effect on serum cholesterol level, non-lowering-cholesterol effects of statin involved improving or restoring endothelial function, decreasing oxidative stress and vascular inflammation as well as enhancing the stability of atherosclerotic plaques[6-8]. According to facilitation of the elevation of ADMA level by some cytokines and anti-inflammatory properties of simvastatin, we examined whether the protective effect of simvastatin on endothelial cells was related to reduction of endogenous ADMA levels.

MATERIALS AND METHODS

Reagents Probucol, asymmetric dimethylarginine (ADMA), phenylephrine and acetylcholine (ACh) were obtained from Sigma. Simvastatin was obtained from Hong-Rui Pharmaceutical Co, Ltd (Jiangsu province, China). DMEM was obtained from Gibco. Nitric oxide (NO) assay kits was obtained from Ju-Li Biological Medical Engineering Institute (Nanjing, China). Tumor necrosis factor-α (TNF-α) was obtained from Sen-Xiong Science and Technology Industrial Co, Ltd (Shanghai, China).

Preparation of LDL and LDL oxidation by copper Native LDL (nLDL) was isolated from pooled plasma of healthy donors through sequential density gradient ultracentrifugation in sodium bromide density solutions in the density range of 1.019-1.063 kg/L as previously described[9]. Then isolated LDL was dialyzed against PBS (pH 7.4) containing 0.01 % EDTA and subsequently filtered through a 0.22 μm filter and stored at 4 ºC. Protein concentration was measured by previously described methods[9].

Oxidation of nLDL was induced by adding 10 μmol/L $CuSO_4$ for 24 h at 37 ºC. The levels of thiobarbituric acid reactive substance (TBARS), reflecting the extent of LDL oxidation, were measured by previously described methods[10]. The levels of TBARS were (5.60±0.92) and (28.19±4.79) μmol/g protein for nLDL and oxLDL, respectively (n=3, P<0.01).

Organ chamber experiments Male Sprague-Dawley rats weighing 180-220 g were obtained from Laboratory Animal Center, Central South University. Animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 86-23, revised 1986).

The rats were anesthetized with sodium pentobarbital (60 mg/kg, ip). After blood samples were collected from artery, the thoracic aorta was rapidly isolated and cut into rings of 3-mm length. The rings were suspended horizontally between two stainless steel wires and mounted in a 5 mL organ chamber filled with warmed (37 ºC) and oxygenated (95 % O_2 and 5 % CO_2) Krebs' solution. The Krebs' solution had the following composition (mmol/L): NaCl 119.0; $NaHCO_3$ 25.0; KCl 4.7; KH_2PO_4 1.2; $MgSO_4 \cdot 7H_2O$ 1.2; $CaCl_2$ 2.5; and glucose 11.0. One of ring ends was connected to a force transducer. The aortic ring was stretched with 2 g resting force and equilibrated for 60 min, and then precontracted with KCl (60 mmol/L). After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min. In order to measure vasodilator responses, rings were contracted with phenylephrine to 40 %-50 % of their maximal contraction. After the contraction stablized, an accumulative concentration-response curve to acetylcholine (3×10^{-9}-1×10^{-6} mol/L) was observed.

Cell culture ECV304 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % (v/v) fetal bovine serum (FBS), benzylpenicillin 100 kU/L and streptomycin 100 mg/L. When the cells had reached subconfluence, cells were passaged into 24-well culture dishes and the conditioned medium was replaced by the serum-free medium. Cells were counted by Trypan blue exclusion and showed > 95 % viability. Cells were treated with nLDL (100 or 300 mg/L) or ox-LDL (30 or 100 mg/L) for 12, 24, or 48 h. For simvasta-tain and probucol, ECV304 cells were exposed to simvastatin (0.1, 0.5 or 2.5 μmol/L) or probucol (5 μmol/L) for 1 h, and then exposed to ox-LDL (100 mg/L) for 48 h in the presence of simvastatin or probucol.

