Oxidized low-density lipoprotein decreases VEGFR2 expression in HUVECs and impairs angiogenesis

MIN ZHANG and LI JIANG

Division of Cardiology, Shanghai Tongren Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200336, P.R. China

Abstract. Atherosclerosis (AS), which is triggered by endothelial cell injury, evolves into a chronic inflammatory disease. Oxidized low-density lipoprotein (ox-LDL) is an important risk factor for the development of atherosclerosis; ox-LDL induces atherosclerotic plaque formation via scavenging receptors. The present study used ox-LDL-treated human umbilical vein endothelial cells (HUVECs) to investigate the effect of ox-LDL on angiogenesis. ox-LDL decreased HUVEC proliferation by MTT, induced apoptosis by Annexin V-fluorescein isothiocyanate (FITC) staining and markedly suppressed HUVEC tube formation by the Matrigel assay in a dose-dependent manner. Angiogenesis has been correlated with monocyte invasion, plaque instability and atherosclerotic lesion formation. In addition, ox-LDL induced the overproduction of reactive oxygen species using DCFH-DA staining and increased caspase-3 activity. Vascular endothelial growth factor receptor 2 (VEGFR2) were detected by quantitative polymerase chain reaction and western blot analysis and has previously been observed to have a key role in angiogenesis. Furthermore, the present study demonstrated that the abundance of VEGFR2 was decreased in ox-LDL-treated HUVECs. These results suggested that ox-LDL impairs angiogenesis via VEGFR2 degradation, thus suggesting that VEGFR2 may be involved in adaptation to oxidative stress and AS.

Introduction

Atherosclerosis (AS) occurs as a result of endothelium injury, and leads to clogged arteries, resulting in heart attacks and strokes (1). It has recently been demonstrated that oxidized low-density lipoprotein (ox-LDL) has a key role in early inflammatory processes and may induce atherosclerotic lesions (2). In the process of atherosclerotic lesion formation, ox-LDL promotes the initiation of monocyte invasion and is taken up by monocytes/macrophages and endothelial cells via scavenger receptors on the cell surface (2). The subsequent accumulation of cholesterol in macrophages and foam cells is an indicator of atherosclerotic lesion formation. In addition, ox-LDL increases the permeability of endothelial cells and promotes their dysfunction (2). Previous studies have demonstrated that ox-LDL may significantly delay endothelium wound healing, and that the expression of numerous key genes in endothelial cells was changed following ox-LDL treatment, thus altering the function of the endothelium (3,4).

At the molecular level, ox-LDL has been demonstrated to promote the expression of adhesion molecules, heat shock proteins and coagulation proteins; to suppress the production of endothelium-derived nitric oxide (NO) and prostacyclin; and to induce the expression of various proinflammatory cytokines and growth factors in vascular cells (5-9). Therefore, inhibiting ox-LDL-induced vascular endothelial cell injury may be a potential therapeutic strategy for AS.

Previous studies have demonstrated that endothelial dysfunction and inflammation are precursors of AS (10,11). Numerous pathological conditions, including dyslipidemia, hypertension and hyperglycemia, have been associated with the overexpression of reactive oxygen species (ROS), which may stimulate endothelial cells and induce inflammatory responses (12,13). The necrosis of injured endothelial cells may result in the release of various pro-inflammatory factors, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1; these pro-inflammatory factors induce the formation of atherosclerotic plaques (14). Therefore, anti-inflammatory responses may be important for the prevention of AS.

Various growth factors and cytokines are involved in angiogenesis, including vascular endothelial growth factor (VEGF). VEGF binds to VEGF receptors (VEGFR) on the surface of cells, which induces the phosphorylation of downstream signaling molecules, including mitogen-activated protein kinase, focal adhesion kinase, Src kinase and signal transducer (15). The main subtypes of VEGFRs are VEGFRs 1-3, which are predominantly located on the surface of healthy tissue cells. However, VEGFRs have been observed to be upregulated during embryonic and tumor angiogenesis. VEGFR1 is a decoy receptor that has a low tyrosine kinase

Correspondence to: Dr Li Jiang, Division of Cardiology, Shanghai Tongren Hospital, School of Medicine, Shanghai Jiao Tong University, 1111 Xianxia Road, Shanghai 200336, P.R. China
E-mail: zm19821982@hotmail.com

activity (16). Conversely, previous studies have suggested that VEGFR2 has a critical role in cell proliferation, migration and tube formation, leading to angiogenesis (17,18).

