

# Berberine combined with atorvastatin downregulates LOX-1 expression through the ET-1 receptor in monocyte/macrophages

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**Abstract.** Studies have shown that the oxidative modification of low-density lipoprotein (oxLDL) plays a major role in atherogenesis. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) mediated the transport of oxLDL into macrophages, which promoted foam cell formation. Targeting LOX-1 may therefore be a promising approach to inhibit atherosclerosis. In the present study, we aimed to investigate the effect of berberine combined with atorvastatin on LOX-1 and explore the underlying molecular mechanism involved. Expression of LOX-1 in monocyte-derived macrophages (MDMs) exposed to berberine (0, 0.1, 1, 10 and 100 nM) and atorvastatin (100 nM) were analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and western blot analysis. Results showed that the expression of LOX-1 was decreased in a dose-dependent manner. Additionally, knockdown of the endothelin-1 (ET-1) receptor significantly blocked the inhibitory effect of berberine on

LOX-1 expression. Body weight (BW), liver weight (LW) and kidney weight (KW) in the model rats were markedly increased at concentrations of berberine  $\geq 1 \mu\text{mol/kg}$ , while heart weight (HW) and spleen weight (SW) remained constant among all groups. Berberine combined with atorvastatin also decreased serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) levels in the rat model as well as inflammation and oxidative stress. Furthermore, plasma ET-1 levels and LOX-1 expression were decreased by berberine combined with atorvastatin treatment, and the inhibitory effect on LOX-1 was impeded by an ET-1 receptor antagonist. The results demonstrated that berberine combined with atorvastatin downregulates LOX-1 expression through ET-1 receptors in monocyte/macrophages *in vitro* and *in vivo*.

## Introduction

Atherosclerosis has been shown to cause cardiovascular diseases that contribute to morbidity and mortality in developed and developing countries (1,2). Atherosclerosis is defined as a complex inflammatory response characterized by the accumulation of lipid in arteries (3,4). Monocyte/macrophages migrate into the intima and engulf modified low-density lipoproteins (LDLs) such as oxidized LDL (oxLDL) or acetyl LDL (Ac-LDL) via scavenger receptors (SRs) and then transform into foam cells (5-7). These are the initial events in the development of atherosclerosis.

Several SRs including SR-AI/II, SR-BI, cluster of differentiation 36 (CD36), and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) mediate the transport of oxLDL into macrophages, which results in lipid accumulation and the transformation of macrophages into foam cells (6,8).

LOX-1 is a type II membrane protein with an extracellular domain and a short cytoplasmic tail (9). LOX-1 has been reported to be expressed in endothelial cells, monocyte/macrophages, platelets, and vascular smooth muscle cells (VSMCs) as well as in renal, pulmonary and neuronal tissues. LOX-1 expression can be induced by oxLDL, free radicals (reactive oxygen species), endothelin-1 (ET-1), angiotensin II, advanced glycation end-products (AGEs) and shear stress (10-12). Furthermore, various pathological conditions including diabetes mellitus, hypertension, myocardial ischemia and

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**Abbreviations:** AGEs, advanced glycation end-products; AMPK, AMP-activated protein kinase; BW, body weight; CD36, cluster of differentiation 36; CRP, C-reactive protein; EGCG, epigallocatechin gallate; ELISA, enzyme-linked immunosorbent assay; ET-1, endothelin-1; GPx, glutathione peroxidase; HDL-C, high-density lipoprotein-cholesterol; HFD, high-fat diet; HW, heart weight; KW, kidney weight; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein-cholesterol; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; LW, liver weight; MDA, malondialdehyde; MDMs, monocyte-derived macrophages; oxLDL, oxidized low-density lipoprotein; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SOD, superoxide dismutase; SRs, scavenger receptors; SW, spleen weight; TC, total cholesterol; TG, triglyceride; VSMCs, vascular smooth muscle cells

**Key words:** berberine, atorvastatin, lectin-like oxidized low-density lipoprotein receptor-1, endothelin-1 receptor, monocyte-derived macrophages

atherosclerosis contribute to the induction of LOX-1 expression (13,14).

ET-1 has been suggested to be involved in the pathogenesis of cardiovascular diseases. It is well known that the plasma level of ET-1 is increased in patients with hypertension and coronary artery disease (15,16). Studies have demonstrated that local ET-1 concentrations are increased in the atherosclerotic plaques (17,18). Furthermore, ET-1 receptor blockade has been shown to reduce the development of atherosclerotic lesions in an atherosclerotic animal model, apoE-KO mice (17). Morawietz *et al* have shown that ET-1 induces LOX-1 mRNA and protein expression in a time- and dose-dependent manner in human endothelial cells and promotes oxLDL uptake (19). ET-1, exclusively expressed in endothelial cells, enhances the oxidative modification of LDL via the ETB receptor, which further increases the uptake of oxLDL in endothelial cells via the LOX-1 receptor leading to the progression of atherosclerosis (20).