Determination of nitrite/nitrate concentration The levels of NO in the serum and in the conditioned medium were determined indirectly as the content of nitrite and nitrate. The levels of nitrite/nitrate were

measured as previously described[11]. Briefly, nitrate was converted to nitrite with aspergillus nitrite reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a spectrophotometer.

Monocyte-endothelial cell adhesion assay Monocytes were isolated from fresh peripheral blood of healthy volunteers by the Ficoll-Paque density centrifugation method as previously described [12]. The collected monocytes were washed twice in Hanks' balanced salt solution (HBSS) and resuspended in DMEM containing 10 % (v/v) FBS, penicillin 100 kU/L and streptomycin 100 mg/L. This preparation method routinely harvested >95 % of monocytes and maintained >95 % viability as assessed by Trypan blue exclusion.

The adhesion of the monocytes to ECV304 cells was evaluated by protein content as previously described methods[12]. The monolayers of ECV304 cells were washed three times with HBSS before addition of monocytes. Monocyte cells were diluted to a final concentration of 1×10^9 cells/L and were added to each well (1 mL) of ECV304 cells. An additional 1 mL of the monocyte suspension was also obtained for determination of protein content. The monocytes were incubated with ECV304 cells monolayer at room temperature for 30 min on a rocking platform and nonadherent monocytes were carefully removed by twice washes with HBSS. ECV304 cells and adherent monocytes were then lysed with NaOH 0.5 mol/L for protein analysis. The adhesion of monocytes to ECV304 cells was estimated by comparing the amount of protein in wells containing ECV304 cells and monocytes minus the the amount of protein in wells containing ECV304 cells alone, divided by the amount of protein in 1 mL of monocyte suspension.

Determination of TNF-α concentration TNF-α levels in the serum and in the conditioned medium were assayed by enzyme-linked immunosorbent assay (ELISA). The values were measured at 405 nm by a microplate reader (Biotek). The standard curve for TNF-α measured by ELISA was linear from 78 ng/L to 10 μg/L; the detection limit was 100 ng/L.

Determination of ADMA concentration The proteins in the conditioned medium and in the serum were removed using 5-sulfosalicylic acid (5-SSA). The level of ADMA was measured by high-performance liquid chromatography (HPLC) as described previously with some modification[13]. HPLC was carried out using a Shimadzu LC-6A liquid chromatograph with Shimadzu SCL-6A system controller and Shimadzu SIC-6A autosampler. *O*-phthaldialdehyde adducts of methylated amino acids and internal standard ADMA produced by precolumn mixing were monitored using a model RF 530 fluorescence detector set at λ^{ex}=338 and λ^{em}=425 nm on a Resolve C_{18} column. Samples were eluted from the column using a linear gradient containing mobile phase A composed of 0.05 mol/L (pH 6.8) sodium acetate-methanol-tetrahydrofuran (81:18:1, v:v:v) and mobile phase B composed of 0.05 mmol/L sodium acetate-methanol-tetrahydrofuran (22:77:1, v:v:v) at a flow-rate of 1 mL/min.

DDAH activity assay The activity of DDAH in ECV304 cells was estimated by directly measuring the amount of ADMA metabolized by the enzyme[14]. In an ice bath, cell lysates were divided into 2 groups, and ADMA was added (final concentration 500 μmol/L). To inactivate DDAH, 30 % sulfurosalicylic acid was immediately added to 1 experimental group. This group provided a baseline of 0 % DDAH activity. The other lysate was incubated at 37 ºC for 2 h before the addition of 30 % sulfurosalicylic acid. The ADMA levels in each group were measured by HPLC as described above. The difference in ADMA concentrations between two groups reflected the DDAH activity. For every experiment, DDAH activity of ECV304 cells exposed to normal conditioned medium was defined as 100 %, and DDAH activity in other conditions was expressed as percentage of the ADMA metabolized compared with the control.

Determination of lactate dehydrogenase (LDH) activity The activity of lactate dehydrogenase (LDH) in the conditioned medium, as an indicator of cell cytotoxicity, was measured spectrophotometrically using a commercially available assay kit.

