The Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 and Cardiovascular Disease

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Abstract:

Lectin-like oxidized LDL receptor-1 (LOX-1), a lectin-like 50-kD receptor for oxidized low-density lipoproteins (ox-LDL), is present primarily on endothelial cells. Oxidatively modified low-density lipoprotein (oxLDL) is implicated in the pathogenesis of atherosclerosis. Endothelial dysfunction is the initial change in the vascular wall that induces morphological changes for atheroma-formation. LOX-1 was identified as the receptor for oxLDL that was thought to be a major cause of endothelial dysfunction. LOX-1 has been demonstrated to contribute not only to endothelial dysfunction, but also to atherosclerotic-plaque formation, hypertension, myocardial infarction and intimal thickening after balloon injury. Studies with transgenic and knockout mouse models have elucidated in part the role of LOX-1 in the pathogenesis of atherosclerosis and cardiac remodeling. Recently, a circulating soluble form of LOX-1(sLOx-1), corresponding solely to its extracellular domain, has been identified in human serum. Circulating levels of sLOX-1 are increased in inflammatory and atherosclerotic conditions and are associated with acute coronary syndrome, with the severity of coronary artery disease, and with serum biomarkers for oxidative stress and inflammation, suggesting that they could be useful marker for vascular injury. Identification and regulation of this receptor and understanding of signal transduction pathways might open new gateways from diagnosis to therapeutics for cardiovascular diseases.

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Introduction:

Atherosclerosis is the narrowing or occlusion of the arteries by a plaque, and is the most important contributor to the growing burden of cardiovascular disease.[1, 2] The major risk factors of atherosclerosis, such as hypertension, diabetes, smoking and free radicals, have been known to induce endothelial dysfunction. Endothelial dysfunction, functional changes in endothelial cells, has been thought to precede morphological changes of atheroma.[3,4] Modification of low-density lipoprotein (LDL) in the arterial wall, particularly by oxidation, is crucial to the cellular uptake of LDL in the early stages of the atherosclerotic plaque development. [5]

The lectin-like oxidized low-density lipoprotein (LDL) receptor LOX-1 (the type D scavenger receptor) was initially cloned from bovine aortic endothelial cells (BAECs) in 1997 by Sawamura et al via its ability to bind LDL oxidized ex vivo by copper (oxLDL).[6] LOX-1 has been demonstrated to actively contribute to all stages of atherogenesis.LOX-1 is expressed not only in endothelial cells (ECs), but also in macrophages, vascular smooth muscle cells and platelets.[7] LOX-1, a membrane glycoprotein that binds oxLDL and acetylated LDL (AcLDL) but not native LDL, differs from the macrophage scavenger receptors because it contributes to the atherosclerotic process mainly through receptor-mediated signaling mechanisms.[8] LOX-1 is a multiligand receptor that can also recognize multiple classes of ligands, such as, neutrophils, apoptotic/aged cells and bacteria,[9] implying versatile physiological functions. In vitro, the basal expression of LOX-1 in endothelial cells is limited; however, it can be rapidly induced by proinflammatory, pro-

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oxidative and mechanical stimuli.[10] In vivo, the basal expression of LOX-1 is also low, but can be enhanced by several pathological conditions, including hypertension,[11] diabetes mellitus,[12] hyperlipidemia,[13] and chronic renal failure.[14] This review summarizes recent findings concerning the structure, function, and regulated expression of LOX-1 in relation to its potential roles in atherosclerosis, hypertension, myocardial infarction and intimal thickening after balloon injury.

