Inhibitory Effects of Simvastatin on Oxidized Low-Density Lipoprotein-Induced Endoplasmic Reticulum Stress and Apoptosis in Vascular Endothelial Cells

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Abstract

Background: Oxidized low-density lipoprotein (ox-LDL)-induced oxidative stress and endothelial apoptosis are essential for atherosclerosis. Our previous study has shown that ox-LDL-induced apoptosis is mediated by the protein kinase RNA-like endoplasmic reticulum kinase (PERK)/eukaryotic translation initiation factor 2α-subunit (eIF2α)/CCAAT/enhancer-binding protein homologous protein (CHOP) endoplasmic reticulum (ER) stress pathway in endothelial cells. Statins are cholesterol-lowering drugs that exert pleiotropic effects including suppression of oxidative stress. This study aimed to explore the roles of simvastatin on ox-LDL-induced ER stress and apoptosis in endothelial cells.

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with simvastatin (0.1, 0.5, or 2.5 μmol/L) or DEVD-CHO (selective inhibitor of caspase-3, 100 μmol/L) for 1 h before the addition of ox-LDL (100 μg/ml) and then incubated for 24 h, and untreated cells were used as a control group. Apoptosis, expression of PERK, phosphorylation of eIF2α, CHOP mRNA level, and caspase-3 activity were measured. Comparisons among multiple groups were performed with one-way analysis of variance (ANOVA) followed by *post hoc* pairwise comparisons using Tukey's tests. A value of $P < 0.05$ was considered statistically significant.

Results: Exposure of HUVECs to ox-LDL resulted in a significant increase in apoptosis (31.9% vs. 4.9%, $P < 0.05$). Simvastatin (0.1, 0.5, and 2.5 μmol/L) led to a suppression of ox-LDL-induced apoptosis (28.0%, 24.7%, and 13.8%, $F = 15.039$, all $P < 0.05$, compared with control group). Ox-LDL significantly increased the expression of PERK (499.5%, $P < 0.05$) and phosphorylation of eIF2α (451.6%, $P < 0.05$), if both of which in the control groups were considered as 100%. Simvastatin treatment (0.1, 0.5, and 2.5 μmol/L) blunted ox-LDL-induced expression of PERK (407.8%, 339.1%, and 187.5%, $F = 10.121$, all $P < 0.05$, compared with control group) and phosphorylation of eIF2α (407.8%, 339.1%, 187.5%, $F = 11.430$, all $P < 0.05$, compared with control group). In contrast, DEVD-CHO treatment had no significant effect on ox-LDL-induced expression of PERK (486.4%) and phosphorylation of eIF2α (418.8%). Exposure of HUVECs to ox-LDL also markedly induced caspase-3 activity together with increased CHOP mRNA level; these effects were inhibited by simvastatin treatment.

Conclusions: This study suggested that simvastatin could inhibit ox-LDL-induced ER stress and apoptosis in vascular endothelial cells.

Key words: Apoptosis; Endoplasmic Reticulum Stress; Endothelial Cells; Oxidized Low-Density Lipoprotein; Simvastatin

INTRODUCTION

Atherosclerosis is the most common pathological cause of cardiovascular diseases.[1] Vascular endothelial cell apoptosis has been reported to contribute to the development of atherosclerosis.[2] Endothelial cells form the lining of blood vessels and regulate the vascular integrity and homeostasis, while increased apoptosis of endothelial cells results in a disruption of the endothelium barrier and creating leaks that

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destroy the vascular wall integrity.[1] Apoptotic endothelial cells are prothrombotic and pro-proliferative and lead to the ensuing atherogenic processes.[3]

Elevation of low-density lipoprotein (LDL) cholesterol levels is a major risk factor for the pathogenesis of atherosclerosis.[4] The production of reactive oxygen species in vascular endothelial cells causes oxidation of LDL and results in increased levels of oxidized LDL (ox-LDL), a key mediator of the initiation and progression of atherosclerosis.[5-9] Ox-LDL induces oxidative stress and cell injury in endothelial cells, which in turn facilitates the progression of atherosclerosis.[1]