Experimental protocols The first series of experiments were designed to evaluate the protective effect of simvastatin on endothelial function *in vivo*. Rats were pretreated with LDL (4 mg/kg, iv) for 48 h before the experiment[24]. For simvastatin and probucol, the rats were pretreated with simvastatin (30 or 60 mg/kg, ig) or probucol (150 mg/kg, ig) once a day for 3 d and then treated with LDL (4 mg/kg) for 48 h in the presence of drugs. Simvastatin and probucol were dissolved in a vehicle containing 10 % Gum acacia and 0.5 % saline carboxymethyl cellulose.

The second series of experiments were designed to further evaluate the protective effect of simvastatin on endothelial cells in cultured ECV304 cells. Cell in-

jury was induced by treatment with LDL (100 or 300 mg/L) or ox-LDL (30 or 100 mg/L) for 12, 24, or 48 h. For simvastatin and probucol, endothelial cells were exposed to simvastatin (0.1, 0.5, 2.5 μmol/L) or probucol (5 μmol/L) for 1 h, and then exposed to ox-LDL (100 mg/L) for 48 h in the presence of simvastatin or probucol. Simvastatin was activated to its lactone form by adding 0.1 mol/L NaOH solution and heating for 2 h and then adjusting pH to 7.4 by HCl 0.1 mol/L.

Statistic analysis Data are expressed as mean±SD. Data were analysed by ANOVA followed by the unpaired *t*-test for multiple comparisons. The significance level was chosen as $P<0.05$.

RESULTS

Vasoconstrictor and vasodilator responses Phenylephrine was added to increase smooth muscle tone in the rat aortic rings. Vasoconstrictor responses to phenylephrine (1 μmol/L) were also significantly increased in the rats treated with LDL. The tension was (1.42±0.06) and (1.05±0.08) g for LDL and control, respectively ($n = 8$, $P<0.05$). As shown in Fig 1, pretreatment with LDL (4 mg/kg) for 48 h significantly decreased vasodilator responses to acetylcholine. Simvastatin (30 or 60 mg/kg) or probucol (150 mg/kg) significantly attenuated inhibition of vasodilator responses to acetylcholine by LDL. However, simvastatin or probucol itself had no effect on vasodilator responses to acetylcholine.

Concentrations of nitrite/nitrate After pretreatment with LDL (4 mg/kg) for 48 h, serum concentration of nitrite/nitrate was significantly decreased. Simvastatin (30 or 60 mg/kg) significantly inhibited the reduced concentration of nitrite/nitrate by LDL. Probucol (150 mg/kg) also markedly attenuated the decreased concentration of nitrite/nitrate by LDL (Fig 2A).

Treatment with ox-LDL 100 mg/L for 48 h caused a significant decrease in concentration of nitrite/nitrate in the medium. Simvastatin (0.5 or 2.5 μmol/L) significantly attenuated the decreased level of nitrite/nitrate by ox-LDL. The decreased level of nitrite/nitrate by ox-LDL was also attenuated by treatment with probucol 5 μmol/L. Simvastatin or probucol itself had no effect on concentration of nitrite/nitrate (Fig 2B).

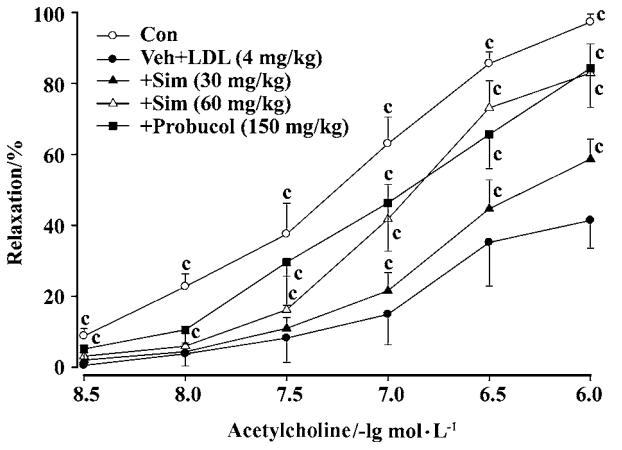

Fig 1. Effect of simvastatin and probucol on vasodilator responses to acetylcholine. Simvastatin and probucol were given orally. The rats were treated with LDL (4 mg/kg) for 48 h. *n*=6-8. Mean±SD. $^cP<0.01$ *vs* LDL.