However, to the best of our knowledge, the cytoprotective effect of VEGFR2 on oxLDL-induced human umbilical vein endothelial cell (HUVEC) injury has yet to be investigated clearly. The present study aimed to investigate the effect of ox-LDL on angiogenesis by exposing HUVECs to ox-LDL and performing endothelial proliferation and angiogenesis assays. In addition, the role of VEGFR2 in AS was determined.

Materials and methods

Chemicals, antibodies and reagents. LDL was purchased from R&D Systems GmbH (Wiesbaden, Germany). Calcein-AM was purchased from Dojindo Molecular Technologies, Inc. (Shanghai, China). Rabbit anti-VEGFR2 polyclonal antibody was obtained from Abcam (1:100; ab39256; Cambridge, UK). Phycoerythrin (PE)-conjugated mouse anti-VEGFR2 monoclonal antibody (1:500; FAB357P; clone 12G5) and purified mouse immunoglobulin (Ig)G (1:500; 550874) were purchased from BD Biosciences (Heidelberg, Germany). Recombinant human VEGF165 (11066-HNAB-500) was obtained from Sino Biological, Inc. (Beijing, China). Rabbit anti-β-actin (1:500; ab16039, Abcam) and rabbit anti-human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:500; SAB2108266) polyclonal antibodies were obtained from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany).

Cell culture and LDL treatment. In total 2×10^5 HUVECs (AllCells, LLC, Shanghai, China) were cultured for 3-5 passages in Dulbecco's modified Eagle's medium (DMEM; PromoCell GmbH, Heidelberg, Germany) supplemented with 10% fetal bovine serum (unless otherwise specified), 0.1 ng/ml human epidermal growth factor, 1 ng/ml basic fibroblast growth factor (bFGF), 90 ng/ml heparin and 1 ng/ml hydrocortisone (all purchased from Sigma-Aldrich Chemie GmbH). Cells were incubated at 37°C for 24 h in a humidified 5% CO_2 incubator at 21% O_2. Ox-LDL was prepared by exposure of native LDL to 5 μM copper sulphate (Sigma-Aldrich Chemie GmbH) at 37°C for 3 h. The reaction was stopped by the addition of 0.25 mM ethylenediaminetetraacetic acid, as described previously (19). Ox-LDL (0-100 μg/ml) was added to the cells alone or in combination with each other at 37°C for 24 h.

Cell proliferation assay. The MTT assay (Sigma-Aldrich Chemie GmbH) was used to assess the proliferation of HUVECs, according to the manufacturer's protocol. Briefly, HUVECs (1×10^4 cells/well) were plated onto 96-well plates and treated with ox-LDL at 37°C for 24 h, after which MTT (20 μl, 5 g/l) was added to media and incubated for 4 h. Subsequently, the supernatant was removed by centrifugation at 1000xg for 10 min at 4°C, and dimethylsulfoxide was added to solubilize the formazan crystals. Absorbance was measured at 560 nm using an ELISA plate reader.

Cell apoptosis assay. The apoptosis of HUVECs induced by ox-LDL starvation was detected by Annexin V-fluorescein

isothiocyanate (FITC) staining assays (Nanjing KeyGen Biotech, Co., Ltd., Nanjing, China). Briefly, 1×10^4 HUVECs were cultured with DMEM supplemented with 10 ng/ml VEGF and in the presence or absence of ox-LDL for 48 h. The cells were incubated with ox-LDL (0-100 μg/ml), VEGF (10 ng/ml) or both for 48 h. HUVECs were then collected and washed with phosphate-buffered saline (PBS) three times, after which Annexin V-FITC and propidium iodide were added to the washed cells (10^6 cells/ml) for 15 min at room temperature in the dark. Subsequently, fluorescence-activated cell sorting buffer was added and the cells were immediately analyzed by flow cytometry.

Endothelial cell wound healing assay. HUVECs (1×10^5 cells/well) were seeded into 12-well plates and were scratched with pipette tips, followed by treatment with 100 μg/ml ox-LDL. Migration of cells into the wound was then observed at 37°C for 24 h. The migrated cells were visualized and imaged under a microscope at various time points. Experiments were performed in triplicate at least three times.

