Deletion of LOX-1 Reduces Atherogenesis in LDLR Knockout Mice Fed High Cholesterol Diet

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Abstract—Atherosclerosis is associated with oxidative stress and inflammation, and upregulation of LOX-1, an endothelial receptor for oxidized LDL (oxLDL). Here, we describe generation of LOX-1 knockout (KO) mice in which binding of oxLDL to aortic endothelium was reduced and endothelium-dependent vasorelaxation preserved after treatment with oxLDL (P<0.01 versus wild-type mice). To address whether endothelial functional preservation might lead to reduction in atherogenesis, we crossed LOX-1 KO mice with LDLR KO mice and fed these mice 4% cholesterol/10% cocoa butter diet for 18 weeks. Atherosclerosis was found to cover 61 ± 2% of aorta in the LDLR KO mice, but only 36 ± 3% of aorta in the double KO mice. Luminal obstruction and intima thickness were significantly reduced in the double KO mice (versus LDLR KO mice). Expression of redox-sensitive NF-κB and the inflammatory marker CD68 in LDLR KO mice was increased (P<0.01 versus wild-type mice), but not in the double KO mice. On the other hand, antiinflammatory cytokine IL-10 expression and superoxide dismutase activity were low in the LDLR KO mice (P<0.01 versus wild-type mice), but not in the double KO mice. Endothelial nitric oxide synthase expression was also preserved in the double KO mice. The proinflammatory signal MAPK P38 was activated in the LDLR KO mice, and LOX-1 deletion reduced this signal. In conclusion, LOX-1 deletion sustains endothelial function leading to a reduction in atherogenesis in association with reduction in proinflammatory and prooxidant signals. (Circ Res. 2007;100:1634-1642.)

Key Words: atherosclerosis ■ oxidative stress ■ inflammation ■ LOX-1

Oxidized form of LDL (oxLDL) is thought to be more important than native LDL in atherogenesis.1 oxLDL injures endothelium and is an important antigen in atherogenesis.2 Production of reactive oxygen species (ROS) is increased in atherosclerotic arteries well beyond the capability of endogenous antioxidants to inactivate them.1 ROS directly injure endothelium, denature nitric oxide (NO), oxidize lipids, and attract inflammatory cells to the site of injury.4,5 The vascular cells in vitro and in vivo internalize oxLDL through receptor-mediated pathways. In smooth muscle cells, monocytes/macrophages, and fibroblasts, this receptor-mediated pathway involves a family of scavenger receptors (SRs), such as class A SR, class B SR type I and CD36, and macrosialin (CD68). However, these SRs are undetectable or expressed in small amount in vascular endothelial cells.6 Sawamura et al7 identified a lectin-like oxLDL receptor-1 (LOX-1) on bovine aortic endothelial cells.8,9 The contributory role of LOX-1 in atherogenesis is supported by several lines of evidence: (1) LOX-1 shows a strong activity in binding, internalizing, and degrading ox-LDL10; (2) oxLDL activates LOX-1 and induces endothelial dysfunction and apoptosis11,12; (3) Besides oxLDL, other mediators of atherosclerosis, such as angiotensin II,13 cyto- kines,14 shear stress,15 and advanced glycation end-products,16,17 upregulate LOX-1; (4) LOX-1 is dynamically up-regulated by proatherogenic conditions, such as diabetes, hypertension and dyslipidemia18–20; and (5) LOX-1 is present in atheroma-derived cells and in human and animal atherosclerotic lesions.1,21,22

We designed this study to test the hypothesis that deletion of LOX-1 will ameliorate oxLDL-mediated endothelial dysfunction and inhibit atherogenesis in the LDL receptor-deficient (LDLR KO) mice model of atherosclerosis.22 The LOX-1 KO mice were prepared on C57BL/6 background (also referred to as wild-type mice).