Natural compounds have been demonstrated to inhibit LOX-1 expression. These compounds include tanshinone II-A (21), curcumin (22), berberine (23), epigallocatechin gallate (EGCG) (24), and resveratrol (25). Berberine is the primary component of rhizoma coptidis and is often used as an anti-inflammatory medicine (26). Berberine has been shown to significantly inhibit low-density lipoprotein-cholesterol (LDL-C) synthesis in human hepatocytes by increasing AMP-activated protein kinase (AMPK) phosphorylation and AMPK activity (27). In addition, berberine significantly decreased the expression of LOX-1 and increased SR-BI expression in a time- and dose-dependent manner (23).

It is well established that atorvastatin (3-hydroxy-3-methyl-glutaryl-coenzyme, a reductase inhibitor) suppresses intracellular cholesterol synthesis and it has been widely used as an anti-inflammatory drug in the treatment of atherosclerosis (28). Atorvastatin has been shown to reduce the activation of transcription factor NF- $\kappa$ B in cultured VSMCs as well as in atherosclerotic lesions in rabbit (29).

In the present study, we aimed to investigate the effect of berberine combined with atorvastatin on atherosclerosis and the underlying molecular mechanism involved. We found that the expression of LOX-1 in monocyte/macrophages treated with berberine (0, 0.1, 1, 10 or 100 nM) combined with atorvastatin (100 nM) was significantly decreased in a dose-dependent manner. Knockdown of the ET-1 receptor by small-interfering (siRNA) transfection significantly reversed the inhibitory effect of berberine on LOX-1 expression in monocyte-derived macrophages (MDMs). A rat model induced with a high-fat diet (HFD) was also used to analyze the regulation of LOX-1 expression. Treatment with berberine combined with atorvastatin markedly influenced physiological parameters, lipid profile, inflammation and oxidative stress in the rat model. In addition, the inhibitory effect of berberine on LOX-1 expression was blocked by an ET-1 receptor antagonist in the rat model.

## Materials and methods

**Cell culture.** MDMs were isolated from peripheral blood monocytes by adherence to plastic as described previously (30). Blood was layered onto Lymphoprep (Axis-Shield,

Dundee, Scotland) and centrifuged for 30 min at 700 g. The white-blood-cell layer was harvested, washed with PBS and suspended in RPMI-1640. Cells were counted and then plated at  $1 \times 10^6$  cells per 140 mm dish in RPMI-1640 with 5% heat-inactivated human serum. After 2 h, the plates were washed three times in RPMI-1640 and then incubated at 37°C overnight. The cells were left to differentiate into MDMs for 7 days, then washed with PBS, treated with 5 mM PBS/EDTA at 37°C for 20 min, harvested gently with a cell scraper, counted and replated on 96- or 6-well trays at  $1 \times 10^4$  and  $1 \times 10^6$  cells per well, respectively, as described previously (31).

**Animals.** One hundred and twenty 8-week-old male Sprague-Dawley rats were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The animals were housed under standard conditions of a 12/12 h light/dark cycle at room temperature with a HFD (MD12033; Mediscience, Ltd., Jiangsu, China), and free access to water. All animal experimental procedures were conducted under the guidelines of the National Health and Medical Research Council for the Care and Use of Animals for Experimental Purposes in China. All efforts were made to minimize suffering.

**Experimental design.** MDMs were plated in triplicate into 12-well cell culture plates (Takara Biotechnology (Dalian), Co., Ltd., Dalian, China). The experimental regime consisted of cells undergoing a preconditioning phase of: i) 100  $\mu$ l vehicle for 4 h; ii) 0.1 nmol berberine and 100 nmol atorvastatin for 4 h; iii) 1 nmol berberine and 100 nmol atorvastatin for 4 h; iv) 10 nmol berberine and 100 nmol atorvastatin for 4 h; v) 100 nmol berberine and 100 nmol atorvastatin for 4 h; vi) transfection with non-specific siRNA followed by the addition of 100  $\mu$ l vehicle for 4 h; vii) transfection with non-specific siRNA followed by the addition of 100 nmol berberine and 100 nmol atorvastatin for 4 h; viii) transfection with specific siRNA targeting the ET-1 receptor followed by the addition of 100 nmol berberine and 100 nmol atorvastatin for 4 h. One hundred and twenty male Sprague-Dawley rats were randomly assigned to 6 groups and fed a HFD for 4 months prior to initiation of mimic atherosclerosis. Subsequently, the rats were exposed to treatment as follows: i) vehicle for 1 month (i.v.); ii) 0.1  $\mu$ mol/kg berberine and 100  $\mu$ mol/kg atorvastatin for 1 month (i.v.); iii) 1  $\mu$ mol/kg berberine and 100  $\mu$ mol/kg atorvastatin for 1 month (i.v.); iv) 10  $\mu$ mol/kg berberine and 100  $\mu$ mol/kg atorvastatin for 1 month (i.v.); v) 100  $\mu$ mol/kg berberine and 100  $\mu$ mol/kg atorvastatin for 1 month (i.v.); vi) 100  $\mu$ g/kg/min BQ-788 (i.v.) followed by 100  $\mu$ mol/kg berberine and 100  $\mu$ mol/kg atorvastatin for 1 month (i.v.).