The endoplasmic reticulum (ER) is the primary intracellular site for folding and assembly of membrane-associated and secreted proteins. Physiological and pathological perturbations may interfere with protein folding processes in the ER and lead to accumulation of unfolded or misfolded proteins, a cellular condition termed ER stress.[8,9] ER stress triggers the unfolded protein response (UPR), a transcriptional induction pathway aimed at restoring normal ER functioning.[10] If UPR is insufficient to recover ER homeostasis, cells undergo apoptosis.[11-13] The UPR is mediated by three ER stress receptors: protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1), and activating transcription factor-6 (ATF6) that representing three branches of the UPR.[14] PERK is an ER transmembrane protein kinase that inhibits protein translation through inactivation of eukaryotic translation initiation factor 2α-subunit (eIF2α), which results in upregulation of CCAAT/enhancer-binding protein homologous protein (CHOP), a critical factor for triggering apoptosis in response to ER stress.[8,15,16] Our previous study has shown that ox-LDL induces apoptosis in vascular endothelial cells largely through the PERK/eIF2α/CHOP ER stress pathway.[8]

Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in the multistep mevalonate cascade for cholesterol synthesis.[17] Statins are widely used in the prevention of cardiovascular disease by lowering cholesterol.[17] Beyond their cholesterol reduction effect, statins have been shown to have pleiotropic effects.[15-17] Accumulating evidences indicated that the inhibition of the mevalonate pathway by statins induced ER stress and apoptosis in cancerous and noncancerous cells.[17-20] In this study, we explored the beneficial effect of simvastatin on ox-LDL-induced ER stress and apoptosis in human vascular endothelial cells.

METHODS

Ethical approval

The study was reviewed and approved by the Medical Research Ethics Committee of the China-Japan Friendship Hospital.

Human umbilical vein endothelial cell culture

Primary human umbilical vein endothelial cells (HUVECs; ATCC PCS-100-010; American Type Culture Collection;

Manassas, VA, USA) were grown in an incubator with vascular cell basal medium (ATCC PCS-100-030) and endothelial cell growth kit-BBE (ATCC PCS-1-040) and 100 U/ml penicillin-streptomycin (Sigma-Aldrich, USA) under humidified atmosphere of 95% air and 5% CO_2 at 37°C.[8]

Preparation of oxidized low-density lipoprotein

Lipoproteins were isolated from plasma obtained from healthy volunteers with their informed consent.[21] Isolated LDL was desalted on an Econo-Pac 10 DG chromatography column (Bio-Rad, Hercules, CA, USA) and sterile filtered (0.22 μm pore size; Millipore, Bedford, MA, USA). The lipoprotein (0.5 mg/ml in sterile phosphate buffer saline) was incubated with 5 μmol/L $CuSO_4$ at 37°C for 20 h to oxidize LDL. The ox-LDL was concentrated for 2 h at 3000 ×g and 8°C by centrifuging in Amicon Centriplus YM-100 tubes (Millipore, Bedford, MA, USA). The oxidation was confirmed by measuring thiobarbituric acid-reactive substances using tetraethoxypropane as a standard.[22] HUVECs were treated with simvastatin (0.1, 0.5, or 2.5 μmol/L; Sigma-Aldrich) or selective caspase-3 inhibitor DEVD-CHO (100 μmol/L; Sigma-Aldrich) for 1 h before the addition of ox-LDL (100 μg/ml) and then incubated for 24 h.[8]