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Materials and Methods

Generation of LOX-1 Gene Knockout Mice

Mouse LOX-1 gene was cloned by screening 129/SV mouse genomic DNA library made with Lambda FIXII by the use of mouse LOX-1 cDNA as a probe. The insert of a phage clone D was digested with SacI, and the resultant 3 fragments of the insert were subcloned into pBluescript SK(-) (Clone D3, D8, and D5). After sequencing and mapping with restriction enzymes, targeting vector was constructed according to the following strategy: (1) replacing a part of LOX-1 gene from the 6th to 8th exon with neomycin resistant gene (Figure 1A), (2) placing homologous part of LOX-1 gene at both sides of neomycin resistant gene (upstream: 3.8 kb of ClaI/ApaI digested fragment, downstream: 2 kb of KpnI/BamHI fragment), (3) attaching thymidine kinase gene for negative selection at the downstream of the LOX-1 gene fragments (Figure 1A). The targeting vector was linearized with XhoI digestion, and introduced by electroporation into $10^6$ of RW-4 ES cells from 129/SvJ mice. Selection of ES clones was performed with 0.2 mg/mL G418, and further screened with Southern blot analysis. Two homologous part of LOX-1 gene at both sides of neomycin resistant gene (upstream: 3.8 kb of ClaI/ApaI digested fragment, downstream: 2 kb of KpnI/BamHI fragment), (3) attaching thymidine kinase gene for negative selection at the downstream of the LOX-1 gene fragments (Figure 1A). The targeting vector was linearized with XhoI digestion, and introduced by electroporation into $10^6$ of RW-4 ES cells from 129/SvJ mice. Selection of ES clones was performed with 0.2 mg/mL G418, and further screened with Southern blot analysis. Two homologously recombined clones were obtained from 368 G418 resistant clones.

ES cells containing the disrupted gene were injected into blastocysts from C57BL/6 mice, and embryos were transferred to uteri of pseudopregnant mice. The chimeric mice were mated with C57BL/6 mice and resultant agouti mice were found to be carrying the disrupted LOX-1 gene. Offspring with the inactivated gene in germ line cells were backcrossed onto a C57BL/6 background 8 times. Homozygous LOX-1--deficient animals have no overt phenotype, and breed normally. The LOX-1 genotypes were verified by PCR analysis of genomic DNA extracted from tail with the primer pair for deleted portion of LOX-1 gene: 5'-GGCAACCCATGGCTGTGCGAGAATGG-3' and 5'-AGGATCTCGTGACCCATGGCGA-3'; and for neomycin resistant gene: 5'-AGGATCTCTCCTGGTGACCGCAGTACCCTGAA-3'.

RT-PCR Analysis of LOX-1 Expression

Total RNA was extracted from the aortic lysate, and 1 µg was subjected to cDNA synthesis with Superscript II reverse transcriptase with random hexamer. Production was then subjected to PCR with LOX-1 primers (see above) producing 453 bp, and for GAPDH for internal standard: 5'-GACCACAGTCCATGACATGACATCACT-3' and 5'-GCCACAGTGGCTGTTGCTGTA-3' producing 273 bp. Thermal cycler condition was 94°C 40 sec, 60°C 1 minute, 72°C 1 minute repeated 35 times for LOX-1 and 25 times for GAPDH. Then, the PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide on UV transiluminator.
Immunofluorescence Staining
Expression of LOX-1 was analyzed in the cryothin sections of aorta with Cy3-labeled anti-mouse LOX-1 monoclonal antibody (1 μg/mL, JTXS8). To confirm the presence of endothelium, immunostaining with biotinylated anti-CD31 (0.1 μg/mL) and avidin-FITC was performed and subjected to laser confocal microscopy.

Analysis of the Uptake of oxLDL
Thoracic aortas excised from mice were incubated for 12 hours at 37°C in DMEM/10% FCS containing 10 μg/mL Dil-labeled ox-LDL. Then washed with PBS 3 times and snap frozen. Cryothin sections were subjected to indirect immunofluorescence staining with biotinylated anti-CD31 and avidin-FITC to visualize endothelium and subjected to laser confocal microscopy.

