Review

LOX-1-Mediated Effects on Vascular Cells in Atherosclerosis

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Key Words

Atherosclerosis • Atherogenesis • Arteries • Lectin-like oxidized low-density lipoprotein receptor-1 • LOX-1 • Oxidized LDL • OxLDL

Abstract

In healthy arteries, expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is almost undetectable. However, in proatherogenic conditions, LOX-1 is markedly up-regulated in vascular cells. In atherosclerosis, LOX-1 appears to be the key scavenger receptor for binding oxidized LDL (oxLDL). Notably, a positive feedback exists between LOX-1 and oxLDL. LOX-1 is involved in mediating of proatherosclerotic effects of oxLDL which result in endothelial dysfunction, proinflammatory recruitment of monocytes into the arterial intima, formation of foam cells, apoptosis of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), as well as in plaque destabilization and rupture. In this review, we consider effects of the LOX-1/oxLDL axis on several types of vascular cells such as ECs, VSMCs, and macrophages.

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Introduction

Atherosclerotic disease is a chronic disorder that affects arterial vessels of medium or large calibers and has a histological manifestation of plaques enriched with lipids. The entrance of low density lipoproteins (LDL), which are rich of cholesterol, into the intima in athero-prone sites of arteries is accompanied by the adhesion of monocytes to the luminal

endothelium [1]. Monocytes attracted by the proinflammatory stimuli coming from the inflamed endothelial cells infiltrate the subendothelial layer where they differentiate to macrophages [1]. Macrophages engulf oxidized LDL (oxLDL) but cannot utilize lipids and, eventually, transform into foam cells. Macrophage activation leads to the liberation of proinflammatory cytokines, increased production of reactive oxygen species (ROS), and development of oxidative stress [2].

Macrophages could bind oxLDL through several scavenger receptors (SRs) such as SR-AI, SR-BI, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), and CD36 [3]. LOX-1 is a membrane glycoprotein that consists of an N-terminal cytoplasmic domain, a transmembrane domain, an extracellular stalk region (responsible for receptor oligomerization), and C-type lectin-like extracellular domain (responsible for interaction with a ligand) [4]. The receptor comprises 273 amino acid residues. LOX-1 precursor has a molecular weight of 40 kDa that is then glycosylated and processed to a 50-kDa mature form [5].

LOX-1 was also shown to be expressed in endothelial cells (ECs) and smooth muscle cells (VSMCs) [6]. In normal conditions, LOX-1 production is minimal but could be markedly increased under proinflammatory signals. Expression of LOX-1 was found to be initiated by proinflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β , interferon- γ , and vasoconstrictors including angiotensin II and endothelin-1 [5].

Expression of LOX-1 is especially elevated in advanced plaques [7]. The application of immunohistochemical approach for analysis of LOX-1 expression in human atherosclerotic plaques demonstrated that the LOX-1 immunopositivity is displayed by a large number of intimal cells, even though the intensity of LOX-1 expression markedly varies in different intimal cells [7] (Fig. 1). LOX-1 levels were observed to be up-regulated in intimal VSMCs and in human carotid-plaque macrophages [7]. Genetic deletion of LOX-1 in LDL receptor-deficient mice resulted in diminished atherosclerosis whereas overexpression of LOX-1 led to advanced disease [8, 9]. Therefore, expression of LOX-1 in vascular cells could suggest for its involvement in atherosclerosis.

In addition to oxLDL, numerous ligands such as acetylated forms and other forms of modified lipoproteins, advanced glycation end-products (AGEs), and inflamed platelets could interact with LOX-1 [5]. Furthermore, LOX-1 interacts with high affinity with moderately oxidized LDL rather than massively oxidized LDL [10]. OxLDL binding to LOX-1 results in fast internalization of lipids to the cell while this process could be interrupted by an antibody specific to oxLDL. After internalization, oxLDL becomes unbound of LOX-1, and both molecules retain in distinct areas of the cytoplasm [11]. The major location of LOX-1 is caveolae/lipid rafts, and function of LOX-1 is influenced by the cholesterol content of the plasma membrane [11, 12]. Reduction in membrane cholesterol levels leads to a more random distribution of LOX-1 in the plasma membrane and decreased binding of oxLDL. Therefore, an accumulation of LOX-1 in special membrane compartments is needed for effective binding of oxLDL followed by endocytosis of complexes between LOX-1 and oxLDL [12].