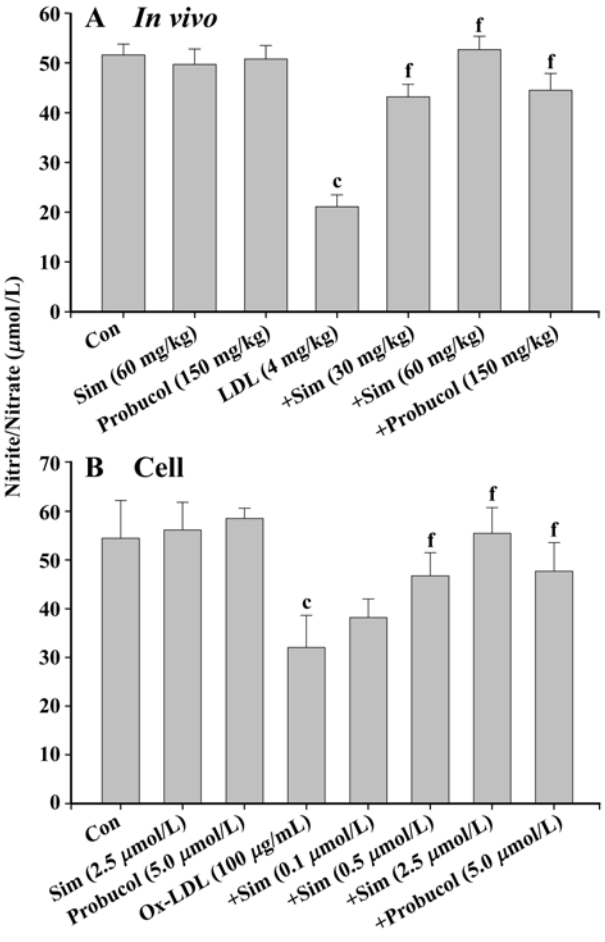

Fig 2. Effects of simvastatin and probucol on concentration of nitrite/nitrate in the serum (A) and in the conditioned medium (B). The rats and the endothelial cells were treated with LDL or ox-LDL at the dose of 4 mg/kg and 100 mg/L for 48 h, respectively. *n*=6-8. Mean±SD. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* ox-LDL.

Adhesion of the monocytes to endothelial cells Treatment with ox-LDL (100 mg/L) for 48 h caused a

significant increase in the adhesion of monocytes to ECV304 cells compared with control (26.7 %±5.6 % *vs* 4.7 %±5.2 %, $P<0.01$). Simvastain (0.1, 0.5 or 2.5 μmol/L) significantly attenuated the increase in adhesion of monocytes by ox-LDL (20.8 %±3.0 %, 14.3 %± 2.8 %, 9.8 %±1.6 % *vs* 26.7 %±5.6 %, $P<0.05$). The increased adhesion of monocytes to ECV304 cells by ox-LDL was also attenuated by treatment with probucol (5 μmol/L) (14.3 %±2.8 % *vs* 26.7 %±3.6 %, $P<0.01$). Simvastatin or probucol itself had no effect on the adhesion of monocytes to ECV304 cells (4.7 %±5.0 %, 4.5 %±8.3 % *vs* 4.7 %±5.2 %, $P>0.05$).

Concentrations of TNF-α After pretreatment with LDL (4 mg/kg) for 48 h, serum concentration of TNF-α was significantly increased. Simvastatin (30 or 60 mg/kg) significantly inhibited the elevated concentration of TNF-α by LDL. Probucol (150 mg/kg) also markedly inhibited the elevated concentration of TNF-α by LDL (Fig 3A).