Evaluation of Endothelial Nitric Oxide Synthase Activity
Aortic rings from wild-type male mice 10 weeks of age were used for vascular reactivity analysis. Resting tension of the aortic ring was adjusted at 0.5 g. Contraction was induced with 5 μmol/L PGF2α. After stable contraction, endothelium-dependent vasorelaxation was induced with acetylcholine (Ach, 10−3 to 3 x 10−7 M). After washout, a second contraction was induced with PGF2α, in the presence of oxLDL 10 μg/mL. In some cases, aortic rings were preincubated with anti-LOX-1 antibody or nonspecific IgG (50 μg/mL) for 30 minutes.

Preparation of LDLR/LOX-1 Double Knockout Mice
Wild-type and homozygous LDLR KO mice were obtained from Jackson Laboratories (Bar Harbor, Maine). LDLR KO and LOX-1 KO mice were bred by brother-sister mating. First generation animals were all LDLR+/−/LOX-1−/−, which were cross-bred with either LDLR KO or LOX-1 KO. The genotype of second generation animals was examined by PCR, and LDLR KO/LOX-1 KO (double KO) mice were selected. They were housed in a room lit from 6:00 AM to 6:00 PM and kept at 21°C. All animal were given a high-cholesterol diet (4% cholesterol/10% cocoa butter) for 18 weeks prior to sacrifice. All experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee.

Analysis of mRNA Expression by RT-PCR
Aortic specimens were derived from animals at 18 weeks. The mRNA isolation and RT-PCR amplification analysis of LOX-1 were performed as described previously.

Analysis of Protein Expression
Standard methodologies were used for determination of protein expression (Western blotting) and localization (immunohistochemistry). Relative to β-actin) intensities of protein bands were analyzed. The intensity of immunohistochemistry stain was calculated by Image-Pro Plus program.

Superoxide Dismutase Activity
Mice liver homogenates were cleared by centrifugation at 15 000g for 30 minutes at 4°C, and supernatants were used for measurements of superoxide dismutase (SOD) activity as described previously.

Analysis of Atherogenesis
After harvesting the animals, fatty deposits (index of atherosclerotic lesion formation) were quantitated as described previously. Briefly, 5 to 7 mice from each group were euthanized and the aortas separated from surrounding tissues. After removal of the adventitial fat, aortas were opened longitudinally from the aorta arch to the iliac bifurcation, and fixed in 10% formalin for 24 hours. The aortas were then rinsed in 70% alcohol and stained with Sudan IV for 15 minutes. Aortas were then mounted and photographed with a camera connected to a dissection microscope. The images were analyzed by Image-Pro Plus (Media Cybernetics). The person performing the staining was blinded to the experimental protocol.

In the second method, entire aorta from the aortic arch above the aortic valves to the iliac bifurcation was stored in formalin and 5µ cross-sections were made at 5 predefined points (proximal ascending aorta, aortic arch, descending aorta, mid thoracic aorta, and abdominal aorta above the renal arteries). The sections were stained with H&E. In each case, the average value in each animal was used for measurement of intima thickness.

