Intracellular Signaling of LOX-1 in Endothelial Cell Apoptosis

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Atherosclerosis in the coronary artery is a major culprit in unstable coronary syndromes and sudden cardiac death. Rupture of the cap covering the atherosclerotic plaque is thought to lead to platelet deposition and formation of an occlusive thrombus resulting in cessation of blood flow. Oxidatively modified LDL (ox-LDL) has been implicated in the pathogenesis of atherosclerosis and plaque rupture by promoting lipid accumulation, proinflammatory response, release of metalloproteinases, and apoptotic cell death.1,2 Ox-LDL leads to endothelial activation, characterized by expression of adhesion molecules, contributing to adherence and migration of inflammatory cells across the endothelial barrier. Endothelial activation is followed by endothelial dysfunction and injury characterized by loss of expression and activity of constitutive endothelial nitric oxide synthase and a state of oxidative stress. Ox-LDL, particularly in large concentrations, induces apoptosis in endothelial cells, macrophages, and smooth muscle cells.3–5

Ox-LDL is taken up by monocytes/macrophages and smooth muscle cells through a variety of scavenger receptors (SRs), such as SR-AI/II, CD36, SR-BI, macroosomal/CD68, SREC, and LOX-1, and exerts its proatherogenic effects on the vessel wall.1,6,7 Several studies have demonstrated that SRs other than LOX-1 are absent or expressed in minimal amounts in endothelial cells.6–8 LOX-1 plays a crucial role in ox-LDL–induced pathological transformation of the vessel wall components. The importance of LOX-1 became evident in studies that showed significant limitation of atherosclerotic lesion formation in the LDL receptor knockout mice with LOX-1 deletion.9 In these studies, there was clear evidence of preservation of endothelial function and reduction in oxidative stress and inflammatory response.

Regulation of LOX-1

OX-1 is a type II membrane protein ox-LDL receptor with a C-type lectin-like extracellular domain and a short cytoplasmic tail.7,10 LOX-1 is minimally expressed in endothelial cells, monocytes, platelets, and smooth muscle cells under physiological conditions but highly induced under pathologic conditions, such as diabetes, hypertension, myocardial ischemia, and atherosclerosis.10 LOX-1 can be induced by ox-LDL, shear stress, cytokines, free radicals, angiotensin II, and advanced glycation end products.10–14 It is interesting that ox-LDL can upregulate its own receptor at transcriptional level in human coronary artery endothelial cells in a time- and concentration-dependent fashion. The upregulation of LOX-1 in response to ox-LDL can be blocked by a specific antibody or antisense to LOX-1 mRNA. Lysophosphotidylcholine, which has been implicated in atherogenesis, also induces mRNA and protein expression of LOX-1.15 The cytokine tumor necrosis factor (TNF)-α, a proinflammatory cytokine increases cell-surface expression of LOX-1 in a concentration-dependent manner, with a peak time to expression of 8 to 12 hours.12 TNF-α also activates the transcription of LOX-1, as measured by nuclear run-off assay. Shear stress in the physiological range (1 to 15 dynes/cm²) has also been shown to upregulate LOX-1 in a time-dependent fashion.13 Chelation of intracellular Ca²⁺ reduces shear stress-induced LOX-1 expression, and the Ca²⁺ ionophore ionomycin enhances LOX-1 expression. The upregulation of LOX-1 in response to shear stress may be important in endothelial cell activation and injury. Furthermore, angiotensin II, a critical player in atherogenesis, also upregulates the expression of LOX-1.14 Interestingly, angiotensin II and ox-LDL synergistically interact to induce ox-LDL uptake and endothelial injury.

In this issue of Circulation Research, Lu et al15 examined whether LOX-1 mediates endothelial cell uptake of L5, an electronegative component of LDL abundant in dyslipidemic but not in normolipidemic human plasma. In cultured bovine aortic endothelial cells, L5 upregulated the expression of LOX-1 and induced apoptosis. Transfection of bovine aortic endothelial cells with LOX-1–specific small interfering RNAs (siLOX-1) minimized baseline LOX-1 production and inhibited L5-induced LOX-1 upregulation. Internalization of labeled L1–L5 was monitored in endothelial cells by fluorescence microscopy. LOX-1 knockdown with siLOX-1 impeded the endocytosis of L5, but not the L1–L4, component. In contrast, blocking LDL receptor stopped the internalization of L1–L4, but not of L5 component. It is important to recognize that although L5 and ox-LDL are chemically different, they competed for endothelial cell entry through LOX-1.

Signaling of LOX-1 Actions

Ox-LDL induces expression of genes for cell injury through several intracellular signaling pathways. The lectin-like domain of LOX-1 seems to be essential for ligand binding.16 In particular, the large loop between the third and fourth cysteine of the lectin-like domain plays a crucial role for ox-LDL binding, as well as C-terminal end residues. Alanine-
Directed mutagenesis of the basic amino acid residues around this region revealed that all of the basic residues are involved in ox-LDL binding. Simultaneous mutations of these basic residues almost abolished the ox-LDL–binding activity of LOX-1. An electrostatic interaction between basic residues in the lectin-like domain of LOX-1 and negatively charged ox-LDL is critical for the binding activity of LOX-1.