Lox-1 Dependent Intracellular Signaling Pathways

LOX-1 is expressed in most cell types relevant to the development of atherosclerotic plaques and the interaction of LOX-1 with its ligands modifies the cell phenotype in pro-atherogenic sense, so that the cell become dysfunctional and more prone to death.[15] (Table 1) Several reports have revealed that elevated plasma levels of oxLDL are associated with coronary artery diseases (CAD).[16] The plasma levels of oxLDL are related to the presence of angiographically detected complex and thrombotic lesion morphology in patients with unstable angina.[17] LOX-1 has been recently shown to form a complex with MT1-MMP under a basal condition. When oxLDL binds to LOX-1 it induces rapid RhoA and Rac1 activation via MT1-MMP, which results in NADPH oxidase activation and eNOS downregulation.[18] The imbalance of NO and oxidative stress resulting from the binding of oxLDL to LOX-1 causes oxLDL-induced endothelial dysfunction leading to atherosclerosis. OxLDL has dual effects on cultured cells depending on its concentration and exposure time: at a lower concentration (from 5 to 10 μg/mL) and shorter exposure time it induces proliferation, whereas at a higher concentration (from 50 to 300 μg/mL) and longer exposure time it induces apoptosis in ECs, macrophages, and SMCs.[19,20] In addition to the Rho and Rac pathways, the following signal transduction pathways have been reported to be activated via LOX-1:p38 mitogen-activated protein kinaseC (MAPK), p44/42MAPK, protein kinase C, protein kinase B, ERK1/2, protein tyrosine kinase and NF-êB.[21] Among them, LOX-1-mediated NF-êB activation by oxLDL is crucial for increasing the expressions of the following adhesion molecules: E- and P-selectins, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1),[22] which brings proinflammatory changes to the vessel wall. Additionally, LOX-1 activation changes endothelial cells and smooth muscle cells prone to apoptosis by increasing the Bcl-2-associated X protein (Bax)/Bcl-2 ratio.[7] (Fig.1)

Table-I

Cellular effects of ligand-LOX-1 interaction on atherogenesis.

Endothelial cells
Alteration of vascular tone
Increased intracellular oxidative stress
Induction of apoptosis
Induction of proliferation and angiogenesis by increasing VEGF † expression
Increased expression of adhesion molecules (VCAM-1*, ICAM-1**, Selectins)
Increased expression of monocyte chemoattractant protein-1
Induction of plasminogen activator inhibitor-1
Reduction of endothelial nitric oxide synthase
Release of matrix metalloproteinases
Smooth muscle cells
Induction of apoptosis
Monocytes
Induction of monocyte adhesion and activation
Increased oxLDL uptake and foam cell formation

† Vascular endothelial growth factor, * vascular cell adhesion molecule-1; ** Intercellular cell adhesion molecule-1.

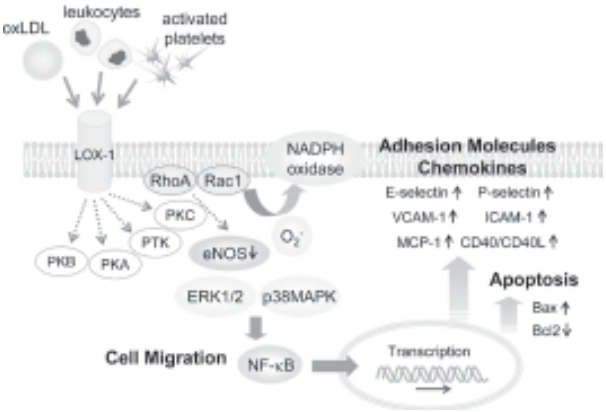

Fig.1. *Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) signaling pathway. MAPK, mitogen-activated protein kinase C; PKC, protein kinase C; PKB, protein kinase B; PTK, protein tyrosine kinase; eNOS, endothelial nitric oxide synthase; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemotactic protein-1.*