At the end of the treatment, body weight (BW) was measured and the animals were anesthetized with 10% chloral hydrate. Blood was collected by cardiac puncture. Organs such as heart, liver, kidneys and spleen were harvested and weighed.

**Knockdown of ET-1 receptor by siRNA.** Scrambled siRNA and siRNA targeting the ET-1 receptor were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Cells were transfected with scrambled or ET-1 receptor siRNA according to the manufacturer's instructions. Briefly, the ET-1 receptor and scrambled siRNAs (30 pmol) were diluted

Table I. List of primers for qPCR analysis.

Gene	Primers
LOX-1	F: 5'-GAA CGT TTG CCT GGG ATT AGT A-3' R: 5'-CTG GTG GTG AAG TTC CAT TTG G-3'
ET-1 receptor	F: 5'-GATACGACAACCTCCGCTCCA-3' R: 5'-GTCCACGATGAGGACAATGAG-3'
$\beta$ -actin	F: 5'-GTG GGG CGC CCC AGG CACCA-3' R: 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'

qPCR, quantitative polymerase chain reaction, F, forward; R, reverse.

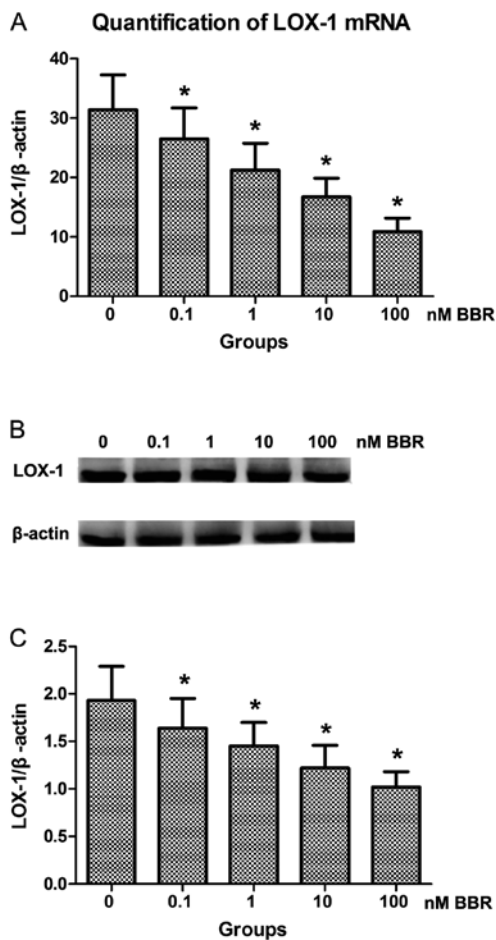


Figure 1. Analysis of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in monocyte-derived macrophages (MDMs). (A) Quantification of LOX-1 mRNA by quantitative polymerase chain reaction (qPCR). (B and C) Detection of LOX-1 protein by western blot analysis. BBR, berberine. \* $P < 0.05$  vs. 0 nM berberine group denotes significant differences.

in 500  $\mu$ l DMEM and mixed with 5  $\mu$ l Lipofectamine RNAi MAX (Invitrogen Life Technologies, Carlsbad, CA, USA). After 15 min incubation at room temperature, the complexes were added to the cells to a final volume of 3 ml medium. The cells were then harvested at the indicated times for further analysis. The efficiency of the ET-1 receptor siRNA was confirmed by western blot analysis of Flag expression.

*Detection of total cholesterol (TC), triglycerides (TGs), LDL-C and high-density lipoprotein-cholesterol (HDL-C).* Blood samples were collected and the levels of TC, TG, LDL-C, and HDL-C were detected with an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). The samples were analyzed in duplicate.