Our group reported that protein kinase C plays an important role in LOX-1 induced intracellular signaling. LOX-1 via downstream signaling pathway of protein kinase C mediates the expression of CD40 and CD40 ligand in endothelial cells in response to ox-LDL. Inhibition of protein kinase C prevents LOX-1–mediated the expression of CD40 and CD40 ligand. These findings indicate that ox-LDL through LOX-1 triggers CD40 signaling pathway that activates inflammatory response in endothelial cells. Other intracellular protein kinases, such as p42/44 mitogen-activated protein kinase (MAPK) play a critical signaling pathway in LOX-1–mediated gene expression. The activation of p42/44 MAPK also plays a critical role in ox-LDL–mediated expression of monocyte chemoattractant protein-1 and adhesion molecules that subsequently lead to enhanced monocyte adhesion to endothelial cells.

Cominacini et al showed that it is the binding of ox-LDL to LOX-1 that initiates nuclear factor κB activation, as well as the increase in intracellular reactive oxygen species formation. These effects of ox-LDL were blocked by a monoclonal antibody to LOX-1. Direct evidence for ox-LDL–mediated intracellular reactive oxygen species formation in endothelial cells through activation of LOX-1 has also been demonstrated. As mentioned earlier, treatment of endothelial cells with ox-LDL results in the activation of p42/44 MAPK and nuclear factor κB and subsequent expression of several genes related to apoptosis.

Many studies have shown that LOX-1 mediates ox-LDL–induced apoptosis. Chen et al examined proapoptotic signaling in endothelial cells in response to ox-LDL. Ox-LDL decreased antiapoptotic proteins c-IAP-1 (inhibitory apoptotic protein-1) and Bcl-2 but did not significantly change cFLIP (Fas-associated death domain-like interleukin-1-β–converting enzyme-inhibitory protein) and proapoptotic protein Fas. Furthermore, ox-LDL activated caspase-9 and caspase-3, which related to the degradation of c-IAP-1 and Bcl-2, and caspase-9 inhibitor blocked ox-LDL–induced activation of caspase-9 and -3 and apoptosis. In contrast, ox-LDL did not activate caspase-8 which related to induction of Fas and degradation of cFLIP, and caspase-8 inhibitor also did not inhibit ox-LDL–induced caspase-3 activity. Importantly, LOX-1 blockade with an antisense and caspase-9 inhibitor both inhibited ox-LDL–induced apoptosis of endothelial cells. These findings suggest that ox-LDL binding to LOX-1 subsequently decreases the expression of antiapoptotic proteins such as Bcl-2 and c-IAP-1 and then activates apoptotic signaling pathway caspase-9 and caspase-3 and finally results in apoptosis.

In this issue of Circulation Research, Lu et al report that L5 via LOX-1 attenuated Akt phosphorylation and suppressed expression of Bcl-2. L5 also selectively inhibited Bcl-xL expression and endothelial nitric oxide synthase phosphorylation, but increased synthesis of Bax, Bad, and TNF-α. Blocking Akt phosphorylation increased LOX-1 expression, suggesting a modulatory role of Akt in LOX-1 synthesis. This finding provides new evidence that the L5 component of dyslipidemic plasma impairs Akt-mediated growth and survival signals via LOX-1. This study provides another link between L5, LOX-1, and endothelial cell apoptosis. This observation is consistent with previous finding that protein kinase B, the cellular homologue of v-Akt, is a key signaling component downstream of phosphatidylinositol-3
kinase. Activation of protein kinase B appears to be vitally important in the expression of endothelial nitric oxide synthase. These observations have obvious implications relative to an important role of LOX-1 in atherogenesis. The Figure incorporates results of many studies linking LOX-1, apoptosis, and atherosclerosis.

**Perspectives**

The key pathological steps involved in the process of a stable atherosclerotic plaque changing into a ruptured plaque remain poorly understood. It is important to elucidate molecular, cellular, and signaling processes that modify plaque composition and lead to plaque destabilization. Identification of these key steps is of immense clinical significance. Understanding LOX-1 activation may also lead to the development of novel diagnostic techniques which will help in the detection of early changes in atherosclerotic plaque composition in vivo. Recent clinical studies have underscored the importance of multiple locations of vulnerable and ruptured atherosclerotic plaques and the diffuse inflammation of the arterial tree in patients with acute ischemic events. LOX-1 appears to be an excellent target to develop novel diagnostic strategy to assess atherosclerotic plaques, as well as therapeutic target to treat atherosclerosis-related disease states.

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None.

**References**


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