Tube formation assay. HUVECs (2×10^4) were seeded into Matrigel-coated wells of a 96-well plate. The cells were incubated with ox-LDL (0-100 μg/ml) and/or VEGF (10 ng/ml) for 24 h, during which cells were maintained in an incubator at 37°C containing 21% O_2. Images were captured at low magnification (magnification, x5) under a microscope and tubes were counted. Only perfectly continuous tubes between two branching points were included. For each condition, three independent experiments were performed, of which mean tube numbers are presented.

Intracellular ROS production. HUVECs (2×10^5 cells/well) were seeded into a 6-well plate and treated with ox-LDL (0-100 μg/ml) for 24 h. The dichlorofluorescein diacetate (DCFH-DA) assay (Sigma-Aldrich Chemie GmbH) was used to measure the intracellular levels of ROS. Briefly, following treatment, the cells were incubated with 10 μM DCFH-DA for 30 min at 37°C. Flow cytometry was used to detect the fluorescence intensity.

Measurement of caspase-3 activity. The activity of caspase-3 was measured using a Caspase-3 Cellular Activity Assay kit (Nanjing KeyGen Biotech, Co., Ltd.), according to the manufacturer's protocol. Briefly, 1×10^4 HUVECs were removed from culture dishes, washed twice with PBS and centrifuged at 10,000 x g for 1 min at 4°C. Cell pellets were then treated for 30 min with ice-cold lysis buffer provided by the manufacturer of the kit. Cell suspensions were then centrifuged at 10,000 x g for 1 min at 4°C and the supernatants were transferred to a clear tube. To each tube, 2X reaction buffer and specific substrate for caspase-3 were added, and the tubes were incubated at 37°C for 4 h. Following incubation, caspase-3 activity was measured at 405 nm using a micrometer plate reader.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). A total of 1×10^4 HUVECs were treated with various concentrations of ox-LDL (0-100 μg/ml) for 24 h or 100 μg/ml ox-LDL for 24-72 h, after which total RNA was extracted from

HUVECs using the RNeasy Midi kit (Qiagen GmbH, Hilden, Germany), according to manufacturer's protocol. Total RNA (1 µl) was reverse transcribed into cDNA using iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). qPCR was performed using Power SYBR® Green PCR Master Mix (Eurogentec, Verviers, Belgium) on the StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The following primers were used: VEGFR2 forward, 5'-CTCTTGGCCGTGGTGCCT TTG and reverse, 3'-GTGTGTTGCTCCTTCTTTCAAC; and GAPDH forward, 5'-CATCTTCCAGGAGCGAGATCC and reverse, 3'-GGTGCAGGTGGCATTGCTGATG. GAPDH was used as housekeeping gene. The PCR cycling conditions were as follows: Denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. All samples and standards were conducted in triplicate. The relative mRNA expression was determined using the $2\Delta\Delta c_q$ method. A negative control without RT enzyme and an RT-minus control (without reverse transcriptase added to the cDNA synthesis reaction) were used.

Western blot analysis. A total of $2x10^5$ HUVECs were treated with ox-LDL (0-100 µg/ml) at 37°C for 72 h, after which HUVECs were washed with PBS and lysed using radioim-munoprecipitation assay (Thermo Fisher Scientific, Inc.) lysis buffer. Cell lysates were centrifuged at 10,000 x *g* for 10 min at 4°C, after which protein concentrations were determined using a Pierce BCA Protein Assay kit (23225; Sigma-Aldrich Chemie GmbH). Total lysate proteins (40 µg) were resuspended in loading buffer and separated by 10% SDS-PAGE, followed by transfer onto a polyvi-nylidene difluoride membrane. For detection of VEGFR2, the membranes were incubated overnight at 4°C with rabbit anti-VEGFR2 and rabbit anti-β-actin polyclonal antibodies (1:400). After washing three times with Tris-buffered saline containing Tween-20, the membranes were blocked with 5% bovine serum albumin for 1 h at room temperature and incubated with peroxidase-conjugated goat anti-rabbit IgG (1:2,000; Abcam) for 1 h and then detected by incubation with chromomeric substrate, 3, 3'-diaminobenzidine.