Western blot analysis

HUVECs were homogenized with lysis buffer containing 2% Nonidet P and a protease inhibitor cocktail (Sigma-Aldrich) by sonication 30 s on ice. The supernatant obtained after centrifugation at 2000 ×g for 15 min at 4° was used for protein concentration determination by the Coomassie Blue method. An equal amount of proteins were blotted onto a polyvinylidene difluoride microporous membrane (Millipore). Membranes were incubated for 1 h with rabbit antihuman PERK polyclonal antibody (H-300; sc-13073; 1:1000 dilution), goat antihuman eIF2α polyclonal antibody (K-17; sc-30882; 1:100 dilution), rabbit antihuman phosphorylated eIF2α polyclonal antibody (sc-101670; dilution 1:1000), or mouse antihuman β-actin monoclonal antibody (ACTBD11B7; sc-81178; 1:1000 dilution) and then washed and incubated for 1 h with 1:5000 dilution of secondary antibodies including bovine anti-rabbit IgG-horseradish peroxidase (HRP; sc-2370), bovine antigoat IgG-HRP (sc-230), or bovine antimouse IgG-HRP (sc-2371). Peroxidase was revealed with an enhanced chemiluminescent kit (GE Healthcare, USA). Three independent experiments were performed.[8]

Real-time quantitative reverse transcription polymerase chain reaction

TRIzol reagent was used to prepare RNA, and SuperScript II reverse transcriptase (Life Technologies; Carlsbad, CA, USA) was used to synthesize cDNA. Real-time quantitative polymerase chain reaction was performed on an Abi-Prism 7700 Sequence Detection System (Thermo Fisher Scientific, USA), with the use of the fluorescent dye SYBR Green Master Mix (Applied Biosystems, Beijing, China) according to the manufacturer's protocol. The primers used are as follows: for CHOP, 5'-GCCTTTCTCCTTTGGGACACTGTCCAGC-3'

(forward) and 5'-CTCGGCGAGTCGCCTCTACTTCCC-3' (reverse); for GAPDH, 5'-CCAGCAAGAGCACA AGAGGAA-3' (forward) and 5'-ATGGTACATGACAA GGTGCGG-3' (reverse). Relative quantification of the mRNA level of Bmi1 was determined using the $2^{-\Delta\Delta Ct}$ method.[23] Each experiment was repeated for three times in duplicates.[8]

Cell apoptosis assay

HUVECs were pretreated with simvastatin (0.1, 0.5, or 2.5 μmol/L) or DEVD-CHO (100 μmol/L) for 1 h before the addition of ox-LDL (100 μg/ml) and then incubated for 24 h. Cell apoptosis was measured at 24 h with a microplate reader-based TiterTACS *in situ* apoptosis detection kit (4822-96-K; R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions.[24] Each experiment was repeated for three times in duplicates.[8]

Caspase-3 activity assay

The caspase-3 activity was determined with the colorimetric CaspACE Assay System (G7351) purchased from Promega (Madison, WI, USA).[25] Briefly, HUVECs were pretreated with simvastatin (0.1, 0.5, or 2.5 μmol/L) or DEVD-CHO (100 μmol/L) for 1 h before the addition of ox-LDL (100 μg/ml) and then incubated for 24 h. Supernatants of cell extracts were inoculated into microtiter wells containing caspase assay buffer, dimethyl sulfoxide, 100 mmol/L dithiothreitol, and colorimetric caspase-3 substrate labeled with the chromophore, *p*-nitroaniline (Ac-DEVD-pNA). The plates were incubated at 37°C for 3 h and absorbance was read at 405 nm with a microtiter plate spectrophotometer.[8]

Statistical analysis

All continuous variable values were expressed as mean ± standard deviation (SD). Statistical analyses were performed with SPSS for Windows version 19.0 (IBM Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was administered to compare the means of multiple groups. A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Simvastatin inhibited oxidized low-density lipoprotein-induced apoptosis and reduced expression of protein kinase RNA-like endoplasmic reticulum kinase and phosphorylation of eukaryotic translation initiation factor 2α-subunit in human umbilical vein endothelial cells

Exposure of HUVECs to ox-LDL (100 μg/ml) for 24 h resulted in a significant increase in apoptosis (31.9%), whereas the apoptosis rate of control group was 4.9%. Treatment of HUVECs with simvastatin (0.1, 0.5, and 2.5 μmol/L) led to a suppression of ox-LDL-induced apoptosis (28.0%, 24.7%, and 13.8%, $F = 15.039$, all $P < 0.05$). Moreover, treatment of HUVECs with selective caspase-3 inhibitor DEVD-CHO (100 μmol/L) also significantly inhibited ox-LDL-induced apoptosis [11.9%, $P < 0.05$; Figure 1].