Simvastatin (0.5 or 2.5 μmol/L) significantly inhibited the elevated concentration of TNF-α by ox-LDL (100 mg/L). Probucol (5 μmol/L) also markedly inhibited the elevated concentration of TNF-α by ox-LDL. However, simvastatin or probucol itself had no effect on concentration of TNF-α (Fig 3B)

Pretreatment with LDL at the dose of 100 or 300 mg/L for 12 or 24 h only caused a slight increase in concentration of TNF-α, while pretreatment with LDL at a higher dose (300 mg/L) for 48 h caused a significant increase in concentration of TNF-α. Pretreatment with ox-LDL at the dose of 30 mg/L for 12 or 24 or 48 h only caused a slight increase in concentration of TNF-α, while pretreatment with ox-LDL at a higher dose (100 mg/L) for 24 or 48 h caused a significant increase in concentration of TNF-α (Tab 1).

Concentrations of ADMA Pretreatment with LDL (4 mg/kg) for 48 h significantly increased the serum level of ADMA. Simvastatin (60 or 120 mg/kg) had no effect on the elevated concentration of ADMA by LDL, while probucol (150 mg/kg) markedly inhibited the elevated concentration of ADMA by LDL (Fig 4A).

Pretreatment with LDL at the dose of 100 mg/L for 12 or 24 h only caused a slight increase in concentration of ADMA, while pretreatment with LDL at a higher dose (300 mg/L) for 24 or 48 h caused a significant increase in concentration of ADMA. Pretreatment with ox-LDL at the dose of 30 mg/L for 12, 24, or 48 h

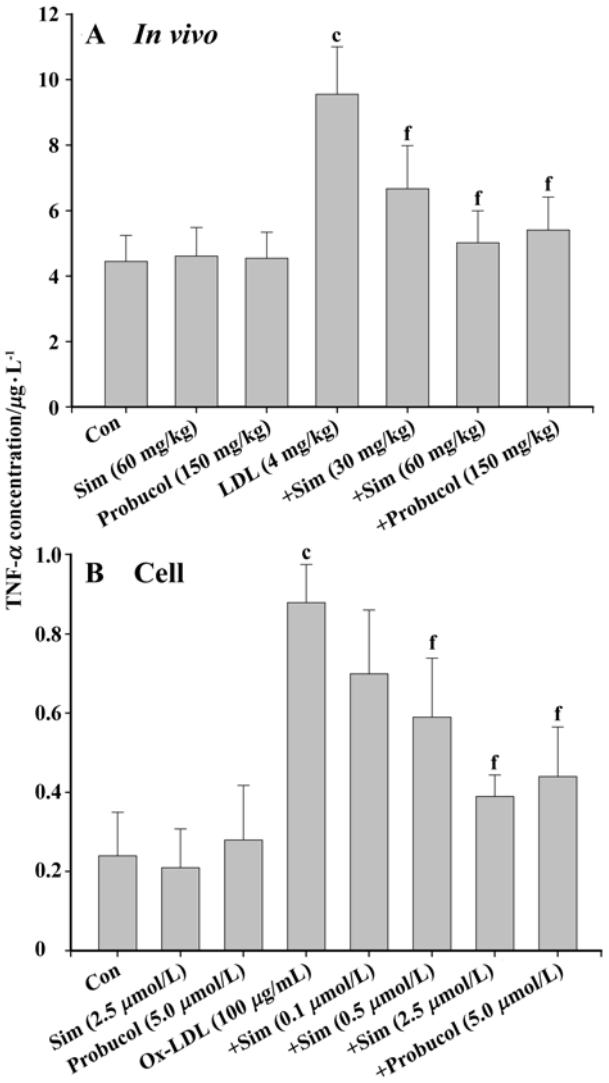

Fig 3. Effects of simvastatin and probucol on concentrations of TNF-α in the serum (A) and in the conditioned medium (B). The rats and the ECV304 cells were treated with LDL or ox-LDL at the dose of 4 mg/kg and 100 μg/L for 48 h, respectively. *n*=6-8. Mean±SD. $^{c}P<0.01$ *vs* control. $^{f}P<0.01$ *vs* LDL or ox-LDL.