*Detection of C-reactive protein (CRP), malondialdehyde (MDA), glutathione peroxidase (GPx) and superoxide dismutase (SOD).* The CRP levels were determined with an ultrasensitive CRP test with a coefficient of variance below 5% (Sigma, St. Louis, MO, USA). A Biochemical Analysis kit (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China) was used to measure MDA content, GPx, and SOD activity according to the manufacturer's instructions.

*Enzyme-linked immunosorbent assay (ELISA) analysis for ET-1.* The levels of ET-1 protein in serum were analyzed using a commercially available ELISA (Yanjin Biotechnology Co., Shanghai, China) according to the manufacturer's instructions. The absorbance was read at 450 nm using a 680XR microplate reader (Bio-Rad, Hercules, CA, USA). All the samples were analyzed in duplicate. The standard curve for ET-1 estimation was conducted by linear regression analysis.

*RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR).* RNA was extracted from MDMs or monocytes using TRIzol RNA-extraction reagent (Gibco-BRL, Rockville, MD, USA) according to the manufacturer's instructions. Total RNA (5  $\mu$ g) for each sample was reverse transcribed into first-strand cDNA for qRT-PCR analysis. qRT-PCR was performed in a final volume of 10  $\mu$ l, which contained 5  $\mu$ l of SsoFast™ EvaGreen supermix (Bio-Rad), 1  $\mu$ l of cDNA (1:50 dilution), and 2  $\mu$ l each of the forward and reverse primers (1 mM). The steps in qRT-PCR were performed as follows: 94°C for 2 min for initial denaturation; 94°C for 20 sec, 58°C for 15 sec, and 72°C for 15 sec; 2 sec for plate reading for 40 cycles; and a melt curve from 65 to 95°C.  $\beta$ -actin was used as a quantitative and qualitative control to normalize the gene expression. Data were analyzed using the formula:  $R = 2^{-[\Delta CT \text{ sample} - \Delta CT \text{ control}]}$ . All of the primers used in this experiment are shown in Table I.

*Western blot analysis.* Cells were homogenized and lysed with RIPA lysis buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% TritonX-100, 1 mM EDTA, 10 mM  $\beta$ -glycerophosphate, 2 mM sodium vanadate and protease inhibitor). Protein concentration was assayed using a micro-BCA protein kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Forty micrograms of protein per lane were separated by 12% SDS-PAGE and electroblotted onto nitrocellulose (Amersham Pharmacia Biotech, Freiburg, Germany). Non-specific binding was blocked by incubating with 5% non-fat milk in TBST buffer at room temperature for 1 h. Immunodetection of LOX-1 and  $\beta$ -actin was conducted using mouse monoclonal anti-LOX-1 antibody (1:1,000; Santa Cruz Biotechnology, Inc.), and anti- $\beta$ -actin (Sigma), respectively. Goat anti-mouse IgG (1:5,000; Sigma) followed by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, NJ, USA) were used for the detection of  $\beta$ -actin.

