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•ORIGINAL ARTICLE•

Lovastatin Down Regulates the Expression of Lectin-like Oxidized Low Density Lipoprotein Receptor (LOX) 1 mRNA on Human Umbilical Vein Endothelial Cells

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MeSH Lovastatin; Endothelium, Vascular; Lipoprotein, LDL; Receptor, Lipoprotein

Aim To investigate whether statins alter the expression of lectin-like oxidized low density lipoprotein receptor (LOX) 1 mRNA and uptake of oxidized low density lipoprotein on cultured human umbilical vein endothelial cells (hUVEC).

Methods Total RNA was extracted with Trizol reagent. Reverse transcription polymerase chain reaction (RT-PCR) was used to quantify mRNA expression of LOX-1 in hUVEC. Ox-LDL was radioiodinated with ^{125}I to detect the uptake of ox-LDL by endothelial cells. A spectrophotometric enzyme assay was performed to determine the activity of LDH.

Results LOX-1 mRNA expression was significantly suppressed to 84%, 69% and 48% of control after hUVEC were incubated with 1, 10, 50 μmol/L of lovastatin for 48 h. LOX-1 mRNA expression was significantly suppressed to 82%, 69% and 52% of control in the cells treated with lovastatin after 24, 48 or 96 h respectively.

Conclusion Lovastatin reduces the expression of LOX-1 mRNA and uptake of ox-LDL without any toxic effects which may contribute to the antiatherosclerotic potential of lovastatin.

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1 INTRODUCTION

Vascular endothelial cell injury elicited by oxidized low density lipoprotein (ox-LDL) is considered to be a key trigger in the initiation and progression of atherosclerosis[1]. Endothelial cells internalize and degrade ox-LDL through a receptor-mediated pathway, which does not involve the classic macrophage scavenger receptors. An endothelium receptor for ox-LDL, LOX-1 (lectin-like ox-LDL receptor-1), was first identified as a critical molecule responsible for ox-LDL uptake by endothelial cells[2]. It is suggested that ox-LDL uptake via LOX-1 in vascular endothelium may cause endothelial activation and/or dysfunction, thus leading to the formation of atherosclerosis[3]. Several large-scale clinical trials of statins therapy have demonstrated that statins diminished cardiovascular events even in normocholesterolemic or hypocholesterolemic patients, and their effects were different from those observed in reduction of plasma cholesterol levels by other drugs[4].

It suggests that the substantial clinical benefit of statins administration may result not only from their lipid-lowering properties but also from their multiple effects on the components of the atherosclerotic lesions. Simvastatin, a HMG-coenzyme A reductase inhibitor, can improve endothelial function after 1 month of[5]. We postulated that statins may prevent atherosclerosis partly by attenuating the endothelial injury caused by ox-LDL. In this study, we examined whether lovastatin altered the expression of LOX-1 mRNA and uptake of ox-LDL in human endothelial cells.

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2 MATERIALS AND METHODS

2.1 Culture of endothelial cells

Human umbilical vein endothelial cells (hUVEC) were isolated from fresh umbilical veins by incubation with 0.25% trypsin and were cultured in M199 medium supplemented with 10% fetal calf serum (Gibco). Human UVEC were identified as endothelium in origin by their cobblestone-like morphology and anti-factor (Ⅷ) related antigen immunofluorescence. Cells at 2nd to 3rd passage were used to perform the following study.

2.2 Isolation, oxidization and labeling of low density lipoprotein

Low density lipoprotein (LDL) was isolated from fresh plasma donated by health volunteers by density gradient ultracentrifugation. LDL was oxidized by exposure to $CuSO_4$ (final concentration, 5 μmol/L) in PBS at 37 ℃ for 24 h. Oxidized LDL was radioiodinated with ^{125}I by the iodine

monochloride method as previously described. It was then purified and extensively dialyzed against Tris-HCl. Lipoprotein preparations were kept in 50 mmol/L Tris-HCl, 0.15 mol/L NaCl, and 2 mmol/L EDTA at pH 7.4. Protein concentration was determined by Lowry's method. The thiobarbituric acid reactive substances content was used to assess the extent of LDL oxidization. MDA of ox-LDL was 182 ± 2.8 versus 5.6 ± 1.6 μmol/g protein in the native LDL preparation.