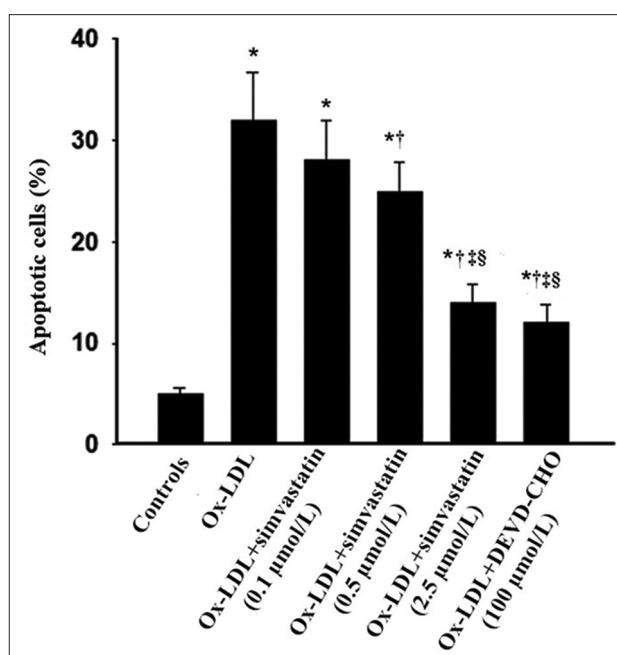Next, we examined the effect of simvastatin and ox-LDL on the expression of PERK in HUVECs. Exposure of HUVECs

Figure 1: Simvastatin inhibited ox-LDL-induced apoptosis in human umbilical vein endothelial cells. Apoptosis rates were expressed as percentages of total cells. Untreated cells were used as a control. *$P < 0.05$, versus control; †$P < 0.05$, versus ox-LDL; ‡$P < 0.05$, versus ox-LDL + simvastatin (0.1 μmol/L); §$P < 0.05$ versus ox-LDL + simvastatin (0.5 μmol/L). Ox-LDL: Oxidized low-density lipoprotein.

to Ox-LDL (100 μg/ml) significantly increased the expression of PERK (499.5%); the expression of PERK of control group was 100%. Simvastatin (0.1, 0.5, or 2.5 μmol/L) treatment blunted ox-LDL-induced expression of PERK (407.8%, 339.1%, and 187.5%, $F = 10.121$, all $P < 0.05$). In contrast, DEVD-CHO (100 μmol/L) treatment had no significant effect on ox-LDL-induced expression of PERK [486.4%, $F = 10.121$, $P > 0.05$; Figure 2].

We also examined the effect of simvastatin and ox-LDL on the phosphorylation of eIF2α in HUVECs. Exposure of HUVECs to Ox-LDL (100 μg/ml) significantly increased the phosphorylation of eIF2α (451.6%), while the phosphorylation of eIF2α of control group was considered as 100%. Simvastatin (0.1, 0.5, and 2.5 μmol/L) treatment blunted ox-LDL-induced phosphorylation of eIF2α (407.8%, 339.1%, and 187.5%; $F = 11.430$; $P < 0.05$). In contrast, DEVD-CHO (100 μmol/L) treatment had no significant effect on ox-LDL-induced phosphorylation of eIF2α [418.8%, $F = 11.430$, $P < 0.05$; Figure 3].