Statistical analysis. Data are expressed as the mean ± standard deviation. Comparisons among groups were performed by one-way analysis of variance followed by Tukey's post-hoc test. Comparisons between two groups were performed by two-tailed unpaired t-tests. Statistical analysis was performed using SPSS 10.0 software for Windows (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Ox-LDL reduces cell viability in a dose-dependent manner. MTT assays were used to evaluate the effect of ox-LDL on the viability of HUVECs. HUVECs were cultured in growth factor-deprived DMEM containing ox-LDL (0-100 µg/ml) for 24 h. Fig. 1 shows that HUVEC proliferation was decreased following treatment with ox-LDL in a dose-dependent manner; the proliferation of HUVECs was significantly decreased following treatment with 100 µg/ml ox-LDL (P<0.05). These results suggest that ox-LDL reduces the viability of HUVECs.
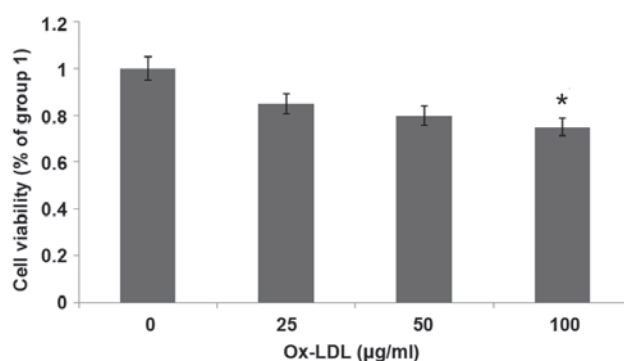

Figure 1. Effect of ox-LDL on the proliferation of HUVECs compared to group 1. HUVECs were treated with 0, 25, 50 or 100 µg/ml ox-LDL for 24 h, after which cell proliferation was assessed using MTT assays. Ox-LDL decreased the proliferation of HUVECs in a dose-dependent manner. Data are expressed as the mean ± standard deviation. *P<0.05, vs. the control (0 µg/ml). Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells.
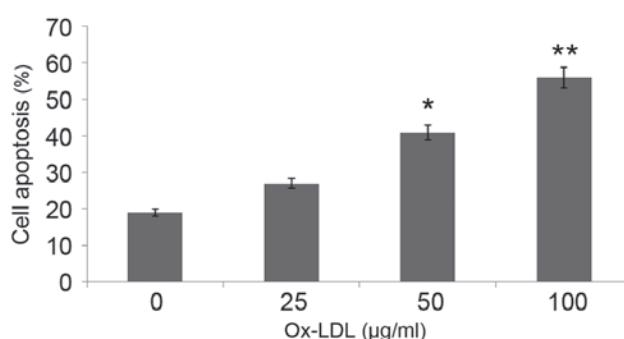

Figure 2. Effect of ox-LDL on the apoptosis of HUVECs. HUVECs were treated with 0, 25, 50 or 100 µg/ml ox-LDL for 48 h, after which the per-centage of cell apoptosis was detected by flow cytometry. Ox-LDL induced the apoptosis of HUVECs in a dose-dependent manner. Data are expressed as the mean ± standard deviation. *P<0.05, **P<0.01, vs. the control (0 µg/ml). Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells.

Ox-LDL induces HUVEC apoptosis in a dose-dependent manner. In order to investigate the effect of ox-LDL on HUVEC apoptosis, serum deprivation-induced apoptosis of HUVECs was assessed by flow cytometry. Serum deprivation induced apoptosis of ~19% of HUVECs, which was significantly increased to 56% following treatment with 100 µg/ml ox-LDL for 48 h (P<0.01; Fig. 2). These results suggest that ox-LDL induces the apoptosis of HUVECs.

Ox-LDL dose-dependently decreases HUVEC migration. Cell migration is an essential process in angiogenesis. The present study performed wound healing assays to investigate the effects of ox-LDL on the migration of HUVECs. ox-LDL (100 µg/ml) markedly inhibited the migration of HUVECs into the wound area (Fig. 3). This effect was significant at 6, 12 and 24 h, as compared with the control (P<0.05; Fig. 3).