Due to the strong proatherogenic role, marked up-regulation in inflammatory conditions, and effects on ECs, VSMCs, and macrophages (i.e., cells that are crucially involved in atherogenesis), LOX-1 can represent a valuable pharmaceutical target in therapy of cardiovascular diseases. In this brief review, we consider proatherosclerotic effects of LOX-1 on macrophages, ECs, and VSMCs (Fig. 2).

Influence of LOX-1 on Endothelial Cells

Compared to the other types of vascular cells, LOX-1 exhibits the most robust and broad proatherogenic and proinflammatory effects on arterial ECs because they are primarily exposed to the action of LOX-1. LOX-1 induces endothelial dysfunction and arterial denudation through several mechanisms including proinflammatory activation of ECs,

Fig. 1. Typical patterns of LOX-1 expression in human atherosclerotic plaque (A, B). In (A), large arrows show cells that intensely express LOX-1, while small arrows show cells that display a relatively low LOX-1 immunopositivity. (A, B): Human aortic specimens; Immunofluorescent analysis utilizing anti-LOX-1 antibody (Abcam; cat# ab60178) and fluorescein isothiocyanate (FITC) visualization. (A, B): Scale bars = 150 μ m.

Fig. 2. A summary of proatherosclerotic effects of oxidized LDL (oxLDL) mediated by lectin-like oxLDL receptor-1 (LOX-1) on macrophages, endothelial cells, and vascular smooth muscle cells (VSMCs).

increased endothelial apoptosis and senescence, elevated recruitment of monocytes to ECs, and decreased vascular dilatation (Table 1 [13-24], Fig. 2) [5].

LOX-1 inhibits endothelial NO production

ECs take up oxLDL mainly via LOX-1 [6]. In ECs, arginase II that decomposes arginine to ornithine and urea controls activity of endothelial nitric oxide synthase (eNOS) by competing for the common substrate L-arginine [25]. LOX-1 mediates stimulatory action of oxLDL on arginase II activation that in turn leads to down-regulation of eNOS activity and impairs endothelial function [26]. In human aortic ECs, oxLDL triggers translocation

Table 1. Proatherogenic activities of LOX-1 in different cell types. Abbreviations: Bax, Bcl2-associated X protein; Bcl2, B-cell lymphoma 2; CCL2, (C-C motif) ligand 2; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin-6; NAIP1, neuronal inhibitory apoptosis protein 1; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1

Cell type	LOX-1 effects	References
EC	Vasoconstriction through decrease of NO production and availability and increase of endothelin-1 production	[13, 14]
	Proinflammatory activation of ECs (induction of chemokine CCL2, IL-6, ICAM-1, VCAM-1, selectin)	[14-16]
	Adhesion of leukocytes to ECs	[16]
	EC apoptosis through inhibition of anti-apoptotic factors Bcl2 and NAIP1 and activation of caspase-3 and caspase-9, which degrade Bcl2 and NAIP1	[17]
	Increased senescence of ECs by inhibition of activation of transcription factor NF-κB, two-fold increase in Bax/Bcl2 ratio, and 3-fold increase in apoptotic response to TNF-α	[18]
VSMC	Induction of proliferation of VSMCs via NF-κB- and JNK-dependent signaling pathways	[19]
	Induction of migration of VSMCs	[9]
	Induction of neointimal formation and intimal hyperplasia of VSMCs	[20]
	Enhanced VSMC apoptosis that is mediated by activation of Bax, caspase-3, and other apoptotic mediators and leads to plaque destabilization	[21, 22]
Macrophage	Stimulation of cholesterol uptake and transformation of macrophages to foam cells	[23]
	Induction of macrophage apoptosis through activation of acid sphingomyelinase/ceramide signaling pathway, followed by the endoplasmic reticulum stress	[24]

of arginase II from mitochondria to cytosol and back through LOX-1- and rho kinase-dependent mechanisms [27]. Mitochondrial processing peptidase was shown to be involved in the activation of arginase II. In ApoE-deficient mice, knockdown of arginase II resulted in decreased plaque progression, reduced oxidative stress, elevated NO production, and improved endothelial function [27]. Indeed, LOX-1 plays an important role in mediating oxLDL-induced endothelial dysfunction.

LOX-1 also mediates oxLDL uptake by ECs by stimulating c-Jun N-terminal kinase (JNK) and protein kinase C (PKC) β2 that leads to the activation of 66-kDa isoform of Shc adaptor proteins (p66Shc) [28]. p66Shc mediates hypertension-associated, cyclic stretch-dependent, endothelial damage [29]. Increased cyclic stretch to the vessel wall leads to endothelial dysfunction through elevated generation of reactive oxygen species (ROS) and reduced NO bioavailability.