Measurement of Plasma Lipids by Nuclear Magnetic Resonance
The lipid components from 50 μL of plasma were isolated by the acetonitrile extraction followed by chloroform:methanol (2:1 volume ratio) extraction. NMR spectra were acquired using a Bruker Avance spectrometer operating at 600.133 MHz for proton and equipped with a 5-mm triple resonance cryoprobe. Spectra were referenced to internal trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (TMSP). A total of 128 transients were collected for plasma extracts with a spectral width of 7183.91 Hz. The pulse width for proton was 8.10 μsec at a power level of 2.60 dB. Gradient-enhanced, phase sensitive 1H-13C HSQC spectra were acquired with a spectral width of 16.0221 ppm in the proton dimension and a width of 166.4892 ppm in the carbon dimension. For the plasma extracts, 104 transients were acquired with 1024 points in the time domain (t1). A relaxation delay of 2.5 sec and 256 t1 increments were used. The pulse width for proton was 11.00 μsec at 2.40 dB, whereas the pulse width for carbon was 16.00 μsec at a power level of −1.40 dB. Spectra were processed using ACD/Labs 1D NMR Manager. All spectra were zero filled to 131 072 points followed by multiplication by a 0.3-Hz line broadening factor. Spectra were then Fourier transformed, phased using the “simple” method, and baseline corrected using the “Sp Averaging” method with a box half width of 61 and noise factor of 3 and autoreferenced to TMSP at 0.00 ppm. The final size in the t2 dimension was 1024, whereas the final size in the t1 dimension was 512. A squared sine bell apodization function and forward linear prediction applied before transformation along the t1 dimension. Integration was done using 2D NMR Manager. Proton and HSQC were also acquired for lipid standards to identify individual lipids in the 2D HSQC spectra.

Statistical Analysis
Data are expressed as mean±SEM. Between-group difference was evaluated by unpaired t test. All other data were analyzed by a 2-way analysis of variance with a Bonferroni post-hoc test. A P<0.05 was considered significant.

Results
Characterization of LOX-1 Knockout Mice
The homologous recombination between targeting vector and mouse LOX-1 gene in ES cells and germ line transmission of the targeted allele are shown in Figure 1B. Lack of LOX-1 in the LOX-1 KO mice was confirmed by RT-PCR analysis and immunostaining of aortic tissues (Figure 1C). LOX-1 was not detected in the LOX-1 KO mice aorta, although the presence of endothelium was confirmed by simultaneous staining of CD31. LOX-1 was clearly present in the wild-type mice. Further, the uptake of oxLDL in endothelium was undetectable in LOX-1 KO mice, but clearly evident in wild-type mice (Figure 1C, right).

Impact of LOX-1 Deletion on Endothelium-dependent Vasorelaxation
Removal of endothelium did not affect PGF2α-induced constriction, but eliminated Ach-induced relaxation (data not
shown). L-NAME, an inhibitor of endothelial nitric oxide synthase (eNOS), had the same effect as endothelium removal, indicating that NO released from endothelium mediates Ach-induced vasorelaxation in this system.

As shown in Figure 2A, basal relaxation in response to Ach was greater in aortic rings from LOX-1 KO mice (versus wild-type mice). Importantly, oxLDL treatment reduced Ach-induced relaxation in wild-type mice aortic rings, whereas SNAP, an NO donor, still induced relaxation. In contrast, Ach-induced relaxation was preserved in the LOX-1 KO mice aortic rings despite treatment with oxLDL. Next, we examined whether these changes in the LOX-1 KO mice are attributable to the deletion of LOX-1 by application of anti–LOX-1 antibody to wild-type mice aortic rings. As shown in Figure 2B, anti–LOX-1 antibody modulated the effects of oxLDL on Ach-induced relaxation, and the vasorelaxation became similar to that with LOX-1 ablation. The effects of oxLDL were abolished by the addition of SOD in the medium (data not shown), in accordance with the previous observation that the binding of oxLDL to LOX-1 induces the formation of ROS.14

High Cholesterol Diet and Increase in Plasma Lipids
Figure 3 (left) depicts the NMR proton spectra of lipids in plasma from different groups of mice. The HDL-cholesterol (0.77 to 0.83) was lower, and both LDL-cholesterol (0.83 to 0.88) and VLDL-cholesterol (0.88 to 0.92) higher in the double KO and LDLR KO mice (P<0.01 versus wild-type mice). There were no differences in LDL- and HDL-cholesterol peaks between the LDLR KO and double KO mice. Figure 3 (right) summarizes the data on the levels of HDL-cholesterol, VLDL-cholesterol, and LDL-cholesterol in different groups of mice.
Expression of LOX-1 and the Extent of Atherosclerosis

All wild-type mice exhibited modest LOX-1 expression. In keeping with previous data, 26 LDLR KO mice exhibited increased expression of LOX-1. The LOX-1 KO and double KO mice, as expected, did not express LOX-1 (Figure 4A).