Simvastatin reduced oxidized low-density lipoprotein-induced CHOP mRNA level and caspase-3 activity in human umbilical vein endothelial cells

Therefore, we examined the effect of ox-LDL and simvastatin on the CHOP mRNA level in HUVECs. As shown in Figure 4, exposure of HUVECs to ox-LDL (100 μg/ml) resulted in approximately 7-fold changes in the mRNA level of CHOP as compared with that in the control ($F = 10.825$, $P < 0.05$). Simvastatin treatment attenuated ox-LDL-induced
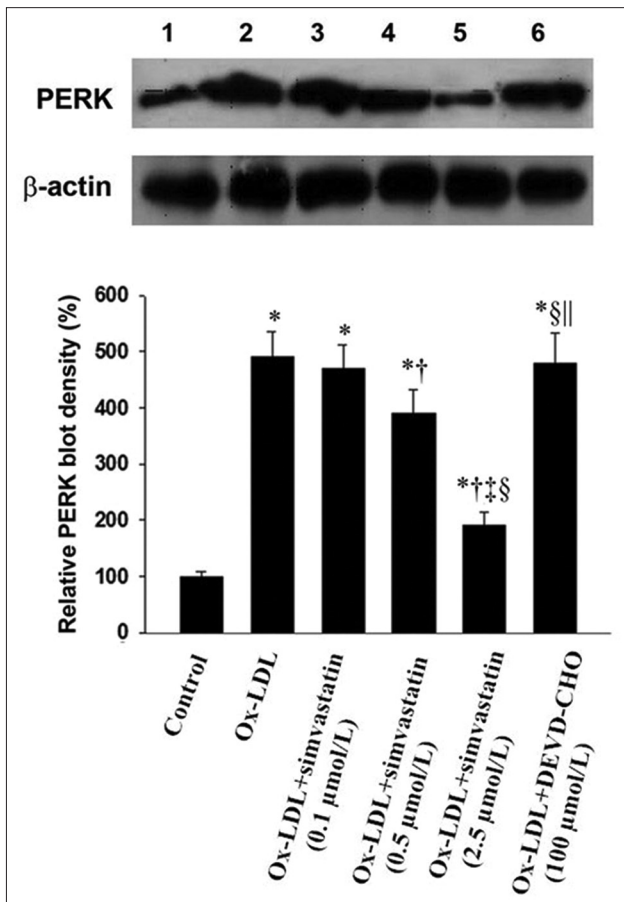
Figure 2: Simvastatin inhibited ox-LDL-induced expression of PERK in human umbilical vein endothelial cells. The β-actin blotting was used as a loading control. *$P < 0.05$, versus control; †$P < 0.05$, versus ox-LDL; ‡$P < 0.05$, versus ox-LDL + simvastatin (0.1 μmol/L); §$P < 0.05$ versus ox-LDL + simvastatin (0.5 μmol/L); ‖$P < 0.05$, versus ox-LDL + simvastatin (2.5 μmol/L). Ox-LDL: Oxidized low density lipoprotein.

Figure 3: Simvastatin inhibited ox-LDL-induced phosphorylation of eIF2α in human umbilical vein endothelial cells. *$P < 0.05$, versus control; †$P < 0.05$, versus ox-LDL; ‡$P < 0.05$, versus ox-LDL + simvastatin (0.1 μmol/L); §$P < 0.05$ versus ox-LDL + simvastatin (0.5 μmol/L); ‖$P < 0.05$, versus ox-LDL + simvastatin (2.5 μmol/L). Ox-LDL: Oxidized low density lipoprotein; eIF2α: Eukaryotic translation initiation factor 2α-subunit.

elevation of CHOP mRNA level ($F = 10.825$, $P < 0.05$), whereas DEVD-CHO (100 μmol/L) treatment had little effect [$F = 10.825$, $P > 0.05$; Figure 4].

As shown in Figure 5, ox-LDL treatment (100 μg/ml) increased the caspase-3 activity in HUVECs by approximately 3-fold compared with that in the control ($F = 18.136$, $P < 0.05$). Treatment with simvastatin inhibited ox-LDL-induced activation of caspase-3 ($F = 18.136$, $P < 0.05$). DEVD-CHO treatment (100 μmol/L) also significantly inhibited ox-LDL-induced caspase-3 activation in HUVECs [$F = 18.136$, $P < 0.05$; Figure 5].