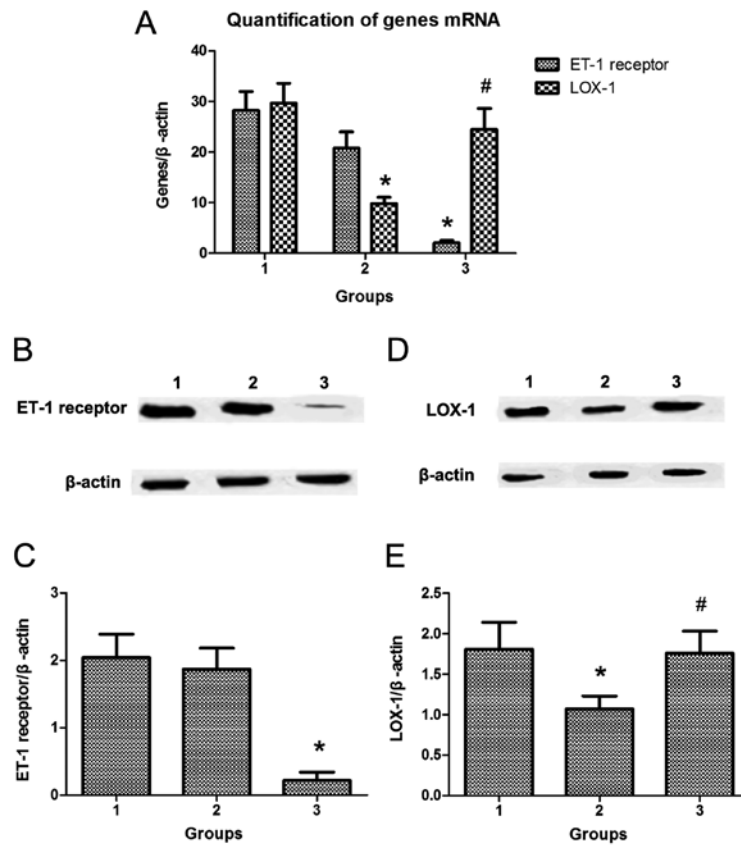


Figure 2. Analysis of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and endothelin-1 (ET-1) receptor expression in monocyte derived macrophages (MDMs). (A) Quantification of LOX-1 and ET-1 receptor mRNA by quantitative polymerase chain reaction (qPCR). (B and C) Analysis of LOX-1 protein by western blotting. (D and E) Analysis of ET-1 receptor protein by western blotting. Lanes: 1, non-specific siRNA + vehicle group; 2, non-specific siRNA + 100 nM berberine group; 3, ET-1 receptor siRNA + 100 nM berberine group. \* $P < 0.05$  vs. non-specific siRNA + vehicle group denotes a significant difference; # $P < 0.05$  vs. non-specific siRNA + 100 nM berberine-treated group denotes a significant difference.

**Statistical analysis.** Results are expressed as means  $\pm$  SD. Statistical significance was analyzed with one-way factorial ANOVA or the Student's two-tailed t-test.  $P < 0.05$  was considered statistically significant. Analyses were conducted using SPSS software (SPSS, Inc., Chicago, IL, USA).

## Results

**Berberine combined with atorvastatin downregulates the expression of LOX-1 in MDMs.** To investigate the effect of berberine combined with atorvastatin on the expression of LOX-1, the two genes were analyzed by qRT-PCR and western blot analysis. The qRT-PCR results showed that the mRNA level of LOX-1 tended to decline as the amount of berberine increased by 10 to 1,000-fold (Fig. 1A). This result was confirmed by western blot analysis (Fig. 1B and C).

**The ET-1 receptor mediates the inhibitory effect of berberine combined with atorvastatin on LOX-1 expression in MDMs.** ET-1 has been shown to regulate the expression of LOX-1 through the ET-1 receptor in endothelial cells. To examine the involvement of the ET-1 receptor in the regulation of LOX-1 expression in MDMs, we transfected ET-1 receptor siRNA into MDMs. Following treatment with berberine and atorvastatin, the expression of LOX-1 mRNA was analyzed by qRT-PCR and western blot analysis. Transfection with siRNA significantly blocked the inhibitory effect of

berberine combined with atorvastatin on LOX-1 mRNA expression (Fig. 2A), which was confirmed by western blot analysis (Fig. 2B and C).

**Berberine combined with atorvastatin influences physiological parameters in model rats.** To explore the effect of berberine combined with atorvastatin on the physiological parameters of the model rats, the BW, heart weight (HW), liver weight (LW), spleen weight (SW), and kidney weight (KW) of rats in different groups were calculated at the end of the treatment. BW, LW and KW were markedly increased at concentrations of berberine  $\geq 1$   $\mu\text{mol/kg}$ , while HW and SW remained constant for all the groups. The BW gains were 7.6, 11.4 and 16.1%, the LW gains were 29.3, 43.9 and 48.8%, and the KW gains were 41.7, 62.5 and 66.7% in the 1, 10 and 100  $\mu\text{mol/kg}$  berberine groups compared to the control (0  $\mu\text{mol/kg}$  berberine group), respectively (Table II).

**Berberine combined with atorvastatin alters serum TC, TG, LDL-C and HDL-C levels in model rats.** To investigate variations in serum lipid profiles in model rats treated with berberine and atorvastatin, serum TC, TG, LDL-C and HDL-C levels were monitored via an automatic biochemistry analyzer at the end of the treatment. Compared to the control group (0  $\mu\text{mol/kg}$ ), treatment with berberine in combination with atorvastatin notably decreased serum TC, TG and LDL-C levels in rats (Fig. 3A-C). However, no significant difference

Table II. Body and organ weight of animals.