2.3 Reverse transcription polymerase chain reaction for human LOX-1 mRNA

After incubation of hUVEC with or without lovastatin (1, 10, 50 μmol/L) for 24, 48 or 96 h, total RNA was extracted by TRIzol (Gibco). 1 μg of total RNA from each sample was reverse transcribed with oligo (dT18) and moloney murine leukemia virus reverse transcriptase (Promega) at 42 ℃ for 1 h. Reverse-transcribed products (2 μL) were amplified with Taq DNA polymerase (Promega) using a primer pair specific to human LOX-1 (sense primer, 5'-TTACTCTCCATGGTGGTGCC-3'; antisense primer, 5'-AGCTTCTTCTGCTTGTTGCC-3'). The expected PCR product was 193 bp. Perkin-Elmer Cetus thermocycler was set to 94 ℃ 40 s for denaturation, 55 ℃ 1 min for annealing and 72 ℃ 1 min for extension for 35 cycles, followed by an extension step at 72 ℃ for 10 min. Human β-actin was used (sense primer, 5'-TCGAATTCTGGAGAAGAGCTATGAGCTGCCG-3'; antisense primer, 5'-TCGGATCCGTGCCACCAGA CAGCACTGTGTTG-3'). The expected PCR product was 201 bp. PCR consisted of 95 ℃ 1 min for denaturation, 50 ℃ 1 min for annealing, and 72 ℃ 1 min for extension for 35 cycles, followed by an extension step at 72 ℃ for 10 min[6]. The RT-PCR-amplified samples were visualized on 1.5% agarose gel stained with ethidium bromide. Relative intensities of bands of interest were analyzed by the GDS-7600 gel documentation system (UVP) and were expressed as ratios of LOX-1 to the β-actin band.

2.4 Uptake of ^{125}I-ox-LDL by hUVEC

Human UVEC were incubated witHuman ox-LDL in the presence or absence of lovastatin (1, 10 or 50 μmol/L) for 48 h. Cells were prechilled for 30 min in HEPES buffer, pH 7.4. ^{125}I-ox-LDL was added to each dish in a final concentration of 10 mg/L. Incubation was carried out at 4 ℃ for 2 h. Cells were washed 3 times on ice with 150 nmol/L NaCl, 50 mmol/L Tris, and 2 mmol/L EDTA, pH 7.4, containing 2 g/L BSA. Cells were then rinsed with cold PBS and lysed at room temperature in 0.5 mol/L NaOH solution. An aliquot of the cell lysate was counted to determine the amount of bound ^{125}I-ox-LDL[6].

2.5 Measurement of lactate dehydrogenase

In order to rule out toxic effects of lovastatin on cultured endothelial cells, lactate dehydrogenase (LDH) activity was measured as described[7]. Cells were lysed with 2% Triton X-100 after collected by centrifugation. 2 mL of 150 mmol/L Tris buffer (pH 8.9) containing 50 mmol/L lactate and 7 mmol/L NAD was added to 0.1 mL of lysed cells or culture supernatant prior to incubation for 30 min at 37 ℃. The absorbance of each sample at 340 nm was measured and the activity of LDH was expressed as units per milliliter. The percent of LDH activity was expressed as follow. Release of LDH(%) = LDH activity in supernatant ÷ total LDH activity in supernatant and cells

2.6 Data Analysis

All data are presented as $\bar{x} \pm s$ of duplicate samples from at least 3 independently performed experiments. Differences were taken as statistically significant at $P < 0.05$.

3 RESULTS

3.1 Effect of lovastatin on the expression of LOX-1 mRNA

As expected, the size of reverse transcription-polymerase chain reaction (RT-PCR) product for LOX-1 is 193 bp and that for β-actin is 201 bp. To study the pharmacological effects of lovastatin on LOX-1 expression, hUVEC were incubated with 1, 10, and 50 μmol/L lovastatin for 48 h. As show in Figure 1, lovastatin decreased the LOX-1 mRNA expression in a dose-dependent manner. LOX-1 mRNA expression was significantly suppressed to 84%, 69%, and 48% of control after hUVEC were incubated with different concentrations of lovastatin for 48 h. To study the the time-course effect of lovastatin on LOX-1mRNA expression, 10 μmol/L lovastatin were incubated with hUVEC for 24, 48 or 96 h. As show in Figure 2, lovastatin decreased the LOX-1 mRNA expression in a time-dependent manner. LOX-1 mRNA expression was significantly suppressed to 82%, 69% and 52% of control in lovastatin-treated cells after 24, 48 or 96 h respectively.

3.2 Effect of lovastatin on the uptake of ox-LDL

LOX-1 was first identified as a critical molecule involved in the ox-LDL uptake by endothelial cells. Endothelial cells can internalize and degrade ox-LDL via LOX-1. As show in Figure 3. Lovastatin reduced the uptake of ox-LDL by hUVEC in a dose-dependent manner. Compared with controls, uptake of ox-LDL was significantly suppressed to 62%, 74% and 57% of control after lovastatin treatment for 24, 48 or 96 h respectively.