LOX-1 induces endothelial apoptosis

LOX-1 is involved in handling oxLDL-induced apoptosis of ECs that could be prevented by statins (lipid-lowering drugs) and LOX-1-specific inhibitors [30]. This scavenger receptor was shown to down-regulate expression of anti-apoptotic proteins Bcl-2 and neuronal inhibitory apoptotic protein (NAIP) [17]. Moreover, LOX-1 stimulates caspase-3 and -9 that cleave both anti-apoptotic proteins [31].

In addition, the electronegative LDL fraction that actually represents oxidized lipids and unfolded apoB protein, a major protein component of LDL particles, was found to be extensively bound by LOX-1 but not the LDL receptor [32]. This LDL fraction through LOX-1 induces apoptosis of ECs by up-regulating expression of several proapoptotic proteins such as tumor necrosis factor (TNF-α), Bax, and Bcl-2-associated death promoter (Bad) and down-regulating B-cell lymphoma-extra-large (Bcl-xL) [33]. Down-regulation of Akt plays a crucial role in mediating proapoptotic effects of the electronegative LDL fraction on ECs since inhibition of phosphorylation of this kinase is associated with increased expression of LOX-1 [17]. Furthermore, electronegative LDL that circulate in blood of smokers were observed to alter the differentiation of endothelial progenitor cells (EPCs) to ECs by suppression of Akt phosphorylation via LOX-1 [34]. Indeed, these data suggest that Akt could be critically involved in modulation of LOX-1 expression.

LOX-1 induces senescence of ECs

OxLDL were shown to induce senescence of EPCs associated with decreased telomerase activity, diminished proliferation capacity, and ability to form capillary-like structures. Pretreatment of cultivated EPCs with atorvastatin or LOX-1-specific antibody delayed EPC senescence [35]. Compared to early cultures, late passage human umbilical vein ECs (HUVECs) exhibited significant increase in Bax/Bcl2 ratio and 3-fold increase in apoptotic response to TNF- α exposure [18]. Therefore, potential mechanism of the involvement of oxLDL in endothelial senescence may be related to enhanced apoptosis.

LOX1 mediates endothelial inflammatory activation and adhesion of leukocytes to ECs

LOX-1 contributes to the recruitment of monocytes to ECs, a key event in early pathogenesis of atherosclerosis. In ECs, oxLDL was shown to markedly increase production of chemokine (C-C motif) ligand 2 (CCL2) (also known as monocyte chemoattractant protein 1 (MCP1)) [16]. LOX-1 is crucially involved in induction of oxLDL-dependent expression of CCL2. In addition, LOX-1 mediates oxLDL-dependent stimulation of proinflammatory transcription nuclear factor (NF)- κ B that in turn drives expression of various adhesion molecules [36]. Finally, LOX-1 itself could act as a cell adhesion molecule for monocytes [37].

Influence of LOX-1 on Macrophages

As mentioned above, macrophages usually express low levels of LOX-1 in the norm. Furthermore, the impact of this receptor in ingestion of oxLDL is too small in healthy arteries [13]. Due to the richness of the arsenal of scavenger receptors, it is not easy to estimate the true input of LOX-1 macrophages. In normal macrophages, the impact of LOX-1 to oxLDL uptake accounts for only 5-10%. Proinflammatory cytokines stimulate LOX-1 expression and suppress expression of other SRs such as SR-AI and CD36 [3]. In proatherosclerotic conditions, when expression of LOX-1 is increased, the contribution of this receptor to oxLDL uptake by macrophages becomes sufficient and accounts for up to 40% [23]. Indeed, LOX-1 could play a crucial role in the transformation of macrophages to foam cells [17].

Notably, this receptor is not expressed in monocytes but could be induced in differentiated macrophages [38]. Macrophages could uptake oxLDL LOX-1 after stimulation by various ligands such as oxLDL, lysophosphatidylcholine (LPC) [23], palmitic acid [39], and increased glucose [40]. In non-stimulated macrophages, LOX-1 does not alter oxLDL uptake suggesting for its role for macrophages activated by proinflammatory stimuli.

Monocytes are able to differentiate to dendritic cells (DCs) that play an important role in priming proinflammatory activation of T cells in atherosclerosis [41]. OxLDL influences maturation and motility of DCs [42, 43]. In proinflammatory DCs, increased levels of LOX-1 expression were observed. Indeed, LOX-1 significantly activates oxLDL uptake by DCs since using an antibody against LOX-1 decreases uptake of oxLDL by nearly two-fold [44].