Regulation of LOX-1 Expression

In the in vitro setting, the basal expression of LOX-1 in endothelial cells is very low. However, it can be rapidly induced by pro-inflammatory, pro-oxidant and mechanical stimuli such as ox-LDL Ang II , cytokine tumor necrosis factor-α (TNF-α) and shear stress ,lipopolysaccharide, phorbol 12-myristate 13-acetate (PMA), heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF).[23] In ECs, glycoxidized LDL and oxLDL significantly increase LOX-1 expression and production of sLOX-1,although the effect of glycoxidized LDL is greater than that of oxLDL. In addition in the same cells, advanced glycation enproducts(AGEs), C- reactive protein (CRP),high glucose and IL-6 induce expression of LOX-1.[24] Furthermore, lysophosphatidylcholine, the main phospholipid component of oxLDL, as well as super-oxide anions, hydrogen peroxide and homo cycsteine up-regulate the expression of LOX-1 mRNA.[25] Transforming growth factor which plays a crucial role in vascular remodeling and the pathogenesis of atherosclerosis induces the expression of LOX-1 in both EC and SMCs. In addition, in SMCs, LOX-1 acts as a receptor for remnant-like lipoprotein particles (RLPs) that up-regulate its expression and induce cell migration, suggesting a proatherogenic role, especially in the settings of post-prandial hyperlipidemia, diabetes and metabolic syndrome.[26, 27] The most widely acknowledged stimuli for LOX-1 expression are shown in Table 2. In the in vivo settings, basal LOX-1 expression is also low, but can be enhanced by several pathological conditions, including hypertension, diabetes mellitus and hyperlipidemia,[28] as well as others which are also presented in Table 2 LOX-1 expression in cardiac myocytes, as well as in vessel walls of falling rat. hearts in vivo, is induced by neurohormonal factors activated in heart failure such as norepinephrine and ET-1. In this regard, since increased protease activity is a key feature of plaque instability that may achieve an enhancement of sLOX-1 release, cardiac myocytes, may be another source of sLOX-1.[29] Since most of these pathological conditions (inflammation, hyperlipidemia, hypertension and diabetes) are directly or indirectly associated with atherogenesis,[30] their presence could have additive or synergistic effects on the regulation of LOX-1 gene expression.

Table-II

In vitro and in vivo stimuli for LOX-1 gene upregulation

In vitro stimuli for LOX-1 upregulation
Angiotensin II (Ang II)
C-reactive protein (CRP)
Endothelin-1 (ET-1)
Glucose
Histamine
Homocysteine
Human cytomegalovirus (HCMV)
Interferon-g (IFN-g)
Interleukin-1h (IL-1h)
Oxidant species
Oxidized-low density lipoprotein (ox-LDL)
Phorbol ester
Shear stress
Transforming growth factor-h (TGF-h)
Tumor necrosis factor-a (TNF-á)
In vivo conditions for LOX-1 upregulation
Atherosclerosis
Diabetes mellitus
Hyperlipidemia
Hypertension
Ischemia reperfusion injury
Transplantation

LOX-1 and C - Reactive Protein (CRP)

The prospective Physicians' Health Study (PHS), high plasma concentration of CRP was associated with a 2-fold increase in risk of stroke, a 3-fold increase in risk of myocardial infarction (MI), and a 4-fold increase in risk of developing peripheral vascular disease.[31, 32] Evidence has shown that CRP and LOX-1 share a range of biological functions.LOX-1 mRNA and proteins are induced by CRP, resulting in increased monocyte adhesion to endothelial cells and oxLDL uptake.[7] Furthermore, it has been shown that CRP-LOX-1binding enhances vascular permeability in vivo in SHR-SP rat's.[33] LOX-1 exhibits binding activity for multiple ligands, all of which are also recognized by CRP.[34] CRP binding to LOX-1 enhances the binding affinity of oxLDL to LOX-1.[35] CRP interaction with LOX-1 might play a role in atherogenetic inflammation, which is relevant to endothelial dysfunction.

Lox-1 Gene: Structure and Association with Coronary Artery Disease (CAD)

LOX-1 has a molecular weight of 50 kDa and is a type II membrane protein belonging to the C-type lectin family. LOX-1 consists of four domains: a short N-terminal cytoplasmic domain, a single transmembrane domain, a connecting stalk region (neck) domain, and a lectin-like extracellular domain at the C-terminus which binds oxLDL.[6] (Fig.2) LOX-1 is encoded by the oxLDL receptor 1 (OLR1) gene, located in the natural killer gene complex on chromosome 12 at p12-p13, which also contains several other families of lectin-like genes, including the CD94 and NKG2 NK receptor genes.[36] The region telomeric of CD94 contains in addition to the LOX-1 gene, the novel human DECTIN-1 and the CLEC-1 and CLEC-2 genes within about 100 kb. The OLR1 gene spans more than 7000 base pairs (bp),and consists of 6 exons interrupted by 5 introns; exons 1-5 range from 102 to 246 bp, whereas exons 6 is relatively long, being 1722 bp . Several groups have analyzed the association of LOX-1 gene polymorphisms with CAD. The 3'-untranslated region (UTR) (T allele), a C-to-T change 188 nucleotides from the stop codon (+188C-T), was associated with a higher risk of acute MI.[37] Furthermore, 7 different single nucleotide polymorphisms (SNPs), 6 of them located within introns 4,5 and 3' UTR comprised in a linkage disequilibrium block, exhibited a significant association with an elevated risk of developing MI.[38] SNP leading to the missense mutation of Lys to Asn at the 167th amino acid residue (K167N) was identified and reported to be associated with an increased risk of MI.[39] Presently, we do not have enough evidence to determine whether the SNPs in the LOX-1 gene are useful to assess the risk and prognosis of CAD.