There were small areas of sudanophilia in the wild-type mice; and the sudanophilic areas were much fewer in the LOX-1 KO mice ($P<0.01$ versus wild-type mice, Figure 4B). The LDLR KO mice showed extensive sudanophilia covering almost 60% to 80% of aortic surface; importantly, the sudanophilic areas were $\approx 50\%$ less in the double KO mice.

There was extensive intimal thickening in the LDLR KO mice with large areas of proliferation, which in several sections appeared to totally occlude the vascular lumen (see an example in Figure 4C). In contrast, the intimal thickening was much less in the double KO mouse with relatively few occlusive lesions. The most advanced atherosclerotic lesion in a double KO mouse is shown in Figure 5C.

Mechanisms of Inhibition of Atherosclerosis

Endothelial continuity was studied by staining for von Willebrand factor. Figure 5 (left) shows endothelial disruption throughout the regions of plaque formation in the LDLR KO mice, particularly in areas with accumulation of macrophages and foam cells. On the other hand, LOX-1 deletion in the LDLR KO mice resulted in maintenance of endothelial continuity and few areas of macrophage accumulation. Figure 5 (right) shows marked reduction in eNOS expression in the LDLR KO mice with large areas of proliferation, which in several sections appeared to totally occlude the vascular lumen (see an example in Figure 4C). In contrast, the intimal thickening was much less in the double KO mouse with relatively few occlusive lesions. The most advanced atherosclerotic lesion in a double KO mouse is shown in Figure 5C.

Atherosclerosis involves oxidative stress and inflammation.3,28 In keeping with this concept, the expression of NF-κB was reduced in the LOX-1 KO mice (Figure 6A), indicating that LOX-1 deletion reduces basal expression of this transcription factor. The LDLR KO mice showed a marked increase in NF-κB expression ($P<0.01$ versus wild-type mice). Importantly, deletion of LOX-1 in the LDLR KO mice reduced NF-κB expression ($P<0.01$ versus LDLR KO mice).

Data on the expression of CD68, a general marker of macrophage infiltration,29,30 are shown in Figure 6A (top right). LOX-1 KO mice exhibited much less CD68 expression ($P<0.01$ versus wild-type mice). There was a marked increase in CD68 expression in the LDLR KO mice ($P<0.01$ versus wild-type mice). In contrast, the LDLR KO mice with LOX-1 deletion exhibited much lower level of CD68 ($P<0.01$ versus LDLR KO mice).

Recent studies have shown reduced levels of the antiinflammatory cytokine IL-10 in atherosclerosis31 and inhibition of atherogenesis in mice with IL-10 upregulation.32 In this study, we found that the LDLR KO mice had reduced levels of IL-10 ($P<0.01$ versus wild-type mice). Importantly, deletion of LOX-1 significantly increased IL-10 expression in the wild-type and LDLR KO mice (Figure 6A, bottom left).

As a marker of antioxidant activity, we measured SOD activity in the mice liver. The LDLR KO mice were found to have less SOD activity (versus wild-type mice). Importantly, deletion of LOX-1 markedly increased SOD activity in the wild-type and LDLR KO mice, despite high-cholesterol diet. Importantly, extracellular SOD (ecSOD) expression was higher in the aortas of LDLR KO mice (versus wild-type mice), probably a response to increased oxidant stress. ecSOD expression increased further with LOX-1 deletion (Figure 6A, bottom right).