3.3 Effect of lovastatin on cell LDH activity

There is no significant increase in LDH release after

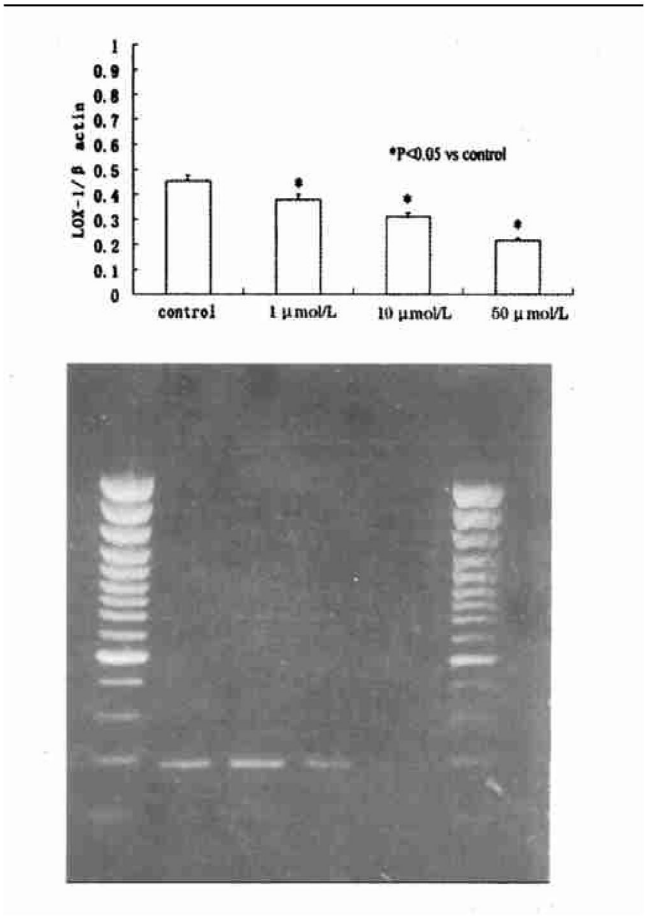

Figure 1. Effects of lovastatin on LOX-1 mRNA expression at different concentrations. Histogram of LOX-1 mRNA expressing valve is the above. Electrophoretogram of specific LOX-1 mRNA separated on EB-stained agarose gel is the below. Lane 1 and 6 are Marker. Lane 2, 3, 4, and 5 are 0, 1, 10, and 50 μmol/L of lovastatins.

Table 1. Percent of released LDH in response to 50 μmol/L of lovastatin

Groups	n	Release rate
Control	4	26.8% ±3.4%
24 h	4	27.2% ±3.9% [a]
48 h	4	27.6% ±3.6% [a]
96 h	4	27.8% ±4.2% [a]

a: P> 0.05, compared with control group

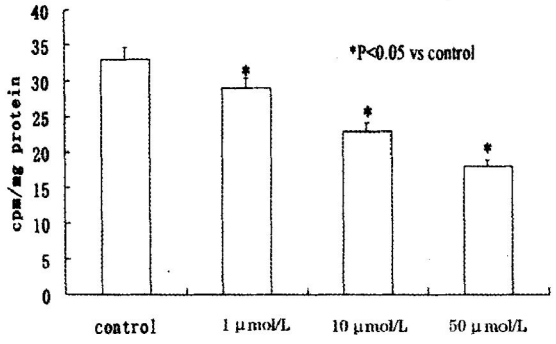

Figure 3. Lovastatin decreases ^{125}I ox-LDL uptake by hUVEC.

