

# LOX-1 biology and targeting LOX-1 in cardiovascular diseases

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## Abstract

Oxidized low-density lipoprotein (ox-LDL) plays a critical role in a variety of cardiovascular diseases, including atherosclerosis, hypertension and myocardial ischemia. Encoded by OLR1 gene, lectin-like ox-LDL scavenger receptor-1 (LOX-1) is the major receptor for ox-LDL at the surface of the cells. LOX-1 activation by ox-LDL, reactive oxygen species (ROS), angiotensin II and inflammatory signals causes many pathophysiological events, such as cell proliferation, apoptosis and autophagy, which are hallmarks of the atherosclerotic lesions. LOX-1 is also involved in mitochondrial DNA damage mediated inflammatory response. All these observations support the possible contribution of LOX-1 to the pathogenesis of cardiovascular disorders, particularly atherosclerosis, indicating that targeting LOX-1 may be an effective strategy for the treatment of cardiovascular diseases. This review summarizes current knowledge of LOX-1 function and possible therapeutic options targeting LOX-1 in cardiovascular diseases.

## Introduction

LOX-1 was initially identified as a main receptor for ox-LDL in endothelial cells by Sawamura *et al.* in 1997 [1]. Further study found that LOX-1 is also expressed in other types of cells, such as smooth muscle cells, macrophages, cardiomyocytes and neurons [2-5]. Under physiological conditions, LOX-1 expression is minimal, but it can be significantly elevated by diverse stimuli, such as reactive oxygen species (ROS), ox-LDL, angiotensin II and high glucose concentration [6-9]. Interestingly, in human primary aortic or umbilical vein endothelial cells and smooth muscle cells, ox-LDL can upregulate its own receptor expression at transcriptional level in a time- and concentration-dependent manner [10,11]. Pretreatment of the specific antibody or siRNA directed at LOX-1 can block the upregulation of LOX-1 in response to ox-LDL. Recently, we reported that LOX-1 has a higher expression in smooth muscle cells than endothelial cells [12], consistent with the concept that smooth muscle cells play a more important role in the development of atherosclerosis. Besides atherosclerosis, LOX-1 expression is also higher in several pathological conditions, including inflammation, hypertension and myocardial ischemia [13].

Atherosclerosis is a chronic inflammatory disease that involves abnormal lipoprotein metabolism, intimal hyperplasia and the release of inflammatory factors [13]. Honjo *et al.* showed that LOX-1 inhibition by pretreatment with its antibody attenuated inflammatory response in rats given lipopolysaccharide (LPS), suggesting the role of LOX-1 in inflammation [14]. Kelly *et al.* also showed that LOX-1 inhibition decreased expression of anti-inflammatory transcription factor peroxisome proliferator-activated receptor- $\delta$  in NRK52E cells [15]. Recently, Shin *et al.* found that LOX-1 activation is involved in NAD(P)H oxidase-dependent superoxide formation and induction of cytokines in human endothelial cell line [16].

More and more evidence indicates that cell death, including

autophagy and apoptosis, is the major modulating factor in the development of atherosclerosis. Autophagy is a highly conserved and cellular self-degradative process that response to stress especially in starvation, responsible for the balancing sources of energy, removing misfolded proteins and recycling of damaged organelles [17]. There is high genome similarity between mitochondria and bacteria because mitochondria originally evolved from bacteria. Similar to bacteria, mitochondrial DNA (mtDNA) often contains inflammatory unimethylated CpG motifs, which triggers immune cells to recognize mtDNA as invading bacteria and induces inflammatory response [18]. Unlike cellular nuclei that have multiple mechanisms for DNA repair, mitochondria appear limited in their ability to rectify all possible forms of DNA damage. Damaged mtDNA are often degraded by autophagy and other biological enzymes [3,14,27]. With cultured human THP-1 macrophages, we reported that LPS induced mtDNA damage could trigger autophagy and NLRP3 inflammasome activation, and LOX-1 inhibition by its siRNA transfection markedly decreased mtDNA damage and attenuated inflammatory response [3]. Similarly, with cultured human endothelial cells, we also showed that escaped damage mtDNA from autophagy could trigger TLR9 expression, while LOX-1 seems to play a critical role in this signaling pathway [6].