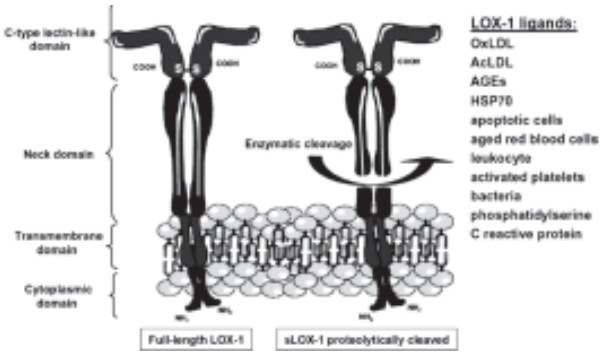

Fig. 2. *Structure of the full-length LOX-1 and its soluble form.*

The extracellular region of the primary structure of LOX-1 consists of C-type lectin-like domain at the C-terminus, followed by a connecting stalk region (neck domain).The full- length LOX-1,in addition to the extracellular domain, displays a single transmembrane spanning region and a short N-terminal cytosolic tail. As illustrated, LOX-1 exists as a disulfide-linked homodimer at cysteine 140 on the cell surface. The circulating form derives form enzymatic cleavage at two potential sites of the full-length cell-surface receptor, between Arg(86)-Ser(87) or Lys(89)-Ser(90) residues located in the membrane proximal extracellular domain, and consists only of a part of the extracellular domain of the receptor (a portion of the neck and the entire C-terminal domain). The ligands of LOX-1 are shown on the right side of the figure.

Role of LOX-1 in the Vascular System: Atherosclerosis and Restenosis

Ox-LDL cause's activation followed by dysfunction of endothelium, which appears in the early stages of atherogenesis.Ox-LDL, stimulates expression of chemokines and adhesion molecules such as MCP-1, E-and P-selectins, VCAM-1 and ICAM-1 on endothelial cells.[40] These molecules facilitate the adhesion of monocytes to endothelium, initiating atherosclerosis. Ox-LDL can cause the release of matrix metalloproteinases (MMPs) without significant effect on tissue inhibitors of metalloproteinase (TIMPs).[41] This may well be the basis of the rupture of soft plaque in acute coronary syndromes. Ox-LDL has also been shown to induce the production of superoxide anion in bovine aortic endothelial cells, while impairing endothelial nitric oxide synthase (eNOS) activation, and consequently the release of nitric oxide (NO) falls.[42] These effects result in increased intracellular oxidative stress, which is a major stimulus for endothelial dysfunction.LOX-1 also plays a critical role in the expression of two smooth muscle/fibroblast directed growth factors, the A and B chains of PDGF and heparin-binding EGF-like protein (HB–EGF), in cultured human endothelial cells.[43] The expression and secretion of these growth factors are associated with the migration and proliferation of smooth muscle cells and fibroblasts, leading to the progression of atherosclerosis. On a high-fat diet, compared with ApoEKO mice, LOXtg/ApoEKO mice showed more