Ox-LDL dose-dependently inhibits angiogenesis. In order to elucidate the potential underlying mechanisms of the effects of ox-LDL on angiogenesis, the tube-forming ability of HUVECs was assessed *in vitro*. HUVECs were cultured
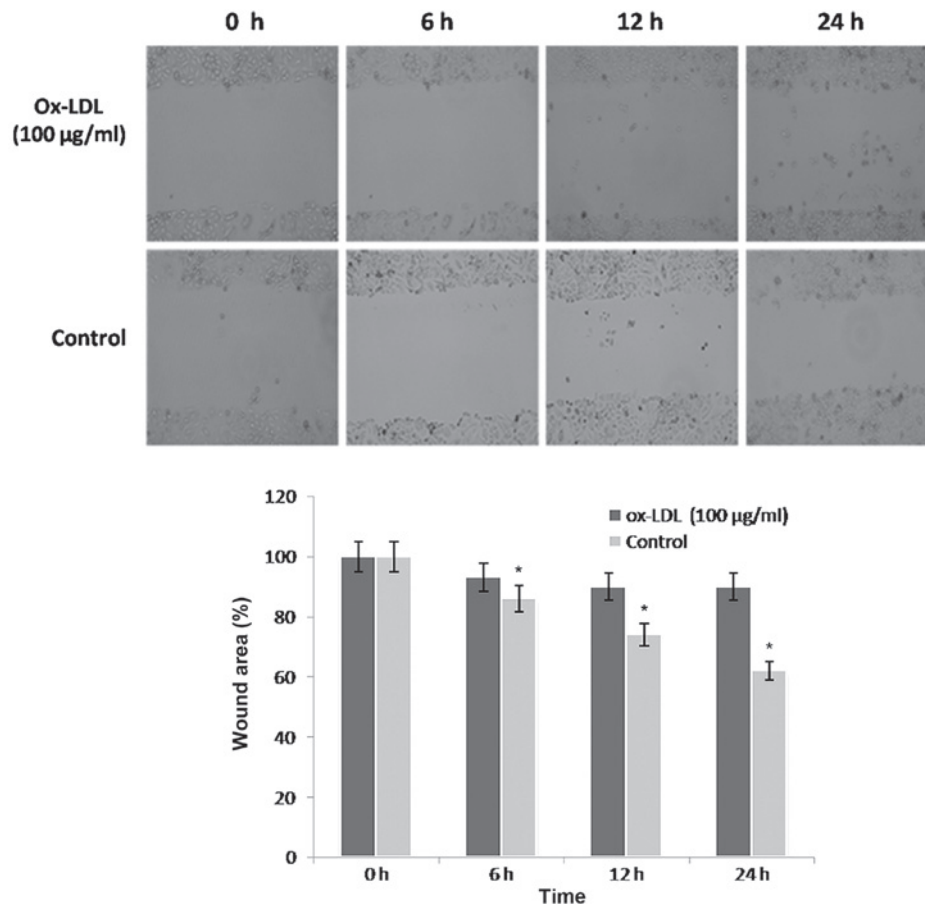
Figure 3. Effect of ox-LDL on the migration of HUVECs. HUVECs were incubated with 100 μg/ml ox-LDL for 24 h and cell migration was detected using wound healing assays. Ox-LDL inhibited the migration of HUVECs into the wound area. Representative images of the wound area were taken at 0, 6, 12 and 24 h following wound induction (magnification, x10). Data are expressed as the mean ± standard deviation (n=3). *P<0.05, vs. the control. Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells.

in DMEM containing 10 ng/ml VEGF and/or ox-LDL (0-100 μg/ml) for 24 h, after which the cells were seeded into matrigel-coated plates and the lengths of tube-like structures were measured. VEGF (10 ng/ml) induced HUVEC tube formation. Conversely, ox-LDL significantly inhibited tube formation by HUVECs in a dose-dependent manner (Fig. 4). These results suggest that ox-LDL is able to suppress HUVEC angiogenesis *in vitro*.

ox-LDL induces overproduction of ROS in HUVECs. The levels of ROS in HUVECs treated with ox-LDL for 24 h were measured using DCFH-DA staining and flow cytometry. As shown in Fig. 5, the levels of ROS were significantly increased in HUVECs following treatment with ox-LDL in a dose-dependent manner, as compared with the control (P<0.05). In particular, treatment with 150 μg/ml ox-LDL increased the levels of ROS in HUVECs by 2.18-fold.

ox-LDL activates caspase-3 in HUVECs. The present study demonstrated that ox-LDL induced HUVEC injury, indicating a potential suppressive effect on HUVEC angiogenesis. Caspases are crucial mediators of programmed cell death (apoptosi). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. Incubation of HUVECs with ox-LDL for

24 h significantly increased caspase-3 activity, as compared with the control (P<0.05; Fig. 6).