Similar to autophagy, apoptosis is another cell death program, a major event in the pathophysiology of atherosclerosis. Endothelial cells apoptosis results in increased vascular permeability to lipids,

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cell proliferation and increased coagulation, while smooth muscle apoptosis may contribute to atherosclerotic plaque destabilization [19]. LOX-1 activation can induce apoptosis in many cell types, including endothelial cells, smooth muscle cells and macrophages, which are three main components of blood vessels [7]. Under physiological conditions with shear stress at 13 dynes/cm<sup>2</sup>, LOX-1 has been shown to regulate apoptosis activity in endothelial cells by influencing apoptotic proteins cytochrome c, Bax, Bcl-2 and Bcl-xL [20]. Lu *et al.* [21] reported that LOX-1 inhibition by its siRNA transfection reduced L5 uptake. L5 is an electronegative component of LDL abundant in dyslipidemic, which is upregulated by LOX-1 activation and induced apoptosis. Takabe *et al.* [22] found that LOX-1 is involved in ox-LDL-induced c-Jun N-terminal kinase (JNK) activation, which is one of the main signaling pathways in endothelial cell apoptosis. We [23] examined the expression of autophagy and apoptosis in human primary aortic endothelial cells and smooth muscle cells treated with ox-LDL or LPS, which further confirmed that LOX-1 is involved in these two types of cell death. As expected, pretreatment with the LOX-1 inhibitor miRNA hsa-let-7g or its siRNA transfection declined protein expression of autophagy and apoptosis. Sun *et al.* [24] also found that LOX-1 is involved in smooth muscle cell apoptosis, which is mediated by ROS and nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation. Compared with apoptosis in endothelial cells and smooth muscle cells, macrophage apoptosis may contribute to enlargement of the lipid core, result in lipid accumulation and transformation into foam cells.

Atherosclerosis is the cause of myocardial ischemia and several other cardiovascular maladies. It appears that ox-LDL is more potent in the development of atherosclerosis than native LDL. The above discussion suggests strongly that ox-LDL initiates and sustains atherogenesis by activation of LOX-1. Indeed, we first described that LOX-1 deletion reduces atherogenesis in LDLR knockout mice fed high cholesterol diet [33]. Besides atherosclerosis, human LOX-1 gene has been also confirmed to be associated with several other cardiovascular diseases. Myocardial ischemia-reperfusion injury results from temporary cessation of coronary blood supply, a clinically relevant problem associated with thrombolysis, percutaneous coronary interventions, and coronary bypass surgery. In mice with myocardial ischemia-reperfusion injury, Kataoka *et al.* [26] found that, compared with normal IgG or saline administration, LOX-1 inhibition by its antibody treatment resulted in a nearly 50% reduction in myocardial infarct size. Pretreatment with LOX-1 antibody in rats with ischemia-reperfusion model, Li *et al.* [27] reported that LOX-1 inhibition prevented adhesion molecule expression and leukocyte recruitment. To further determine the role of LOX-1 in ischemia-reperfusion injury to the heart, Hu *et al.* [28] subjected wild type and LOX-1 knockout mice to 60 min of left coronary artery occlusion followed by 60 min of reperfusion, which found that LOX-1 knockout resulted in a significant reduction in myocardial injury and in accumulation of inflammatory cells. Nucleotide polymorphisms (SNPs), a missense mutation of LOX-1 protein, is also involved in myocardial infarction [29].

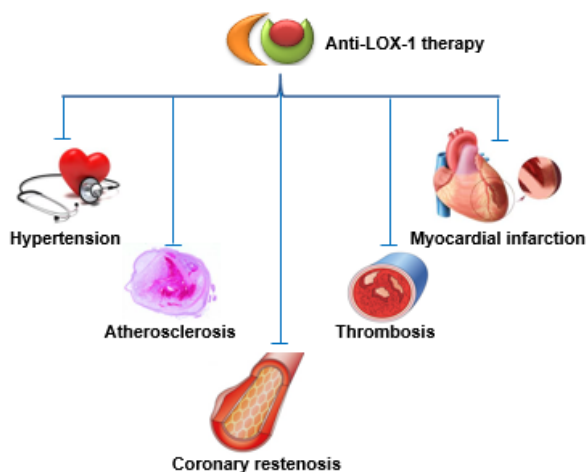
Besides myocardial ischemia-reperfusion, hypertension is another major factor in the development of atherosclerosis. Renin-angiotensin system (RAS) activation has been suggested to play an important role in the pathogenesis and evolution of hypertension. As the key product of the RAS activation, angiotensin II has two main receptors: type 1 receptor (AT1R) and type 2 receptor (AT2R). *In vitro*, angiotensin II elevated LOX-1 expression in both endothelial cells and smooth muscle cells, but this elevation can be blocked by angiotensin-converting enzyme inhibitors and AT1R blockers [30,31]. *In vivo*, compared with

WT mice, LOX-1 knockout mice reveal decreased blood pressure and attenuated cardiac hypertrophy following long-term angiotensin II infusion [32].