pronounced oxLDL accumulation, oxidative stress detected by 8-hydroxy-2'-deoxyguanosine (8-OH-dG), increased expression of ICAM-1 and VCAM-1, and infiltration of macrophages in the heart vessels. Furthermore, atheroma like lesions in the intramyocardial vessels increased 10-fold in LOXtg/ApoEKO mice compared with ApoEKO mice LOX-1-deficient mice by deleting a part of the lectin-like domain that is essential for ligand binding. LOX-1-deficient mice were resistant to oxLDL-induced impairment of endothelium-dependent vasorelaxation.When crossed with atherosclerosis-prone LDL receptor (LDLR) KO mice; the formation of atherosclerotic lesions was significantly reduced in the aorta of LOX-1/LDLR double KO mice. The serum cholesterol levels were comparable between the mice. The expression of NF-êB and infiltration of CD68-positive cells were decreased whereas anti-inflammatory cytokine IL-10 expression and superoxide dismutase activity were increased in the double KO mice.[44] LOX-1 gene deletions also resulted in less arterial collagen accumulation in LDL KO mice. These gain-of-function and loss-of-function approaches clearly demonstrate that LOX-1 plays a role in the development of atherosclerosis.LOX-1 is likely to mediate inappropriate arterial remodeling, which is one of the causes of atherosclerosis and restenosis. In a balloon-injured carotid artery, strong LOX-1 expression was observed at first in injured medial smooth muscle cells, then in proliferating intimal smooth muscle cells, and finally in the regenerated endothelial cells. The reactive intimal thickening, ROS generation and leukocyte infiltration were attenuated by the administration of anti-LOX-1 antibody.[45] Gene silencer pyrrole-imidazole (PI) polyamide targeting the rat LOX-1 gene promoter (PI polyamide to LOX-1) inhibits neointima thickening and preserves the re-endothelialization after balloon injury.[46]

Role of LOX-1 in Thrombosis and Myocardial Infarction (MI)

Thrombosis is usually the event that leads to myocardial ischemia and stroke, and platelets are the usual initiators in this process.[47] Kakatani et al.[48] found LOX-1 antibody decreases arterial thrombus formation in the rats, suggesting a contributory role of LOX-1 in ox-LDL-mediated platelet activation and thrombosis. Firstly, ox-LDL is present in the atherosclerotic tissues of rupture-prone segments; secondly, the degree of myocardial ischemia is characterized by the oxidative state, which facilitates the oxidation of native-LDL,[49] and thirdly, an in vitro study showed that perfusion with ox-LDL significantly decreases myocardial contraction in the isolated rat heart.[50] LOX-1 is also readily detectable in cardiomyocytes under prooxidative conditions. (**Fig. 3**) In vitro, a blockade of LOX-1 inhibits NF-êB activations and diminishes apoptosis, which suggests that LOX-1 plays a role in oxidative stress in cardiomyocytes. In vivo, the expression of LOX-1 in cardiomyocytes is prominently induced by ischemia–reperfusion. Treatment with anti-LOX-1 antibody prevents cardiac remodeling in a rat model of myocardial ischemia–reperfusion. It also reduces the size of myocardial infarct and improves left ventricular function by inhibiting apoptosis and lipid oxidation in cardiomyocytes.[51]

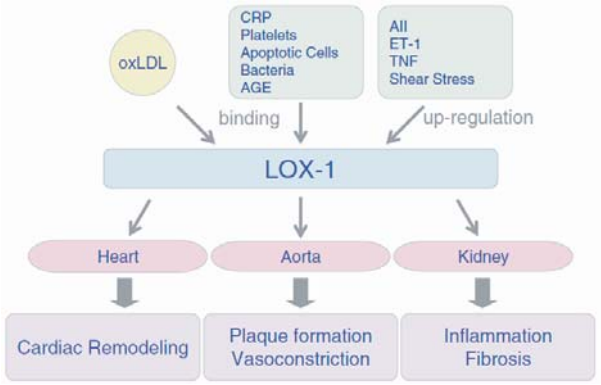

Fig. 3. *LOX-1 is bound and/or upregulated by the various factors indicate. The functions of LOX-1 contribute to cardiac remodeling, plaque formation, vasoconstriction and renal injury. The coordination of these triggers the onset of multiple organ damage.*