Tab 1. The level of TNF-α in the medium in the different treated groups. *n*=6-8. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

	TNF-α/μg·L^{-1}		
	12 h	24 h	48 h
Control	0.28±0.10	0.30±0.06	0.28±0.08
LDL (100 mg/L)	0.28±0.09	0.33±0.09	0.43±0.17
LDL (300 mg/L)	0.38±0.10	0.49±0.13^{b}	0.70±0.11^{b}
Ox-LDL (30 mg/L)	0.31±0.05	0.36±0.04	0.40±0.17
Ox-LDL (100 mg/L)	0.37±0.09	0.61±0.18^{c}	0.95±0.14^{c}

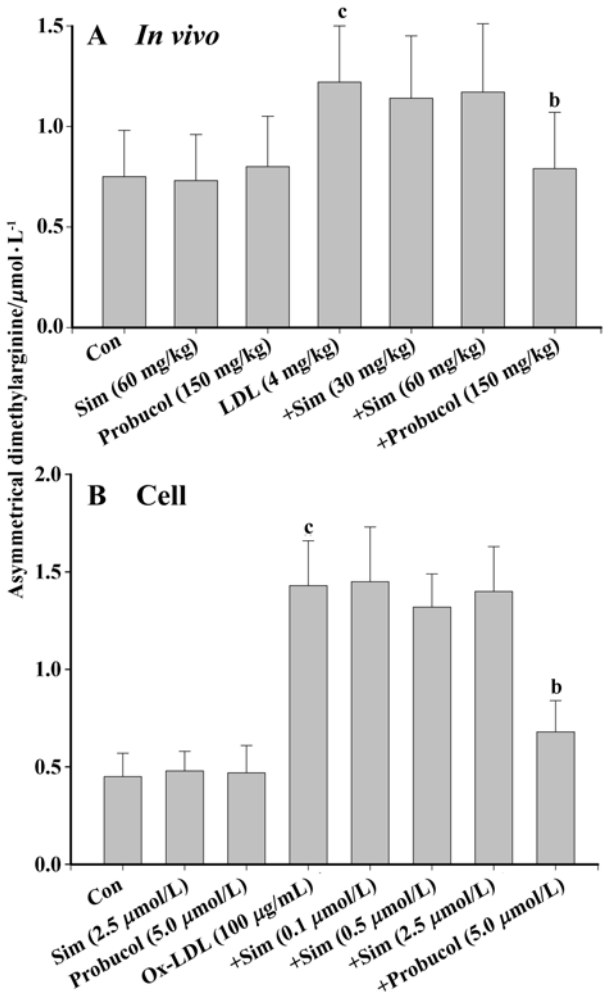

Fig 4. Effects of simvastatin and probucol on concentration of ADMA in the serum (A) and in the conditioned medium (B). The rats and the ECV304 cells were treated with LDL or ox-LDL at the dose of 4 mg/kg and 100 mg/L for 48 h, respectively. *n*=6-8. Mean±SD. ^{c}P<0.01 *vs* control. ^{f}P<0.01 *vs* LDL or ox-LDL.

Tab 2. The level of ADMA in the medium in the different treated groups. *n*=6-8. Mean±SD. ^{b}P< 0.05, ^{c}P<0.01 *vs* control.