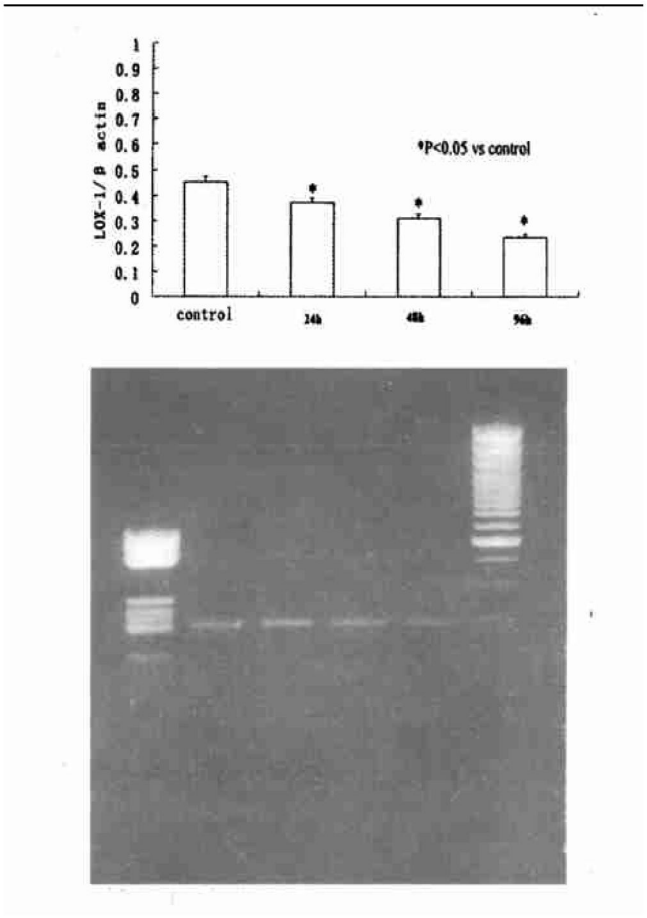

Figure 2. Time-course effects of 10 μmol/L of lovastatin on LOX-1 mRNA expression. Histogram of LOX-1 mRNA expressing valve is the above. Electrophoretogram of specific LOX-1 mRNA separated on EB-stained agarose gel is the below. Lane 1 and 6 are Marker. Lane 2, 3, 4, and 5 are 0, 24 h, 48 h, and 96 h of lovastatins treatment.

hUVEC incubated with 50 μmol/L of lovastatin for up to 96 h, rendering a toxic effect of lovastatin on cultured hUVEC very unlikely (Table 1).

4 DISCUSSION

Endothelial activation and/or dysfunction caused by ox-LDL is strongly linked to the formation of atherosclerosis. Oxidized LDL uptake is at least partly through LOX-1 in vascular endothelial cells. LOX-1 was found to be expressed in the endothelial cells of animal models such as WHHL rabbits[8] and spontaneously hypertensive rats[9].

Moreover, it is also expressed in human atherosclerotic lesions[10]. The expression of endothelial LOX-1 can be induced by shear stress[11] and tumor necrosis factor-α (TNF-α)[12] an inflammatory cytokine present in atherosclerotic arterial wall. It was also reported that ox-LDL could induce the apoptosis of endothelial cells through LOX-1[13]. All these data suggest that LOX-1 may be involved in atherosclerosis. Our study shows that lovstatin

has the ability to reduce the expression of LOX-1 mRNA and down regulate the uptake of proatherogenic ox-LDL in endothelial cells. ox-LDL and its lipid constituents impair endothelial production of nitric oxide[14] and induce the endothelial expression of leukocyte adhesion molecules[15] and smooth muscle growth factors[16], which are involved in atherosclerosis. Given that, the effects of statins on reduction of the expression of LOX-1 could protect endothelium from injury by ox-LDL. Our finding suggests a new mechanism of stains might inhibiting the initiation and progression of atherosclerosis. Although pathophysiological consequences of ox-LDL uptake by vascular endothelial cells through LOX-1 still need to be fully clarified, statins regulation of LOX-1 may contribute to statin's well-documented protection effect against cardiovascular diseases.

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洛伐他汀能下调人脐静脉内皮细胞血凝素样氧化型低密度脂蛋白受体 mRNA 的表达

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[主题词] 洛伐他汀; 内皮, 血管; 脂蛋白, 低密度; 受体, 脂蛋白, 低密度

[摘 要] **目的** 探讨洛伐他汀对人脐静脉内皮细胞表达血凝素样氧化型低密度脂蛋白受体 mRNA 和摄取氧化型低密度脂蛋白的影响。**方法** 用 TRIzol 试剂常规方法提取人脐静脉内皮细胞总 RNA, 逆转录聚合酶链反应检测血凝素样氧化型低密度脂蛋白受体 mRNA 含量, 检测^{125} I 标记的氧化型低密度脂蛋白放射活性法来确定内皮细胞摄取率, 分光光度法测量乳酸脱氢酶含量。**结果** 人脐静脉内皮细胞与 1、10 和 50 μmol/L 洛伐他汀孵育 48 h 后, 血凝素样氧化型低密度脂蛋白受体 mRNA 的表达分别为对照组的 84%、69% 和 48%; 与 10 μmol/L 洛伐他汀孵育 24、48 和 96 h 后, 血凝素样氧化型低密度脂蛋白受体 mRNA 的表达分别为对照组的 82%、69% 和 52%。洛伐他汀使人脐静脉内皮细胞对氧化型低密度脂蛋白的摄取减少, 对乳酸脱氢酶含量没有影响。**结论** 洛伐他汀能降低血凝素样氧化型低密度脂蛋白 mRNA 的表达和对氧化型低密度脂蛋白的摄取。

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