It has been proposed that LOX-1 is an attractive target for the therapy of a number of cardiovascular disease states. Some investigators have described substrate mimics or natural inhibitors that inhibit LOX-1 activity, albeit in large concentrations. With an antagonist of LOX-1 receptor that modified oxidized phospholipid named PLAzPC, Falconi *et al.* [33] found that PLAzPC has high inhibitory activity against ox-LDL binding to LOX-1. Further chemical analysis showed that PLAzPC completely disables the hydrophobic component of the ox-LDL recognition domain, indicating that PLAzPC may be an attractive therapeutic molecule to anti-LOX-1. Similar to ox-LDL, acetyl-LDL also can be degraded by scavenger receptors, including LOX-1. With cultured macrophages, Yoshiizumi *et al.* [34] found that, as an analogue of LOX-1, 2,4-bis(benzenesulfonic acid) sodium salt showed significant inhibitory activity against uptake of acetyl-LDL by macrophages. Statins are a class of cholesterol lowering drugs that are often prescribed by doctors to anti-atherosclerosis. With human coronary artery endothelial cells, we found that two different statins, simvastatin and atorvastatin significantly inhibited ox-LDL induced LOX-1 expression [35]. Further study showed that both simvastatin and atorvastatin inhibited LOX-1 mediated adhesion molecule expression, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Consistently, with molecular docking simulation, Biocca *et al.* [36] found that four types of statins, atorvastatin, fluvastatin, lovastatin and pravastatin could bind C-type lectin-like recognition domain of LOX-1, and further impaired LOX-1 receptor activity. Amati *et al.* [37,38] utilized Schizophyllan (SPG), a polysaccharide that belongs to the  $\beta$ -(1-3) glucan family, to inhibit LOX-1 gene and proposed it as a possible treatment for atherosclerosis. Using RAW 264.7 cells, they found that even the lowest concentration of SPG tested was able to inhibit the expression of LOX-1. In the apolipoprotein E knockout mice, they found that, compared with phosphate-buffered saline group, mice treated with SPG had a 63% reduction in the LOX-1 protein level in aortas. Nishizuka *et al.* [46] identified inhibitors from food extracts and found that procyanidins inhibited ox-LDL uptake in CHO cells expressing LOX-1 as well as the uptake of ox-LDL. Recently, using virtual screening techniques, our group identified several small molecule inhibitors of LOX-1 and tested their inhibitory potential using differential scanning fluorimetry and cellular assays. Two of these molecules significantly reduced the uptake of ox-LDL by human endothelial cells, LOX-1 transcription and the activation of extracellular-signal-regulated kinases 1/2 (ERK1/2) and p38 mitogen-activated protein kinases (MAPKs) in human endothelial cells. In addition, these molecules suppressed ox-LDL-induced vascular cell adhesion molecule 1 (VCAM-1) expression and monocyte adhesion onto human endothelial cells demonstrating their therapeutic potential.

### Clinical implications

LOX-1 biology and LOX-1 based therapy have been studied for near 20 years, the existing evidence indicates that LOX-1 is a potential target to treat ox-LDL mediated athero-thrombosis. Some cardiovascular diseases, such as hypertension, atherosclerosis, myocardial infarction, thrombosis and coronary stenosis that shown in Figure 1, are mediated by LOX-1 activation. LOX-1 inhibition by its antibody, chemical analogue or siRNA transfection markedly improves cellular functions and reduces the probability of formation of atherosclerotic lesion, hypertension and myocardial ischemia injury, suggesting that



**Figure 1.** Potential targets for LOX-1 based therapy for cardiovascular diseases, such as hypertension, myocardial infarction, atherosclerosis, thrombosis and coronary restenosis.

targeting LOX-1 is becoming an attractive strategy for modulating the progression of these cardiovascular diseases, which may translate into clinical applications.

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## Conflict of interest

None declared.

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