	ADMA/μmol·L^{-1}		
	12 h	24 h	48 h
Control	0.33±0.04	0.36±0.06	0.50±0.06
LDL (100 mg/L)	0.38±0.06	0.49±0.07	0.68±0.09^{b}
LDL (300 mg/L)	0.46±0.05	0.66±0.06^{c}	1.07±0.17^{c}
Ox-LDL (30 mg/L)	0.42±0.08	0.48±0.08	0.62±0.11
Ox-LDL (100 mg/L)	0.52±0.15^{b}	0.73±0.09^{c}	1.39±0.22^{c}

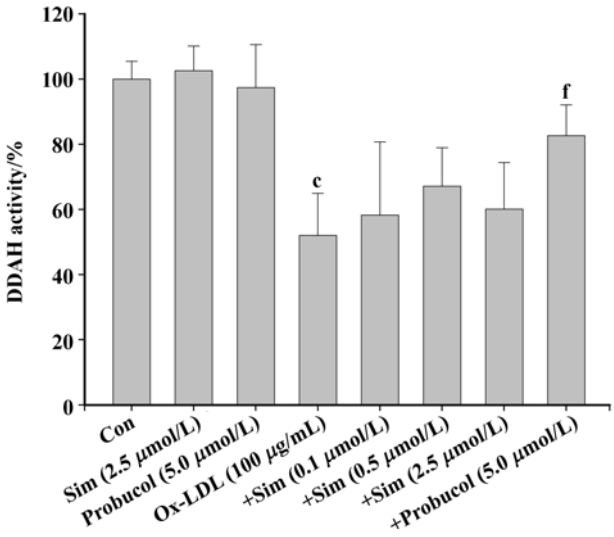

Fig 5. Effect of simvastatin and probucol on the activity of ECV304 DDAH. The ECV304 cells were treated with ox-LDL (100 mg/L) for 48 h. Mean±SD. ^{c}P<0.01 *vs* control. ^{f}P <0.01 *vs* ox-LDL.

only caused a slight increase in concentration of ADMA, while pretreatment with ox-LDL at a higher dose (100 mg/L) for 24 or 48 h caused a significant increase in concentration of ADMA (Tab 2).

Simvastatin (0.1, 0.5 or 2.5 μmol/L) had no effect on concentration of ADMA by ox-LDL, while probucol (5 μmol/L) significantly inhibited the elevated level of ADMA by ox-LDL. simvastatin or probucol itself had no effect on concentration of ADMA (Fig 4B).

Activity of DDAH DDAH activity was significantly decreased in the endothelial cells treated with ox-LDL (100 mg/L) for 48 h. Simvastatin (0.1, 0.5 or 2.5 μmol/L) had no effect on the decreased activity of DDAH by ox-LDL, while probucol (5 μmol/L) markedly attenuated the inhibition of endothelial DDAH activity by ox-LDL. However, simvastatin or probucol itself had no effect on the activity of DDAH (Fig 5).

Activity of LDH Treatment with ox-LDL (100 mg/L) for 48 h significantly increased the activity of LDH in the medium compared with control (83.6 %± 14.3 % *vs* 31.5 %±9.5 %, P<0.01). Simvastatin (0.5 or 2.5 μmol/L) significantly inhibited the elevated activity of LDH by ox-LDL (53.1 %±16.5 %, 45.9 %±16.8 % *vs* 83.6 %±14.3 %, P<0.01). The increased activity of LDH by ox-LDL was also inhibited by treatment with probucol (5 μmol/L) (54.0 %±12.4 % *vs* 83.6 %±14.3 %, P<0.05). However, simvastatin or probucol itself had no effect on the activity of LDH (31.5 %±12.2 %, 29.9 %±8.3 % *vs* 31.5 %±9.5 %, P>0.05).