Ox-LDL regulates VEGFR2 expression at the post transcriptional level. The present study demonstrated that ox-LDL inhibited VEGF-induced angiogenesis of HUVECs. In order to investigate the underlying mechanisms, the mRNA and protein expression levels of VEGFR2 in ox-LDL-treated HUVECs were determined by RT-qPCR and western blotting, respectively. The mRNA expression levels of VEGFR2 were increased in HUVECs treated with various concentrations of ox-LDL for 24 h or with 100 μg/ml ox-LDL for various durations; however, the difference was not significant, as compared with the control (P>0.05; Fig. 7). Conversely, the protein expression of VEGFR was markedly decreased in HUVECs treated with ox-LDL, as compared with the control (Fig. 8). These results suggest that ox-LDL regulates VEGFR2 expression at the protein, but not the mRNA level.

Discussion

Epidemiological studies have examined the incidence of AS being 79.9% in Chinese people >60 years old. AS is serious but is not yet a global health emergency. Ox-LDL-induced vascular endothelial damage has been demonstrated to be a
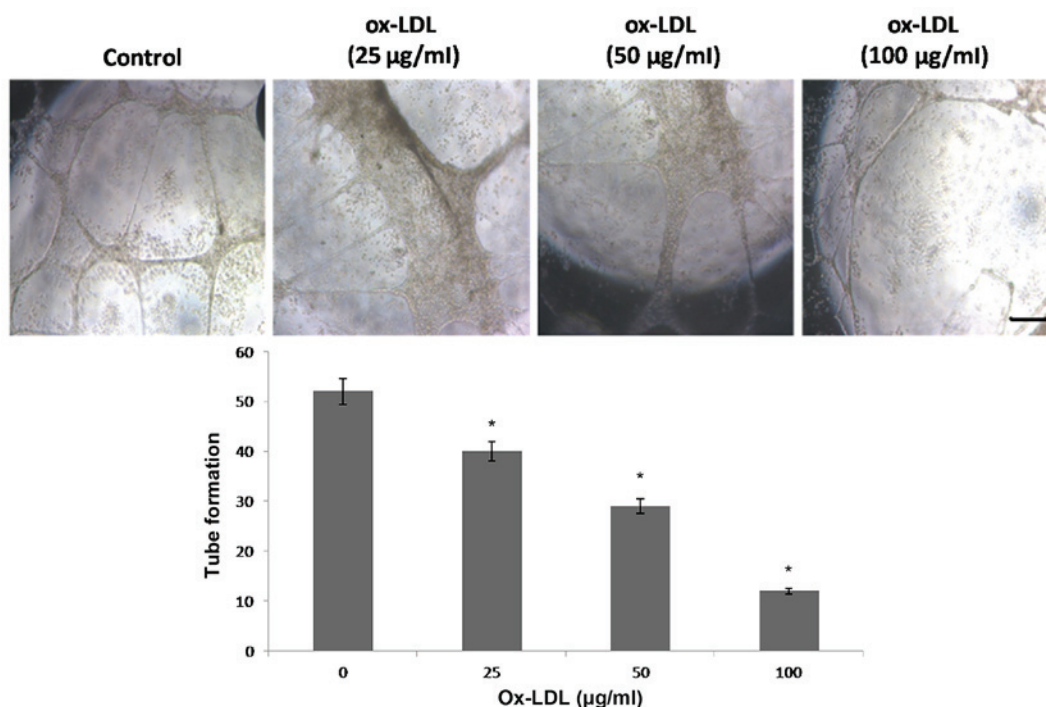
Figure 4. Effect of ox-LDL on tube formation by HUVECs. HUVECs were incubated with 10 ng/ml vascular endothelial growth factor or 0, 25, 50 or 100 μg/ml ox-LDL for 24 h and angiogenesis was assessed by tube formation assays. Representative microphotographs are shown for three different doses (scale bars, 200 μm). Data are presented as the mean ± standard deviation. *P<0.05 vs. the control. Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells.
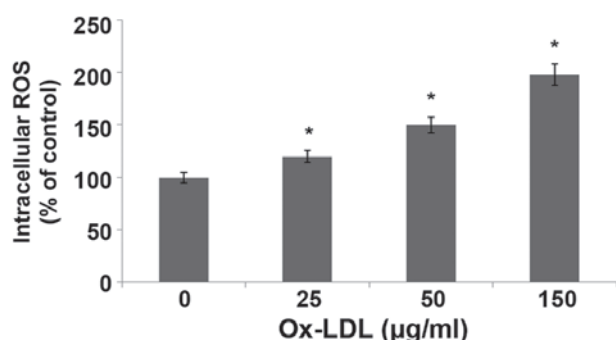