DISCUSSION

Our present study suggested that statins could inhibit ox-LDL-induced PERK/eIF2α/CHOP ER stress signaling in vascular endothelial cells; this effect led to inhibition of ox-LDL-induced vascular endothelial apoptosis and caspase-3 activation.[8,26-28]

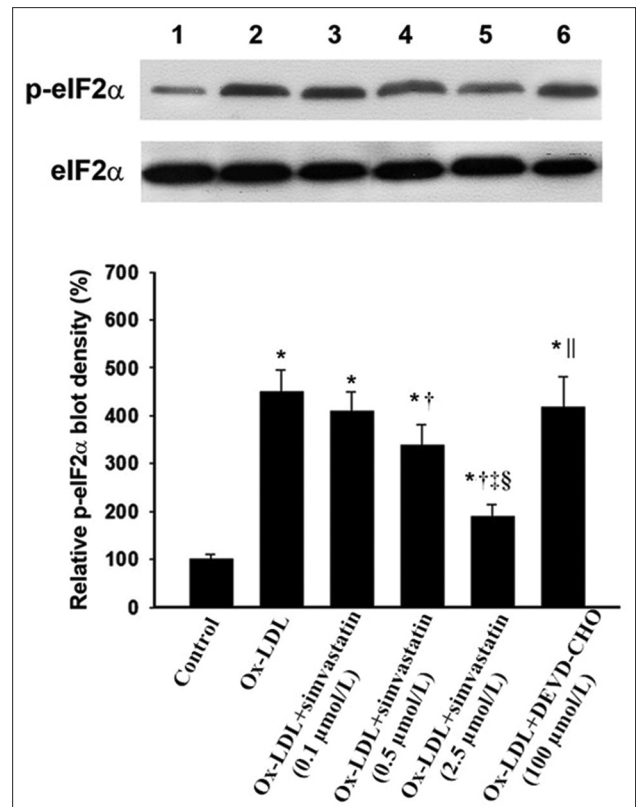Both oxidative stress and endothelial cell dysfunction contribute to the development of atherosclerosis.[29] Ox-LDL is the key factor to induce oxidative stress and endothelial apoptosis in EC, which is essential for the initiation and progression of atherosclerosis.[6,7] Our previous study has shown that ox-LDL induced apoptosis in vascular endothelial cells largely through the PERK/eIF2α/CHOP ER stress pathway.[8] Statins are effective cholesterol-lowering drugs that exert pleiotropic effects including inhibitory effects on inflammation, oxidative stress, and tissue repair.[30] In the present study, we provided the evidence that statins could inhibit ox-LDL-induced ER stress and apoptosis in vascular endothelial cells. Our previous study showed that ox-LDL treatment at 100 μg/ml for 24 h effectively induced ER stress and apoptosis in HUVECs, similar to the results of other studies.[8,31,32]