DISCUSSION

ADMA, an endogenous nitric oxide synthase inhibitor, was present in blood of both humans and animals. There was growing evidence that endothelial dysfunction in some cardiovascular diseases such as hypercholesterolemia, heart failure, and hypertension was associated with the elevation level of ADMA, suggesting that the endogenous inhibitors of NOS might be a novel predictor of endothelial dysfunction[15-17].

ox-LDL possesses numerous biological effects, including induction of endothelial dysfunction, activation of endothelial adhesiveness, monocyte differentiation and adhesion, and smooth muscle cell proliferation, suggesting that ox-LDL plays a key role in the development of atherosclerosis[18-21]. Recently, several lines of evidence showed that ox-LDL might be an important factor contributing to the elevation of ADMA level. For example, the level of ADMA was significantly increased in hypercholesterolemic animals and patients[17,22]. Our previous work had shown that chronic hypercholesterolemia or a single injection of LDL caused a marked increase in the level of ADMA in rabbits or rats[23,24]. In cultured endothelial cells, ox-LDL also significantly increased the content of ADMA[25]. The results of the present study confirmed previous observations that LDL or ox-LDL induced a significant increase in the level of ADMA in the rats treated with LDL or cultured endothelial cells treated with LDL or ox-LDL.

ADMA was synthesized by protein arginine methyltransferases (PRMTs), which utilized *S*-adenosylmethionine methyl group donor, and was degraded by DDAH, which hydrolyzed ADMA to *L*-citrulline and dimethylamine asymmetric dimethylarginine[25-27]. DDAH were widely distributed in tissues including endothelial cells. The elevated level of ADMA induced by LDL or ox-LDL was related to the increased activity of PRMTs and the decreased activity of DDAH[25,26]. More recently, it was reported that the inflammatory cytokine TNF-α raised ADMA levels by the reduction of DDAH activity[25]. The results of the present study were consistent with previous observations that ox-LDL elevated ADMA levels and decreased DDAH activity, concomitantly with an increase in the level of TNF-α[26,28]. These findings suggested that facilitation of the elevation of ADMA level by ox-LDL might be related to reduction of DDAH activity by increasing TNF-α production.

Interactions of ADMA with cytokines were shown in the inflammatory processes of atherosclerosis. Preincubation of ADMA with endothelial cells increased the expression of monocyte chemotactic protein-1 (MCP-1) and potentiated the adhesiveness of endothelial cells, and the effects of ADMA were attenuated by anti-MCP-1 antibody or *L*-arginine[5]. Studies in clinic also found that the level of ADMA was significantly raised in hypercholesterolemic humans, concomitantly with the elevated mononuclear cell adhesiveness[4,29].

Statins, an important lowering-cholesterol drug, was able to inhibit HMG-CoA reductase and decrease *L*-mevalonic acid synthesis, an intermediate of the cholesterol biosynthetic pathway, finally reduce cholesterol biosynthesis. Statins were able to improve endothelial function in cardiovascular diseases such as hypercholesterolemia, atherosclerosis and hypertension partially from its non-lowering cholesterol effects[30-32]. However, the mechanism responsible for the beneficial effect of statins on endothelial cells remained unclear. Recently, there was abundant evidence to suggest that statins had potent anti-inflammatory effects, such as reduction of cytokines and adhesion molecules production in hypercholesteroletic individuals[33]. According to facilitation of the elevation of ADMA by cytokines and reduction of inflammatory reaction with statins, we postulated that the protective effect of statins on endothelial cells might be related to reduction of ADMA level. However, in the present study, treatment with simvastatin did not affect the increased level of ADMA but decreased activity of DDAH induced by LDL. More recently, studies in clinic showed that atorvastatin and simvastatin had no effect on plasma concentrations of ADMA in patients with hypercholesterolemia[34,35]. Evidence presented here and from clinic studies did not support the hypothesis that improvement of endothelial function with statins was related to reduction of ADMA.

Many pathologic processes, including atherosclerosis, ischemia reperfusion, hypertension, and diabetes, were associated with excessive generation of oxygen free radicals, and superoxide anions and other oxyradical species, which could inactivate NO[36-38]. Recent investigations showed that the statins decrease lipid peroxidation production and matrix metalloproteinase activity, which decreased generation of oxygen free radical resulting in reduction of NO activation[39-41]. However, the mechanism by which statins acted on endothelial cells to improve endothelial function awaited further investigation.

In conclusion, the protective effect of simvastatin on endothelial cells might be due to reduction of inflam-

matory reaction, but not alternation of ADMA level.

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