Influence of LOX-1 on Vascular Smooth Muscle Cells

Expression of LOX1 in VSMCs could be stimulated by multiple signals including angiotensin II and oxLDL [45]. Expression of LOX-1 in aorta could be induced by vascular wounding as was shown in a balloon-injury model in rabbits [19] and rats [20]. Moreover, a colocalization LOX-1 and oxLDL was shown in VSMCs in human restenotic plaques thereby indicating the involvement of LOX-1 in oxLDL-dependent VSMC proliferation and restenosis [19].

In cultured rat VSMCs, oxLDL-induced expression of LOX-1 was shown to activate VSMC growth and proliferation (Table 1). After balloon angioplasty, elevated LOX-1 expression was also detected in the neointimal zones of atherosclerotic human coronary arteries. Furthermore, LOX-1 was colocalized with proliferating cell nuclear antigen (PCNA) [19].

These findings suggest that LOX-1 mediates oxLDL-dependent VSMC proliferation and is involved in neointima formation after vessel wounding. In apolipoprotein E (apoE)-deficient mice, LOX-1 knockout results in substantial decrease in VSMC proliferation and migration [9]. In LDL receptor (LDLR)-deficient mice, genetic deletion of LOX-1 causes great reduction in intra-plaque collagen deposits [46].

Increased levels of oxLDL were found to stimulate LOX-1 expression in VSMCs followed by VSMC apoptosis [47]. OxLDL-induced apoptosis of arterial VSMCs could therefore be involved in atherosclerotic lesion vulnerability and rupture [5]. In VSMCs, proapoptotic effects of OxLDL were shown to be related to down-regulation of B-cell lymphoma 2 (Bcl-2), an antiapoptotic protein and up-regulation of proapoptotic regulator Bcl-2-associated X protein (Bax). In human lesions, LOX-1 colocalizes with Bax suggesting for the contribution of LOX-1 in oxLDL-mediated plaque rupture [7].

LPC is known to be a product of hydrolysis of oxLDL mediated by phospholipase A2 [48]. This bioactive lipid exhibits a strong proatherogenic effect. VSMCs exposed to LPC start to express LOX-1 followed by increased uptake of oxLDL [49]. Indeed, LOX-1 could mediate oxLDL-induced transformation of oxLDL to foam cells [50]. In summary, oxLDL up-regulation shows a variety of proatherogenic effects in VSMCs including apoptosis, increased VSMC proliferation and migration, and enhanced foam cell formation, which in turn promotes plaque destabilization, neointima formation, and increase in lipid-rich lesion necrotic core.

Conclusion

In atherosclerosis, LOX-1 could represent a major mediator of proatherogenic activity of oxLDL in vascular cells especially in ECs. OxLDL-induced up-regulation of LOX-1 results in increased uptake of oxLDL by vascular cells and enhances proatherosclerotic and proinflammatory effects of oxLDL. Indeed, a positive feedback exists between oxLDL and its receptor [3]. An essential progress was achieved in deciphering of signaling pathways mediated by LOX1. However, many puzzles should be resolved to enrich our knowledge of the proatherogenic role of LOX-1. For example, it is interesting to know more about detailed mechanisms by which LOX-1 contributes to oxLDL-induced transformation of VSMCs to foam cells. The oxLDL/LOX-1 axis represents one of the key pathogenic tools in induction of atherosclerosis and therefore represents a promising target for anti-atherosclerotic therapy.

Abbreviations

AGEs (advanced glycation end-products); Akt (Protein kinase B); apo (apoprotein); apoE (apolipoprotein E); Bad (Bcl-2-associated death promoter); Bax (Bcl-2-associated X protein); Bcl-2 (B-cell lymphoma 2); Bcl-xL (B-cell lymphoma-extralarge); CCL2 (chemokine (C-C motif) ligand 2); DCs (dendritic cells); ECs (endothelial cells); eNOS (endothelial nitric oxide synthase); EPCs (endothelial progenitor cells); HUVECs (human umbilical vein ECs); JNK (c-Jun N-terminal kinase); LDL (low density lipoprotein); LOX-1 (oxidized low-density lipoprotein receptor-1); LPC (lysophosphatidylcholine); MCP1 (chemotactic protein 1); NAIP (neuronal inhibitory apoptotic protein); NF- κ B (nuclear factor- κ B); oxLDL (oxidized LDL); p66Shc (66-kDa isoform of Shc adaptor proteins); PKB (Protein kinase B); PKC (protein kinase C); ROS (reactive oxygen species); SRs (scavenger receptors); TNF- α (tumor necrosis factor- α); VSMCs (vascular smooth muscle cells).

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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