

LOX-1 and its Implications on Cardiovascular Diseases a Possible New Perspective Target on Atherosclerosis

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Abstract

The receptor for oxidized low density lipoprotein (LOX-1), encoded by the gene ORL1, is a scavenger receptor that plays a key role in the pathogenesis of atherosclerosis. The activation of LOX-1 is associated with apoptosis of endothelial cells, smooth muscle cells and macrophages. This process is an important mechanism in atherosclerotic plaque destabilization and thus in the development of acute coronary syndromes. Genetic association of independent studies have associated variants of the gene ORL1 a different susceptibility to myocardial infarction. These polymorphisms (SNPs) are located in intronic sequences of the gene ORL1 and regulate the expression of a new isoform of splicing, the LOXIN characterized by the lack of exon 5. It is able to counter cytotoxicity induced by Ox-LDL by reducing the degree of apoptosis by 40%.

Keywords: Low density lipoprotein (LOX-1); Coronary artery disease; Atherosclerosis

Introduction

Atherosclerosis is characterized by the accumulation of lipids and fibrous elements in the arteries, and is the most important contributor to the growing burden of cardiovascular diseases. The major risk factors of atherosclerosis, such as hypertension, diabetes, smoking and free radicals, have been known to induce endothelial dysfunction. It has been suggested that others “non traditional” risk factors, such as oxidative modification of LDL (ox LDL), have an important rule on atherosclerosis [1] development. Oxidized low-density lipoprotein, acting on their receptor (Lectin-like oxidized low density lipoprotein receptor-1, LOX-1), induce endothelial dysfunction [2]. Transgenic mouse models for LOX-1 over expression or gene knockout suggests that LOX-1 contributes to atherosclerotic plaque formation and progression. LOX-1 activation by oxidized LDL (low-density lipoprotein) binding stimulates intracellular signaling, gene expression and production of superoxide radicals.

Role of LDL in the Pathogenesis of Atherosclerosis

Atherosclerosis is the narrowing of the arteries for the presence of a plaque, consists of cholesterol, platelet, monocytes/macrophages, calcium, protein aggregates and other substances. When this phenomenon occurs at the level of atherosclerotic coronary arteries is called Coronary Artery Disease.

The initial event is the formation of fatty streaks that consists in the accumulation of low density lipoproteins in the intima, where LDL elimination capacity is limited due to the lack of a microvasculature of this region. In addition, the proteoglycans of the extracellular matrix have a high affinity for LDL, which involves binding to the matrix with formation of the fatty streaks.

LDL in the intima undergoes a series of changes including aggregation, oxidation and degradation of their components. They can be largely explained as an oxidative attack on the particle LDL, possibly completed by oxygen free radicals generated by tissue macrophages. The oxidation of LDL leads to the release of modified lipids such as lysophosphatidylcholine. Many of these species may act as lipid signaling molecules, activating the smooth muscle cells and endothelial cells. (13) This leads to the expression of leukocyte adhesion molecules and vascular cell adhesion molecule-1 (VCAM-1) which is a receptor

for monocytes and T lymphocytes. In addition, aggregates of oxidized cholesterol induce complement activation, which generates additional chemotactic signals. Once present in the intima, monocytes differentiate into macrophages, this process is triggered by the colony stimulating factor for macrophages (M-CSF) produced by activated vascular cells. Thanks to its ability to internalize oxidized LDL, the macrophage accumulates cholesterol in itself becoming a foam lipid cell of lipids.

Brown and Goldstein, awarded the Nobel Prize for their discoveries on the receptors of lipoproteins and cholesterol metabolism, have observed that macrophages are not able to absorb significant amounts of unmodified native LDL, while they can internalize large amounts of oxidized LDL by a series of scavenger receptors. The uptake of ox-LDL by macrophages leads to the presentation of fragments of these antigen-specific T cells. This induces an autoimmune response that leads to the production of proinflammatory cytokines, including interferon- γ , tumor necrosis factor α (TNF- α), interleukin 1, that can activate endothelial cells inducing them to express adhesion molecules and stimulate procoagulant activity; in addition can directly stimulate the activation of macrophages by activating proteases, endocytosis, production of nitric oxide (NO) and cytokines, also act on smooth muscle cells by inducing NO production and inhibiting growth and expression of collagen and elastin. Thus, the LDL particles may be seen as endogenous auto antigens. The lipid streaks has no clinical relevance, because for the most part disappears spontaneously. One possibility for the transition from fatty streaks to fibrous plaque, characterized by the presence of a fibrous cap that separates the core of the lesion to the endothelial surface, is the release of PDGF (Platelet Derived Growth Factor) and bFGF (basic Fibroblast Growth Factor) by platelets and

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macrophages, stimulated by hemodynamic stress and/or inflammatory activation. PDGF stimulates the migration and division of smooth muscle cells and the formation of the fibrous cap. The lipid core is so physically separated from the endothelial surface and stabilizes the plaque. Obviously, the price of this change is made by a narrowing of the lumen of the artery.

Ox-LDL, which Mechanism of Action?