Figure 5. Effect of ox-LDL on HUVEC ROS production. HUVECs were treated with 0, 25, 50 or 150 μg/ml ox-LDL for 24 h, after which the levels of ROS were detected by DCFH-DA assays. Ox-LDL increased ROS production in HUVECs in a dose-dependent manner. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control (0 μg/ml; n=3). Ox-LDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; HUVECs, human umbilical vein endothelial cells.
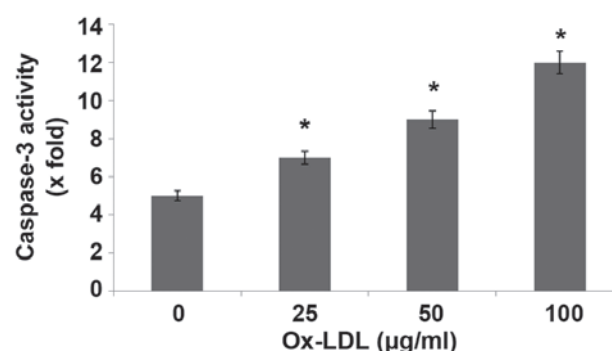
Figure 6. Effect of ox-LDL on the activity of caspase-3 in HUVECs. HUVECs were treated with 0, 25, 50 or 100 μg/ml ox-LDL for 24 h, after which caspase-3 activity was assessed using commercially available kits. Ox-LDL increased caspase-3 activity in HUVECs in a dose-dependent manner. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control (0 μg/ml; n=3). Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells.

driving force in the initiation and development of AS (20). A key therapeutic strategy for AS is the promotion of angiogenesis. Previous studies have used various strategies to induce angiogenesis, including the delivery of VEGF (21), viral vectors (22-24) or plasmids (25,26); however, few have shown success, which may be due to the fact that the molecular mechanisms underlying angiogenesis are largely unknown. Using proliferation, migration and tube formation assays, the present study demonstrated that ox-LDL impaired angiogenesis by HUVECs in vitro. Previous studies have associated hypercholesterolemia with impaired angiogenesis, and hypercholesterolemia has been shown to enhance oxidative

stress resulting in impaired inflammation in vivo (27-30). In the present study, ox-LDL impaired the ability of HUVECs to undergo angiogenesis by decreasing the viability and migratory and tube-forming abilities of the cells. Therefore, the present study provides novel insights into the effects of hypercholesterolemia on angiogenesis.

In the present study, ox-LDL exposure increased ROS production and caspase-3 activity in HUVECs. In addition, ox-LDL was observed to induce the apoptosis of HUVECs. As an executioner caspase, caspase-3 exhibits negligible activity until it is cleaved by an initiator caspase following induction of cell apoptotic events. Therefore, under normal
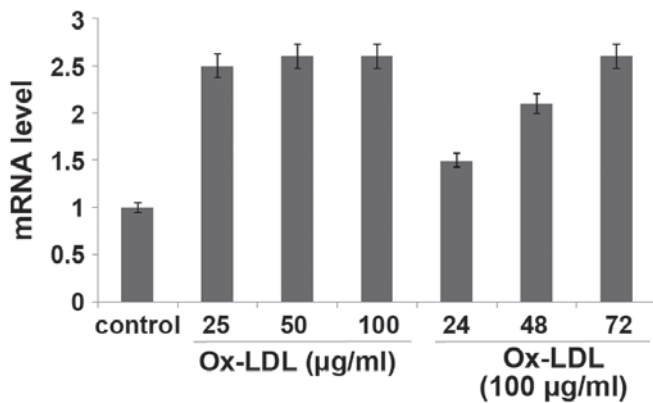
Figure 7. Effect of ox-LDL on the mRNA expression levels of VEGFR2 in HUVECs. HUVECs were treated with various concentrations of ox-LDL (25-100 μg/ml) or 100 μg/ml ox-LDL for various durations (24-72 h). The mRNA expression levels of VEGFR2 in HUVECs were determined by reverse transcription-quantitative polymerase chain reaction. Ox-LDL did not significantly alter the mRNA expression levels of VEGFR2 in HUVECs, as compared with the control. Ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells; VEGFR2, vascular endothelial growth factor receptor 2.