Next, we studied mitogen-activated protein kinase (MAPK) signaling in the presence of LOX-1 deletion. MAPK is a major signal in vascular inflammation.33 As shown in Figure 6B, protein levels of both p38 and p44/42 MAPks were not different in different groups of mice. Phosphorylated p38 MAPK levels was higher in the LDLR KO mice ($P<0.01$ versus wild-type mice), but not in the double KO mice. The levels of phosphorylated p42/44 MAPK were similar in all mice groups.

**Discussion**

Because endothelial dysfunction precedes morphological atherosclerotic changes, endothelial dysfunction has been hypothesized to be a key early lesion in atherogenesis.4,34
Among various insults to vascular endothelial cells, oxLDL has been postulated to be a major antigen.\textsuperscript{34,35} Ox-LDL is taken up in endothelial cells mostly via LOX-1.\textsuperscript{7,8} LOX-1 activation in endothelial cells initiates a cascade of events that are intimately involved with atherogenesis.\textsuperscript{11,12,21} The present study provides the missing link in this hypothesis, namely (1) endothelial dysfunction is mediated by LOX-1, and (2) LOX-1 is involved in the biology of atherosclerosis.\textsuperscript{36}

We show that Ach-mediated relaxation was greater at baseline in LOX-1 KO mice, probably reflecting the enhanced expression of eNOS in LOX-1 KO mice. Further, we demonstrate that the action of oxLDL is mediated, in large part, by LOX-1 activation. Pretreatment with anti-LOX-1 antibody protected the wild-type mice aortic rings from the adverse effects of oxLDL. Our data are supported by the recent description of the important role of LOX-1 in endothelial dysfunction in Apo-E KO mice.\textsuperscript{36}

We also show that LOX-1 is causally involved in atherogenesis. We used LOX-1 KO mice backcrossed to C57BL/6 mice 8 times, and examined in 2 different conditions, namely, wild-type mice and LDLR KO mice all fed high cholesterol diet. Under these conditions, we observed a marked reduction in atherogenesis by LOX-1 ablation. SR-A and CD36 knockout mice all once were initially reported to reduce atherogen-

![Figure 4](https://example.com/figure4.png)

**Figure 4.** A, Expression of LOX-1 in various groups of mice. Note the absence of LOX-1 gene in the LOX-1 KO and double KO mice. B, Extent of atherosclerosis in different mice groups. LOX-1 KO mice have less atherosclerosis than wild-type mice, and LOX-1 ablation in the LDLR mice reduces atherosclerosis. Left panel shows representative aortas from each group and the bar graphs show summary (±SEM) data in each group. C, Intima thickness is significantly reduced in the LOX-1 KO mice (versus wild-type mice) and the double KO mice (versus LDLR KO mice). Left panel shows representative aortic sections. Arrows show regions of marked intimal thickening. Bar graphs show summary (±SEM) data in each group.
upregulation of the antiinflammatory cytokine IL-10 reduces oxidant stress and inflammation. Recent studies show that the LDLR KO mice attenuated these abnormal signals related to atherosclerosis in vivo and to correlate with atherosclerotic disease entities.34,36 We stained aortic sections for von Willebrand factor as a measurement for endothelial integrity. This phenomenon needs to be confirmed in animals with lipid metabolism more akin to man. Further, LOX-1 is a multi-ligand receptor, and lipoprotein recognized by LOX-1 in vivo may not be pro-atherogenic, and its deletion limits atherogenesis despite high cholesterol diet, at least in part, by preserving endothelial function and integrity. The LOX-1 molecule that bridges the two phenomena, ie, endothelial dysfunction and atherogenesis, should be a good target for prediction, prevention and treatment of atherosclerosis-related diseases.