The Ox-LDL is one of the key steps in the beginning of atherogenesis. Numerous studies have shown that the Ox-LDL is present in the atherosclerotic plaque and how antioxidants may be useful in reducing the progression of plaque. The ox-LDL has several atherogenic properties:

1. Are phagocytized by macrophages through scavenger receptors, promoting formation of foam cells within the vascular wall
2. Modify endothelial functions by inducing the expression of numerous adhesion molecules such as proteins, protein-1 chemotactic for monocytes, growth of smooth muscle cells and other factors responsible for the recruitment of monocytes / macrophages in the endothelial intima.
3. Attenuate endothelium-dependent vasodilation by reducing the production of nitric oxide (NO).

These activities of ox-LDL are mediated by a class of receptors called scavenger. As well known from studies in vitro and in vivo, the constituents of the vessel wall are able to internalize large amounts of ox-LDL through an active process mediated by membrane receptors. In particular, a family of scavenger receptors, including SR-A receptors (type I and II), CD36, CD68, and MARCO, is present in vascular smooth muscle cells, monocytes, macrophages and fibroblasts. However, these receptors are not present or are expressed in small amounts in vascular endothelial cells, which represent the first cell line affected by ox-LDL.

LOX-1 Receptor and Regulation of its Expression

LOX-1 is a 50 kDa type II transmembrane glycoprotein comprising 273 amino acids. The protein contains a short N-terminal cytoplasmic domain, a single transmembrane domain and an extracellular domain comprising a neck domain followed by a C-terminal C-type lectin-like ligand-binding domain [3]; this is consistent with its function as a ligand-binding domain and an initiator of internalization and phagocytosis [4].

LOX-1 has been demonstrated to actively contribute to all stages of atherogenesis. LOX-1 is expressed not only in endothelial cells, but also in macrophages [5], vascular smooth muscle cells [6] and platelets [7].

Murase et al. described a soluble form of LOX-1 (sLOX-1), corresponding to its extracellular domain only [8]. Plasma sLOX-1 is associated with variation in the LOX-1 gene in older men and women [9].

LOX-1 has interesting specificity because it binds ox LDL and, with lower affinity, acetylated LDL, while it does not bind native LDL [10].

The LOX-1 promoter is constitutively active only at a low level though its expression can be induced by ox LDL [11], Ang II [12], fluid shear stress [13], Homocysteine [14], C reactive protein (CRP) [15] and others. LOX-1 expression in cardiac myocytes, as well as in vessel walls of failing 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 [16].

Recently it has been observed that CRP is not only a promoter of LOX-1 expression but also it is a novel ligand: the treatment of human endothelial cells with CRP led to the activation of proinflammatory genes including IL-8, ICAM-1, and VCAM-1. The inductions of these genes by CRP were LOX-1 dependent, as demonstrated by their attenuation in cells transfected with LOX-1 small-interfering RNA. Our study identifies and characterizes the direct interaction between LOX-1 and CRP and suggests that this interaction may mediate CRP-induced endothelial dysfunction [17].

LOX-1 Signaling

LOX-1 binding to ox LDL enhances nitric oxide (NO) catabolism as a result of superoxide generation, and decreases NO release via attenuated endothelial NO synthase (eNOS) activity. LOX-1 has been recently shown to form a complex with MT1-MMP under a basal condition. When ox LDL 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 ox LDL to LOX-1 causes ox LDL-induced endothelial dysfunction leading to atherosclerosis. In addition to those, others signal transduction pathways have been reported to be activated via LOX-1, inducing proinflammatory changes to the vessel wall [19-21].

LOX-1 gene: Structure, Polymorphism and Alternative Transcripts

LOX-1 is encoded by the ox LDL receptor 1 (OLR1) gene, located in the natural killer gene complex (NKC) on chromosome 12p12-p13, which also contains several others families of lectin-like genes [22].

The OLR1 gene spans more than 7000 base pairs and consists of 6 exons interrupted by 5 introns. Exon 1 encodes the 5'- untranslated region (UTR) and cytoplasmatic domain, exon 2 encodes the remainder of the cytoplasmatic domain and the transmembrane domain, exon 3 encodes the neck domain, and exon 4,5 and 6 encode the lectin-like domain and the 3'UTR of LOX-1 protein [23].

Some studies have shown common genetic variation in the OLR1 gene to be associated with the risk of coronary artery disease (CAD) [24].

Three common LOX-1 single nucleotide polymorphisms (SNPs) have been identified in intron 4 (G → A), intron 5 (T → G) and 3'UTR (T → C) [25]. Contemporaneously, the involvement of LOX-1 in atherosclerosis and acute myocardial infarction (AMI) was confirmed by defining OLR1 genetic variation in an association study of intragenic SNPs [24].

It was also observed that the LOX-1/3'UTR SNP genotype and allele frequencies differed significantly between the control and the AMI groups in which the subjects with the T/T or C/T genotype were at higher risk of developing AMI [24]. Despite the positive results on OLR1 polymorphisms in CAD or AMI subjects, other similar studies were unable to confirm these putative genetic risks [26]. In particular, regarding a functional SNP, the G → C transition at position 501 in exon 4 which produces a single amino acid change (K167N) in the ligand-binding domain and affects markedly LOX-1 receptor activity, different conclusions have been reported [27,28].