Figure 8. Effect of ox-LDL on the protein expression of VEGFR2 in HUVECs. HUVECs were treated with 25, 50 or 100 μg/ml ox-LDL for 72 h, after which the protein expression levels of VEGFR2 were analyzed by western blotting. β-actin served as a housekeeping protein. Ox-LDL decreased the protein expression levels of VEGFR2 in HUVECs. Ox-LDL, oxidized low-density lipoprotein; VEGFR2, vascular endothelial growth factor receptor 2; HUVECs, human umbilical vein endothelial cells.

conditions, healthy cells express full length, inactive caspase-3. As is shown in the present study, exposure of HUVECs to ox-LDL increased caspase-3 activity, thus suggesting that the rate of apoptosis was increased. These results support the hypothesis that ox-LDL is able to induce the apoptosis of HUVECs. This is significant since atherosclerosis has previously been associated with progressive endothelial cell loss (31). Therefore, the present study provides a potential mechanism by which ox-LDL exposure may enhance oxidative injury, in particular via induction of ROS production and activation of caspase-3.

Increasingly, it has been suggested that VEGFR2 drives the angiogenic response. VEGFR2 abundance and activation of the downstream signaling pathway in endothelial cells has been reported to be decreased under hypoxic conditions, such as those encountered in coronary heart disease, peripheral occlusive artery disease and ischemic stroke; and this was consistent with the results of the present study. In addition, previous studies demonstrated that ox-LDL impaired endothelial cell proliferation and migration via decreasing bFGF expression (32) and activating NO synthase/Akt signaling pathway (33). Furthermore, it has previously been shown that ox-LDL exposure decreased the expression of VEGFR1 in human macrophages (34), and internalized, ubiquinated and proteolytically degraded forms of VEGFR1 have been detected following ox-LDL exposure. However, whether ox-LDL affects the role of VEGFR2 in the angiogenic response pathway has yet to be elucidated.

The ox-LDL-mediated increase in oxidative stress-induced damage of HUVECs is suspected to underlie the pathogenesis of AS. The present study aimed to investigate the effects of ox-LDL on HUVEC apoptosis and the underlying mechanisms. Using endothelial proliferation and tube formation assays, the present study provided new evidence that ox-LDL exposure markedly affected HUVEC angiogenesis. The western blotting data confirmed that VEGFR2 was degraded
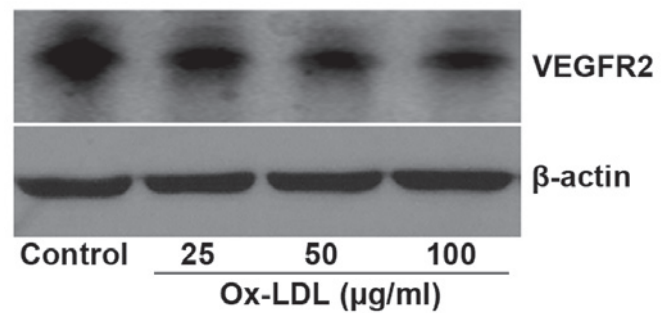
following ox-LDL exposure, and this decrease in VEGFR2 expression may have inhibited angiogenesis by limiting the activation of signaling pathways downstream of VEGFR2. The results demonstrated that VEGFR2 receptor function was significantly affected following ox-LDL exposure.

A limitation of the present study was that only the *in vitro* effects of ox-LDL on HUVECs were analyzed; the dose-dependent association between ox-LDL and HUVEC response may be more complex *in vivo*. Further studies are required to assess the effect of ox-LDL on vessel formation in animal models, and to develop therapeutic strategies for repairing angiogenesis under hypoxic conditions.

In conclusion, the present study aimed to investigate the molecular mechanisms underlying the involvement of VEGFR2 in the regulation of oxidative stress and HUVEC injury in AS. The results of the present study suggested that ox-LDL was able to alter endothelial cell survival and function, and that downregulation of VEGFR2 expression may underlie the development of AS.

Acknowledgements

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