The wild-type mice in our study had modest LOX-1 expression as well as atherosclerosis—most likely a result of high levels of LDL-cholesterol in response to 4% cholesterol/10% cocoa butter diet (Figure 3). On the other hand, LOX-1 KO mice had no LOX-1 expression and minimal atherosclerosis despite the same lipid levels. In a previous study in Apo-E KO mice,26 we showed that lowering of LDL-cholesterol with an HMG CoA reductase inhibitor lowers LOX-1 expression. The basal expression of LOX-1 may be responsible for the basal state of oxidant stress and inflammation. In keeping with this suggestion, LOX-1 deletion reduced the expression of NF-κB and CD 68 as well as phosphorylated p38-MAPK.

Nuclear translocation of p65 has been observed in human and experimental atherosclerosis.39,40 The canonical pathway of NF-κB activation that involves p65 is activated in atherosclerosis and results in selective upregulation of major proinflammatory mediators of the disease.41 These findings suggest that the increased expression of p65 in our study is indicative of its nuclear translocation, resulting in increased inflammatory stress.

Data on LOX-1 deletion in the LDLR KO mice are particularly noteworthy. The double KO mice had much less atherosclerosis than the LDLR KO mice; the latter showed a marked increase in NF-κB and CD68 expression and a dramatic reduction in SOD activity and IL-10 expression. Deletion of LOX-1 in the LDLR KO mice attenuated these abnormal signals related to oxidant stress and inflammation. Recent studies show that upregulation of the antiinflammatory cytokine IL-10 reduces atherosclerosis in the LDLR KO mice,32,42 and IL-10 deficiency increases atherosclerosis in Apo-E KO mice.43 Likewise, enhancement of antioxidant mechanisms has salutary effect on atherogenesis.44

Atherogenesis in LDLR KO mice was associated with enhanced phosphorylation of p38 MAPK, but not of p44/42 MAPK, suggesting activation of proinflammatory pathways.33 A previous in vitro study showed that oxLDL-mediated activation of LOX-1 is associated with p38 MAPK activation, and pretreatment of cells with antisense to LOX-1 blocks p38 MAPK activation.45 Others have shown that p44/42 MAPK phosphorylation is also mediated by LOX-1.46 However, we did not observe any changes in p44/42 MAPK phosphorylation, and the difference in the 2 studies may be a reflection of study design. The present study suggests a link between LOX-1 activation, oxidant stress, and atherogenesis in vivo. It is of note that the deletion of LOX-1 was not associated with any significant alteration in the levels of LDL-cholesterol.

We also demonstrated that LOX-1 deletion resulted in maintenance of endothelial continuity and eNOS expression in the LDLR KO mice, further confirming the importance of endothelial dysfunction in atherogenesis.34,36 We stained aortic sections for von Willebrand factor as a measurement for endothelial integrity and attributed LOX-1 deletion to the preservation of endothelial integrity. This phenomenon needs to be confirmed at multiple stages of atherosclerotic process and/or in the primary cultured endothelial cells. The preservation of endothelial integrity may be secondary to the reduction in atherosclerotic process.

**Study limitations**

The lipoprotein metabolism in mice is quite different from that in man because of the lack of cholesterol ester transfer protein, presence of apoB100 in chylomicron/remainder instead of apoB48, and relative importance of Apo-E for the uptake of VLDL/LDL in the liver. Therefore, the results in mice will need to be confirmed in animals with lipid metabolism more akin to man. Further, LOX-1 is a multi-ligand receptor, and lipoprotein recognized by LOX-1 in vivo may not be produced in vitro, although its presence has been demonstrated in vivo and to correlate with atherosclerotic disease entities.

In summary, our data suggest that LOX-1 expression is important in atherogenesis, and its deletion limits atherogenesis despite high cholesterol diet, at least in part, by preserving endothelial function and integrity. The LOX-1 molecule that bridges the two phenomena, ie, endothelial dysfunction and atherogenesis, should be a good target for prediction, prevention and treatment of atherosclerosis-related diseases.
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Disclosures

None.

References


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