Groups	No.	BW (g)	HW (g)	LW (g)	SW (g)	KW (g)
0	20	392.5±22.8	2.3±0.7	4.1±0.7	0.9±0.2	2.4±0.6
0.1	20	401.3±27.5	2.5±0.5	4.6±0.6	1.1±0.3	2.9±0.7
1	20	422.7±28.7 <sup>a</sup>	2.4±0.5	5.3±0.8 <sup>a</sup>	1.2±0.3	3.4±0.8 <sup>a</sup>
10	20	437.6±29.4 <sup>a</sup>	2.6±0.6	5.9±0.6 <sup>a</sup>	1.1±0.2	3.9±0.8 <sup>a</sup>
100	20	455.9±29.7 <sup>a</sup>	2.5±0.8	6.1±0.9 <sup>a</sup>	1.3±0.4	4.0±1.1 <sup>a</sup>

BW, body weight; HW, heart weight; LW, liver weight; SW, spleen weight; KW, kidney weight. <sup>a</sup>P<0.05 vs. 0 μmol/kg berberine group denotes a significant difference.

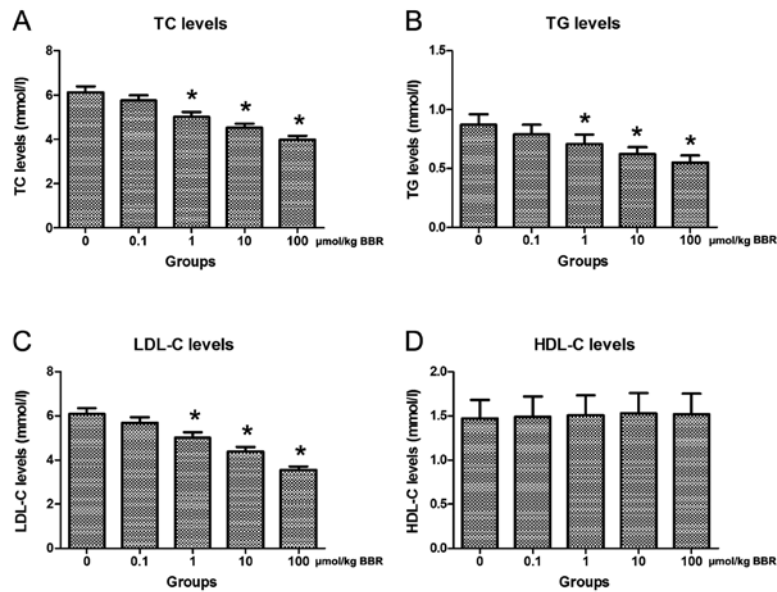


Figure 3. Determination of lipid profiles in animal sera. (A) Plasma total cholesterol (TC) levels; (B) plasma triglyceride (TG) levels; (C) low density lipoprotein-cholesterol (LDL-C) levels; (D) high-density lipoprotein-cholesterol (HDL-C) levels. BBR, berberine. \*P<0.05 vs. 0 μmol/kg berberine group denotes significant differences.

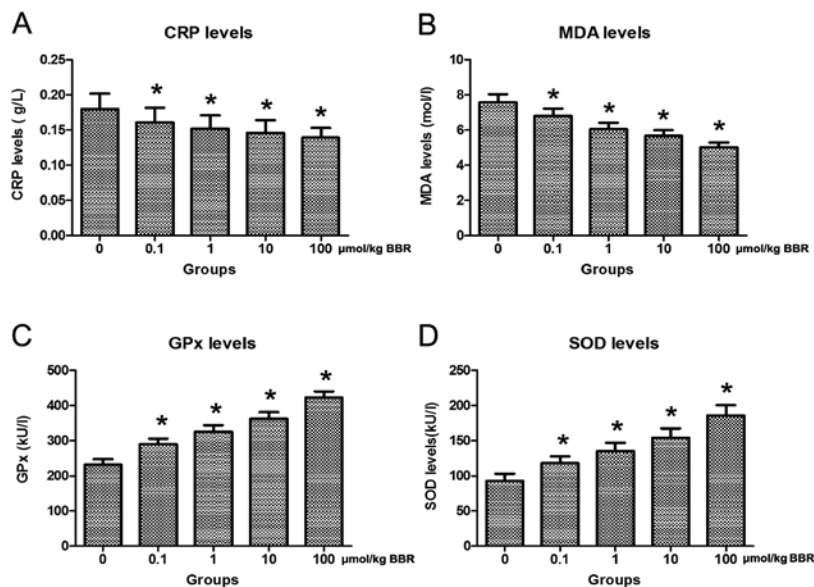


Figure 4. Determination of content of C-reactive protein (CRP) and malondialdehyde (MDA) as well as activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD). (A) Plasma CRP levels; (B) plasma MDA levels; (C) plasma GPx activity; (D) plasma SOD activity. BBR, berberine. \*P<0.05 vs. 0 μmol/kg berberine group denotes significant differences.

