THE LOX-1 SCAVENGER RECEPTOR AND Atherosclerotic Plaque Rupture

Ravinder S. Vohra, Jane E. Murphy, John H. Walker, Sreenivasan Ponnambalam, and Shervanthi Homer-Vanniasinkam. 1Leeds Vascular Institute, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, UK; 2Endothelial Cell Biology Unit, Faculty of Biological Science, University of Leeds, Leeds LS2 9JT, UK

Introduction

Atherosclerosis is a chronic systemic inflammatory disease; the end-stage of which is plaque rupture. This depends on plaque composition and vulnerability rather than the severity of stenosis [1]. Vulnerable plaques are associated with an intense inflammatory response and a thin fibrous cap [2]. Remodeling and degradation of the extracellular matrix (ECM) and fibrous cap are maintained by the products of inflammatory cells [3]. The lectin-like oxidized low-density lipoprotein scavenger receptor (LOX-1) is an increasing focus of attention for molecular, cellular, and clinical studies. Recent genetic studies have identified a strong link between several single nucleotide polymorphisms on the LOX-1 gene and the risk of acute coronary syndrome (ACS) [4]. We review how the LOX-1 receptor and its ligand, oxidized low-density lipoprotein (oxLDL), are implicated at critical stages of plaque destabilization and rupture.

The Inflammatory Response

Atherosclerosis is characterized by the accumulation of low-density lipoproteins (LDLs) and inflammatory cells within the vessel wall. Native LDLs are modified by reactive oxygen species (ROS) (e.g. superoxide, H₂O₂, hydroxyl, and peroxynitrite radicals) generated by the vascular tissues [5]. OxLDL stimulates the overlying endothelial cells (ECs) to produce several adhesion molecules and pro-inflammatory mediators [1]. These pro-inflammatory molecules recruit monocytes and lymphocytes to the sub-endothelial region of the vessel wall. The expression of cell surface receptors such as LOX-1 are also elevated by inflammatory mediators and oxLDL [6].

LOX-1 was first identified and cloned by Sawamura and colleagues as a mammalian endothelial receptor for oxLDL [7]. The LOX-1 gene is located on human chromosome 12 within a cluster of lectin-like natural killer (NK) genes involved in immune recognition [8] and encodes a 273 amino acid membrane protein (Figure 1). Recognition of oxLDL by LOX-1 can result in multiple cellular responses within the vasculature [6]. This class E scavenger receptor has subsequently been detected on other cell types including macrophages, vascular smooth muscle cells (SMCs), and platelets [9].

Overexpression of LOX-1 in transgenic mouse models (LOXtg) has raised questions about its functional role in chronic inflammation and atherosclerosis. Mice lacking ApoE but with elevated LOX-1 levels displayed accelerated intramyocardial vasculopathy with LOX-1 prominently detected in coronary vessels and cardiomyocytes and correlation with enhanced oxLDL uptake [10]. These LOXtg mice also displayed elevated levels of intercellular adhesion molecule type-1 (ICAM-1), vascular cell adhesion molecule type-1 (VCAM-1) and macrophages within the vascular wall [10]. This model needs to be interpreted cautiously as LOX-1 levels were also elevated within regions within the myocardium that normally do not express LOX-1.
even in pathophysiological conditions. One possibility is that LOX-1 also functions as an adhesion molecule on the endothelium by contributing to both oxLDL and inflammatory cell accumulation within the vasculature. Indeed *in vitro* evidence further supports a role of LOX-1 as an adhesion molecule [11-13].

*In vitro* studies suggest that oxLDL-mediated activation triggers an endothelial LOX-1-linked signaling pathway leading to pro-inflammatory responses. LOX-1 activation stimulates ROS production and activates nuclear factor κB (NF-κB) [14]. NF-κB activation and translocation into the nucleus results in increased transcription of genes encoding pro-inflammatory and adhesion molecules, e.g. tumor necrosis factor-α, ICAM-1, and VCAM-1 [15]. A different signaling pathway in vascular smooth muscle cells (VSMCs) may also be regulated by LOX-1 activation. VSMC differentiation, migration, and proliferation regulate the formation of the fibrous cap and ECM of atherosclerotic plaques [3]. The CD40-CD40L receptor-ligand system regulates this pathway in VSMCs by inducing pro-inflammatory gene expression including cytokines, proteinases, and adhesion molecules [16]. Recent findings suggest that LOX-1 activation elevates the CD40-CD40L pathophysiological response in VSMCs [17].

**Remodeling and Apoptosis**

The intense pro-inflammatory response during atherogenesis is closely linked to the deposition of the extracellular matrix (ECM) and the formation of the fibrous cap within the vascular plaque. This fibrous cap can be destabilized by different molecules released into the vasculature including cytokines, proteases, coagulation factors, and free radicals [2]. One such class of secreted proteins is the matrix metalloproteinases (MMPs) which can degrade components of the ECM such as collagen. LOX-1 activation by oxLDL stimulates the p38 mitogen-activated protein kinase (MAPK) intracellular signaling pathway, increasing endothelial MMP gene expression, secretion, and activity *in vitro* [18].