The ER responds to ER stress by activating UPR. If UPR is insufficient to recover ER homeostasis, cells undergo apoptosis.[8,9] The PERK branch of the UPR is strongly protective at modest levels of signaling but can contribute signals to cell death pathways if under irreversible ER stress, for its downstream effector CHOP has a proapoptotic activity and is critical for triggering apoptosis in response to ER stress.[8,9,26] Among various ER responses, eIF2α phosphorylation primarily protects cells from stress by
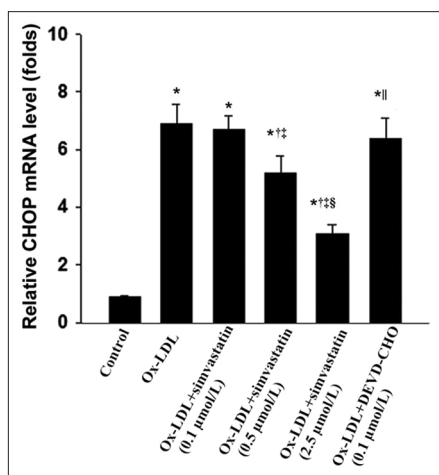
Figure 4: Simvastatin inhibited ox-LDL-induced expression of CHOP mRNA in human umbilical vein endothelial cells. The mRNA level of CHOP was measured by real-time reverse transcription polymerase chain reaction in HUVECs and was expressed as fold changes to that of untreated controls cells (designated as 1). $*P < 0.05$, versus control; $^{\dagger}P < 0.05$, versus ox-LDL; $^{\ddagger}P < 0.05$, versus ox-LDL + simvastatin (0.1 µmol/L); $^{\S}P < 0.05$ versus ox-LDL + simvastatin (0.5 µmol/L); $^{\parallel}P < 0.05$, versus ox-LDL + simvastatin (2.5 µmol/L). Ox-LDL: Oxidized low-density lipoprotein; HUVECs: Human umbilical vein endothelial cells.

Figure 5: Simvastatin inhibited ox-LDL-induced caspase-3 activity in human umbilical vein endothelial cells. The caspase-3 activity was measured with the colorimetric CaspACE Assay System (Promega) in HUVECs and expressed as OD at 405 nm. $*P < 0.05$, versus control; $^{\dagger}P < 0.05$, versus ox-LDL; $^{\ddagger}P < 0.05$, versus ox-LDL + simvastatin (0.1 µmol/L); $^{\S}P < 0.05$ versus. ox-LDL + simvastatin (0.5 µmol/L); $^{\parallel}P < 0.05$, versus ox-LDL + simvastatin (2.5 µmol/L). Ox-LDL: Oxidized low density lipoprotein; HUVECs: Human umbilical vein endothelial cells; OD: Optical density.

attenuating global translation and specifically upregulating chaperone proteins; however, it can lead to apoptosis if under prolonged and severe ER stress.[33] In our study, ox-LDL treatment resulted in activation of PERK/eIF2α/CHOP signaling as well as significant apoptosis in HUVECs, suggesting that ox-LDL was an effective inducer of irreversible ER stress in endothelial cells.[8] The severe ER stress induced by ox-LDL was reflected in induced expression of PERK, increased phosphorylation of eIF2α, elevated CHOP mRNA level, and upregulated caspase-3 activity, which culminated in triggering cell apoptosis. Simvastatin treatment inhibited each step in this process, starting from inhibiting ox-LDL-induced PERK expression. This provided a mechanistic explanation for the protective effect of simvastatin on ox-LDL-induced ER stress and endothelial apoptosis observed in this study. In addition, our results showed that simvastatin at 0.5 and 2.5 µmol/L significantly inhibited ox-LDL-induced PERK/eIF2α/CHOP signaling and apoptosis in HUVECs, suggesting that statin was a potent inhibitor of ox-LDL-induced ER stress and apoptosis in vascular endothelial cells.

Statins inhibit HMG-CoA reductase, which converts HMG-CoA to mevalonate.[34,35] In cancerous cells and noncancerous cells, statins have been reported to inhibition of the mevalonate pathway and induction of ER stress.[17-20,36] Inconsistent with these studies, our study has demonstrated that simvastatin treatment significantly inhibited ER stress in the ox-LDL-treated EC. The seemingly discrepancy might be due to the presence of chronic ox-LDL treatment and the endothelial cell stress. In addition, it was possible that the chronic ox-LDL treatment might have altered the signaling
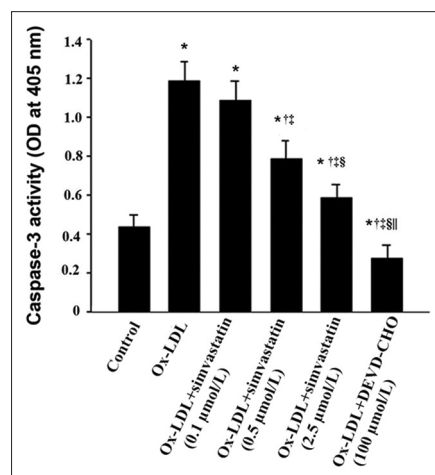
downstream of simvastatin. Further studies are warranted to uncover the underlying mechanisms of actions.