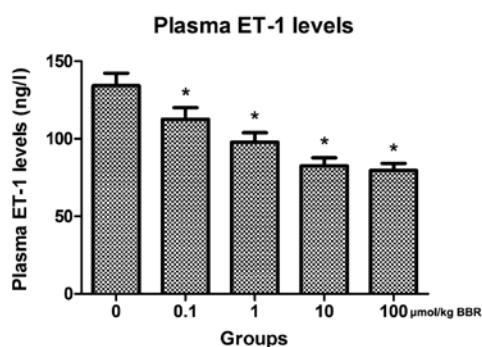


Figure 5. Determination of plasma endothelin-1 (ET-1) levels in animals. BBR, berberine. \* $P < 0.05$  vs. 0  $\mu\text{mol/kg}$  berberine group denotes significant differences.

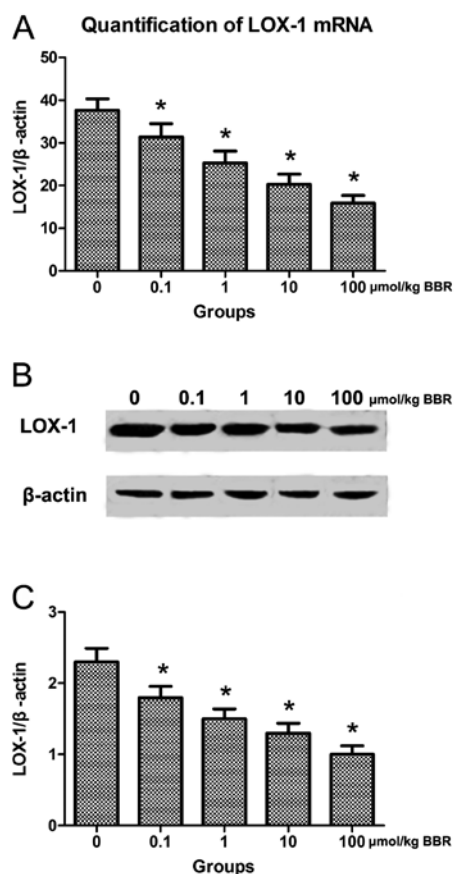


Figure 6. Analysis of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in rat monocytes. (A) Quantification of LOX-1 mRNA by quantitative polymerase chain reaction (qPCR). (B and C) Detection of LOX-1 protein by western blot analysis. BBR, berberine. \* $P < 0.05$  vs. 0  $\mu\text{mol/kg}$  berberine group denotes significant differences.

in the serum level of HDL-C was detected among rats in the different groups (Fig. 3D).

*Berberine combined with atorvastatin attenuates inflammation and oxidative stress in model rats.* To validate whether treatment with berberine in combination with atorvastatin affected inflammation and oxidative stress in model rats, serum CRP, MDA, GPx and SOD were measured using commercial kits. The results showed that treatment with berberine in combination with atorvastatin distinctly reduced serum CRP

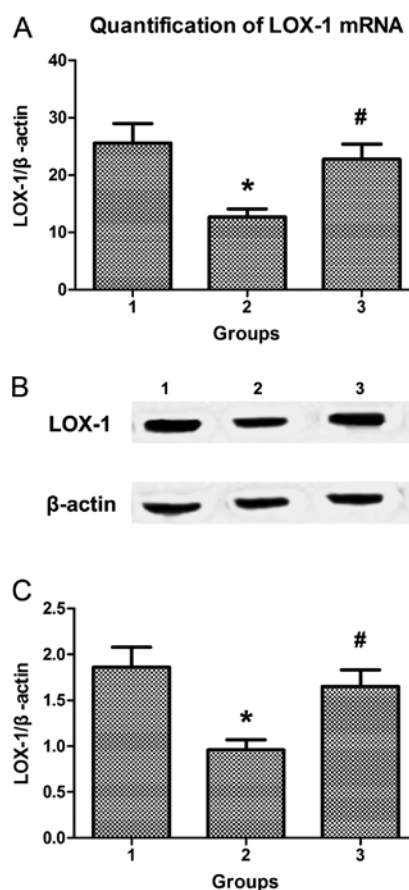


Figure 7. Analysis of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in animal monocytes. (A) Quantification of LOX-1 mRNA by quantitative polymerase chain reaction (qPCR). (B and C) Detection of LOX-1 protein by western blot analysis. Lanes: 1, vehicle group; 2, 100  $\mu\text{mol/kg}$  berberine group; 3, BQ-788 + 100  $\mu\text{mol/kg}$  berberine group. BBR, berberine. \* $P < 0.05$  vs. vehicle group denotes significant differences; # $p < 0.05$  vs. non-specific siRNA + 100 nM berberine-treated group denotes a significant difference.

and MDA levels and promoted serum GPx and SOD levels in the model rats (Fig. 4).