The prone shoulder and fibrous cap of the atherosclerotic plaque displays elevated levels of LOX-1 and oxLDL [19]. Anti-LOX-1 antibodies inhibit the VSMC apoptosis triggered by high oxLDL levels which induce Bax/Bcl-2 pathway activation [20] thus implicating a role for LOX-1 in regulating the stability of the plaque. This hypothesis is further supported by the correlative distribution of apoptotic cells and LOX-1 within the shoulders of atherosclerotic plaques [19]. Thus another aspect of LOX-1 function may be to sense high oxLDL levels in smooth muscle, thus contributing to plaque vulnerability and destabilization.

LOX-1 levels are modulated by several therapeutic agents, highlighting the close link between scavenger receptor function and pro-inflammatory vascular disease. Aspirin inhibits LOX-1-mediated p38 MAPK activation and MMP activity [18]. The important class of cholesterol-lowering drugs called statins (3-hydroxy-3-methylglutaryl coenzyme A analogs) also modulates the LOX-1-mediated responses such as NF-κB activation and expression of genes involved in cell adhesion [21]. Statin-treated hyperlipidemic rabbits display significantly reduced LOX-1 mRNA and protein levels and a reduction in the surface area ratio of lipid core : total lesion [22]. Both aspirin and statins could thus antagonize LOX-1-mediated effects including endothelial dysfunction, foam cell formation, and MMP-induced plaque destabilization.

**Post-translational Processing**
The shedding of endothelial membrane proteins as soluble fragments into extracellular fluids are potential vascular disease biomarkers [23]. Soluble circulatory polypeptides derived from adhesion proteins, e.g. VCAM-1 and ICAM-1, are generated by post-translational processing [2]. LOX-1 also undergoes post-translational proteolytic cleavage within the “neck” region of the extracellular domain releasing a 187-residue polypeptide containing the C-type lectin-like domain known as sLOX-1 [24,25]. This phenomenon is closely linked to pro-inflammatory states as human patients with ACS display elevation of serum sLOX-1 levels [26]. In patient serum samples, sLOX-1 levels transiently peak before appearance of traditional biomarkers for cardiovascular damage such as cardiac troponin T [26]. The sLOX-1 levels subsequently decline to baseline levels after 24 hours. Elevation in thrombin and MMP activities are implicated in the processing of membrane LOX-1 to generate sLOX-1 [26]. Increased pro-atherogenic oxLDL levels and resulting elevation in MMP activity could generate sLOX-1 from apoptotic cells, VSMCs and endothelial cells leading to a destabilized plaque (Figure 2). Thus sLOX-1 may be a prognostic indicator of plaque destabilization rather than myocardial injury. Although sLOX-1 shows potential as a vascular disease biomarker, the variability and events leading to the generation of this proteolytic polypeptide require detailed study.

Conclusions

The LOX-1 molecule is a vascular receptor that mediates oxLDL recognition resulting in diverse cellular and tissue processes. Such phenomena include the initiation of atherogenesis, cell-endothelium, and cell-cell interactions. A recurring theme is that LOX-1 stimulates several intracellular signaling pathways activating transcription factors and changes in gene expression. Many of the downstream molecules linked to LOX-1 activation have also been previously associated with endothelial dysfunction and destabilization of atherosclerotic lesions. Surprisingly, little has been done to analyze the role of LOX-1 in membrane trafficking and processing of pro-atherogenic oxLDL which has implications for the clearance of this potentially hazardous substance from vascular tissues. Mammalian sLOX-1 is a potentially exciting biomarker in vascular disease prediction. Technical hurdles exist in clearly identifying and monitoring sLOX-1 species present in circulating fluids from pathological or normal individuals. The generation of a wider range of antibody and ligand-based assays to monitor membrane-bound or sLOX-1 in the vascular tissues or in body fluids could allow better diagnosis of different vascular and pro-inflammatory diseases.

Acknowledgments

The work in our laboratory is supported by the British Heart Foundation, Medical Research Council, Heart Research UK, BBSRC, the Leeds Vascular Surgical Fund, and the National Health Service.

References


Please address correspondence to:
Shervanthi.Homer-Vanniasinkam
E-mail: Shervanthi.Homer-Vanniasinkam@leedsth.nhs.uk
Tel: 0044-113-392-2628
Fax: 0044-113-392-2624
Figure 1. Schematic representation of the structure and domain organization of human LOX-1. LOX-1 is a type II membrane protein with a short cytoplasmic N-terminus (~40 residues), a single transmembrane domain (TMD) and the rest of the molecule comprising the extracellular domain. This extracellular domain has a coiled-coil 'neck' region of ~80 residues (in humans) and a conserved C-type lectin-like domain (~130 residues).
Figure 2. Plaque destabilization and rupture. OxLDL-activated imbalance of proto-oncogenes and tumour suppressor genes, such as Bax and Bcl-2 respectively, result in apoptosis of vascular SMCs in the shoulder region of the atherosclerotic plaque. Local release of MMPs degrade the ECM, further destabilizes the plaque, which again is partly mediated by oxLDL recognition by the LOX-1 scavenger receptor. MMPs maybe responsible for proteolytic cleavage of LOX-1 receptor on ECs and activated platelets within a thrombus or plaque, thus releasing sLOX-1.