LOX-1 and Hypertension

Chronic kidney disease is a risk factor for cardiovascular diseases and the enhanced progression of renal dysfunction has been reported in patients with obesity and hypertension. Several reports showed the association between LOX-1 and renal dysfunction. (**Fig. 3**) Nagase et al.[11] Reported that LOX-1 mRNA expression is minimal in the aorta from normal rats, but is markedly upregulated in spontaneously hypertensive rats,

suggesting a correlation between LOX-1 and hypertension. This concept is supported by the in vitro observations that Ang II upregulates LOX-1 gene expression in endothelial cells and ACE inhibitors markedly decrease LOX-1 gene expression.[52] LOX-1 is a key modulator of the development of angiotensin II–induced hypertension.[53]All these findings suggest that LOX-1 may contribute to the pathogenesis of hypertension which is induced by the activation of RAS.

LOX-1 as A Clinical Diagnostic Tool

Soluble LOX-1

A number of membrane proteins are cleaved into soluble molecules by proteolysis at the membrane-proximal site of the extracellular domain, which is known as ectodomain shedding. LOX-1 is also released in a soluble form from the cell surface into culture media. There are 2 cleavage sites that have been identified to yield soluble LOX-1(sLOX-1; i.e., Arg86-Ser87 and Lys89-Ser90 bonds of bovine LOX-1).[54] **Fig.2**A member of the A Disintegrin and Metalloproteinase (ADAM) family, ADAM10, contributes to the proteolysis of LOX-1.[55] A community-based cohort study, measured LAB (apolipoprotein B) and sLOX-1 using ELISAs with recombinant LOX-1 and monoclonal anti–apolipoprotein B antibody and with 2 monoclonal antibodies against LOX-1, respectively - Higher LOX index [LOX index = LAB x sLOX - 1] values were associated with an increased risk of CHD.[56] Recently, it has been reported that the serum sLOX-1 level is specifically elevated in acute coronary syndrome.[57] Peak values of sLOX-1 are observed earlier than those of Troponin T in acute coronary syndrome. Furthermore, the sensitivity and specificity of sLOX-1 in acute coronary syndrome are significantly better than high sensitivity CRP.

LOX-1 Ligand Assay

A receptor-based assay to determine the levels of modified LDL as LOX-1 ligands has been reported.[58] Although clinical assessment is yet to be carried out; the LOX-1 ligand level is elevated in hyperlipidemic animals in association with the extent of atheroma-formation. The circulating amount of oxidation-related moiety on LDL has been shown to be effective in predicting or diagnosing metabolic syndrome and cardiovascular diseases. The oxidized phospholipid: apo B-100 ratio and Lp(a) lipoprotein levels are strongly associated with the presence and extent of coronary artery disease. [59, 60]

Imaging

Taking an advantage of the relatively selective expression of LOX-1 in atheroma, the methodology to obtain images of atheroma utilizing the anti-LOX-1 antibody was reported. 99mTc-labeled anti-LOX-1 antibody administered into MI prone Watanabe heritable hyperlipidemic rabbits accumulated in each aortic segment, which was a significantly higher level than that in control rabbits. High-density accumulation is also observed at collagen-rich and neointimal lesions. This nuclear imaging technique might be useful for the diagnosis of plaque vulnerability because of the similarity of the histologic characteristics of atherosclerotic plaques in humans and WHHLMI rabbits. 99mTc-LOX-1-mAb could be useful as a potential imaging probe for predicting lesions prone to spontaneous rupture and monitoring the effects of timely treatment in patients with advanced atherosclerosis.[61] Clinical application of LOX-1 would be diagnostic rather than therapeutic, while it warrants further investigations.

Conclusion:

Identification of LOX-1 and a definition of its biologic role in pathophysiologic states provide a new clue to the reason for the uptake of ox-LDL by vessel wall components. Internalization of ox-LDL leads to a cascade of events that may induce a variety of diseases characterized by endothelial dysfunction, activation and injury. Activation of endothelial cells by ox-LDL through LOX-1 may be a key event in atherosclerosis, hyperlipidemia, hypertension, and myocardial infarction. As such, therapies targeting LOX-1 may be effective strategies for treating atherosclerotic and hypertensive patients. LOX-1 showed unexpectedly diverse ligand specificity with versatile functions, which points to a new avenue of research.

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