*Berberine combined with atorvastatin decreases plasma ET-1 level and the expression of LOX-1 in monocytes in model rats.* To explore variations in plasma ET-1 levels, ELISA was performed on rats in each group. Treatment provoked a marked decrease in the plasma ET-1 level compared to the control group (Fig. 5). Additionally, the expression of LOX-1 in monocytes was analyzed by qRT-PCR and western blot analysis. Compared to the control group, berberine in combination with atorvastatin significantly downregulated the expression of LOX-1 in monocytes (Fig. 6).

*An ET-1 receptor antagonist abolishes the inhibitory effect of berberine combined with atorvastatin on LOX-1 expression in monocytes from model rats.* To examine whether the ET-1 receptor was involved in the regulation of LOX-1 expression by berberine and atorvastatin, model rats were preconditioned with an ET-1 receptor antagonist prior to oral uptake of berberine and atorvastatin. Compared to the control, treatment with berberine in combination with atorvastatin led to the downregulation of LOX-1 expression in monocytes. By contrast, ET-1 receptor

antagonist preconditioning eliminated the inhibitory effect of berberine combined with atorvastatin and resulted in an increased expression of LOX-1 in monocytes (Fig. 7).

## Discussion

Numerous studies have shown that the oxidative modification of oxLDL is extremely relevant in atherogenesis (32,33). oxLDL can be vigorously absorbed by macrophages via receptor-mediated endocytosis, which promotes foam cell formation (34). These receptors may include SR-AI/II, SR-BI, CD36, and LOX-1. LOX-1 is responsible for binding, being internalized, and proteolytically degrading oxLDL but not acetylated LDL, and thus mediates foam cell formation in atherosclerotic plaques (35).

Mounting evidence has shown that LOX-1 expression may be induced by several proinflammatory and proatherogenic stimuli (36). Anti-inflammatory drugs have been identified that decrease LOX-1 expression and regress the progression of foam cell formation. Berberine, as a primary component of rhizoma coptidis, has been found to be involved in decreasing lipid deposition and inhibiting the formation of foam cells in the wall of the aorta (23). In this study, we demonstrated that berberine combined with atorvastatin treatment suppressed LOX-1 expression in MDMs in a dose-dependent manner, consistent with the results of Guan *et al* (23).

ET-1 is a peptide that plays an important role in the pathophysiology of cardiovascular disease by causing vascular damage (37). In human endothelial cells, LOX-1 mRNA and protein expression were induced by ET-1 (38). When the ET-1B receptor was blocked by an antagonist, the induction of LOX-1 mRNA by ET-1 was inhibited (38). Notably, in rat MDMs, we found the ET-1 receptor plays a crucial role in the regulation of LOX-1 expression. Transfection of specific siRNA targeting this receptor into MDMs blocked the reduction in LOX-1 expression induced by berberine. In a rat model, injection of berberine and atorvastatin resulted in a decrease in ET-1 plasma levels. Furthermore, reduction of LOX-1 expression in monocytes was also induced by treatment with berberine and atorvastatin. However, preconditioning with the ET-1 receptor antagonist markedly blocked the inhibition of LOX-1 expression caused by treatment with berberine and atorvastatin. These results indicated that berberine may reduce LOX-1 expression through ET-1 receptors both *in vitro* and *in vivo*.

Treatment with berberine in combination with atorvastatin also influenced physiological parameters in the rat model. The results showed that the gains in BW, LW and KW were significantly increased as the amount of berberine increased. The progression of atherosclerosis is intimately associated with variations in the lipid profile. In this study, the levels of TC, TG and LDL-C in the rat model were decreased following treatment with berberine and atorvastatin. Thus, berberine combined with atorvastatin may be an efficient therapeutic method to treat atherosclerosis.

Previous studies have demonstrated that HFD induces inflammation and oxidative stress in rat models (39,40). HFD is sufficient to trigger NADPH oxidase-related oxidative stress as well as an inflammatory response, represented by increased PGE2 levels (41), increased COX-1, and in particular COX-2 expression (42), and promote NF- $\kappa$ B activation. In this study,

berberine in combination with atorvastatin distinctly reduced CRP and MDA levels as well as elevating GPx and SOD levels in serum. Thus, berberine may play a major role in reducing the inflammation and oxidative stress induced by HFD.

In conclusion, our study has demonstrated that berberine in combination with atorvastatin effectively downregulated LOX-1 expression through the ET-1 receptor *in vitro* and *in vivo*. This study may provide new evidence towards identifying the mechanism of berberine in attenuating foam cell formation and atherosclerosis progression.

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