The UPR is mediated by three ER stress receptors: PERK, IRE1, and ATF6, representing three branches of the UPR.[14] In this study, we only explored the effect of simvastatin on ox-LDL-induced PERK/eIF2α/CHOP signaling. The ATF6 and the IRE1 branches of UPR were not examined. In future studies, we will explore whether simvastatin and ox-LDL regulate the ATF6 and/or the IRE1 ER stress pathways. In addition, it will be important to verify the effects of other clinically applied statins besides simvastatin on ox-LDL-induced ER stress and apoptosis in vascular endothelial cells.

In conclusion, this study has shown that simvastatin could inhibit ox-LDL-induced ER stress and apoptosis in HUVECs, which might add new insights into the pharmacological effects of statins.

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Conflicts of interest

There are no conflicts of interest.

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辛伐他汀抑制氧化型低密度脂蛋白诱导的内质网应激及内皮细胞凋亡

摘要

背景： 氧化型低密度脂蛋白可诱导氧化应激与内皮细胞凋亡，在动脉粥样硬化的发生与进展过程中起到重要作用。既往研究表明氧化型低密度脂蛋白可通过内质网应激的PERK/eIF2α/CHOP途径诱导血管内皮细胞凋亡。他汀类药物除降低血脂水平外，还具有抗炎、抗氧化应激等多重非调脂功能。本研究将就辛伐他汀在氧化型低密度脂蛋白诱导的血管内皮细胞内质网应激和细胞凋亡过程中的作用进行探讨。

方法： 对人脐静脉内皮细胞使用辛伐他汀（0.1, 0.5, 2.5 μmol/L）及100 μmol/L的DEVD-CHO（特异性caspase-3抑制剂）孵育细胞1小时后，加入含100 μg/ml氧化型低密度脂蛋白的培养液孵育内皮细胞24小时，以未处理的细胞作为对照组；采用流式细胞仪检测内皮细胞凋亡率，采用Real-time PCR法检测CHOP mRNA的表达，采用比色法检测内皮细胞内caspase-3活性，采用western-blot法检测细胞内PERK蛋白表达及eIF2α蛋白的磷酸化。多组间比较采用单因素方差分析。

结 果： 与对照组（4.9%）相比，氧化型低密度脂蛋白培养内皮细胞24 小时显著增加内皮细胞凋亡率（31.9%），$P<0.05$，辛伐他汀（0.1，0.5，2.5 μmol/L）可减轻氧化型低密度脂蛋白诱导的内皮细胞凋亡（28.0%，24.7%，13.8%，$F =15.039$，相对于对照组，$P<0.05$）。以对照组为100%，氧化型低密度脂蛋白可显著增加PERK表达（499.5%,$P<0.05$）和eIF2α磷酸化水平（451.6%,$P<0.05$），而辛伐他汀（0.1，0.5，2.5 μmol/L）可抑制氧化型低密度脂蛋白诱导的PERK表达（477.4%，397.0%，194.0%，$F = 10.121$，相对于对照组，$P <0.05$）和eIF2α磷酸化水平（407.8%，339.1%，187.5%，$F = 11.430$，相对于对照组，$P <0.05$）；而DEVD-CHO对于氧化型低密度脂蛋白诱导的PERK表达和eIF2α磷酸化水平无影响。另外，氧化型低密度脂蛋白可显著增强caspase-3活性，增加CHOP mRNA水平，而辛伐他汀可抑制此氧化型低密度脂蛋白诱导的上述效果。

结论： 辛伐他汀可抑制ox-LDL诱导的内皮细胞凋亡，其机制与辛伐他汀抑制内质网应激PERK/eIF2α/CHOP/caspase-3通路有关。