The SNPs give rise to a splicing variant lacking exon 5, named

LOXIN, which lacks two-thirds of the ligand binding domain of LOX-1. The LOX-1/LOXIN mRNA ratio is 33% higher in monocyte-derived macrophages of the subjects homozygous for the risk allele compared with those homozygous for the non-risk allele [29]. On this basis, it has been developed a genetic kit named “LOXIN test” that allows the rapid identification of ORL1 genotypes and therefore establish the susceptibility risk to atherosclerosis and myocardial infarction [30]. Flow cytometry and immunofluorescence studies have shown that the new isoform Loxin can reduce the cytotoxicity induced by ox-LDL, reducing them by 40% degree of apoptosis. These data are supported by in vivo experiments, conducted on patients with previous myocardial infarction, have shown that macrophages of these patients express a lower amount of Loxin and are more susceptible to apoptotic damage induced ox-LDL [29].

LOXIN probably works as a dominant negative form dimerizing with the native form of LOX-1 to protect cells from the damage by ox LDL. LOXIN, when expressed in the absence of LOX-1, shows diminished plasma membrane localization and is deficient in ox-LDL ligand binding. When co-transfected with the full-length counterpart LOX-1, the two isoforms interact to form LOX-1 oligomers and their interaction leads to a decrease in the appearance of LOX-1 receptors in the plasma membrane and a marked impairment of ox-LDL binding and uptake. Co-immunoprecipitation studies confirmed the molecular LOX-1/LOXIN interaction and the formation of non-functional hetero-oligomers. It has been suggested that hetero-oligomerization between naturally occurring isoforms of LOX-1 may represent a general paradigm for regulation of LOX-1 function by its variants [31].

LOX-1 and Atherosclerosis

The close relationship between LOX-1 and atherosclerosis has been supported by many studies. Firstly, LOX-1 is highly expressed in vivo in large arteries (aortic, carotid, thoracic, coronary arteries and veins) which are the predilection sites of atherosclerosis. LOX-1 is expressed in macrophages, SMC and vascular endothelial cells which are the three most important cells involved in the development of atherosclerosis [2,32]. Secondly, ox-LDL plays an important role in the pathogenesis of atherosclerosis [33]; as the major receptor for ox-LDL, LOX-1 mediates most of the toxic effects of ox-LDL. Thirdly, upregulated LOX-1 expression was found in atherosclerotic lesions in humans and experimental animal models. In aorta without atherosclerosis, LOX-1 expression was undetectable while in carotid arteries endothelial cells covering early atherosclerotic lesions were more frequently positive for LOX-1 expression than those in advanced atherosclerotic lesions and endothelial cells in the intimal neovasculature of advanced lesions also expressed LOX-1. Furthermore, macrophages and SMC in the intima of advanced atherosclerotic plaques are positive for LOX-1 suggesting that LOX-1 might play a role in the early stages of atherosclerosis [34]. Fourthly, upregulated LOX-1 mediated a series of pathophysiological effects in atherosclerosis: LOX-1 functions as a cell-adhesion molecule mediating the platelet-endothelium interaction and is involved in endotoxin induced inflammation which may initiate and promote atherosclerosis [35]. LOX-1 expression was well colocalized with Bax expression in the rupture-prone shoulder areas of human atherosclerotic plaques in vivo and played an important role in ox-LDL-induced apoptosis by modulating Bax/Bcl-2 or decreasing the expression of antiapoptotic proteins Bcl-2 and c-IAP-1 [6].

Clinical Tools of LOX 1

It is evident that LOX-1 is a potential target of preventive therapy of atherosclerosis, being a crucial link in the pathogenesis of the disease

and for its chemical and biochemical features full of potential binding sites with molecules that can block its action. Many in vivo and in vitro studies suggest that blocking LOX-1 can lead to improvements in the evolution of atherosclerotic disease, and that the development of drugs targeted against this protein must be the way to follow. However we should not forget that some questions remain open:

- *In vivo* studies show that LOX-1 is expressed in many organs richly vascularized as in placenta, lung, bone marrow and spinal cord, as well as in hippocampus, testis, heart, ovary and muscle [22]. The role of LOX-1 in these locations is yet to be discovered.
- The current data tell us that LOX-1 receptor acts as a scavenger against gram-positive and gram-negative as well as cellular debris, can we say that will not occur any side effects by inhibiting the function of LOX-1?
- It has been demonstrated a close relationship between LOX-1 and the atherosclerotic process but it is possible to state with certainty that it is the keystone of atherosclerosis and that is not only one of the processes involved, as surely important?

A promising new road was opened by the discovery of the existence of a soluble isoform of LOX-1 receptor, identified by Murase et al. sLOX-1, formed by a proteolytic cut of the transmembrane region. That isoform have a molecular mass of 35 KDa and it includes the lectin domain. What is the exact function of this protein is not yet clear. Recent study of Hayashida et al. demonstrated that this isoform increase during acute coronary syndrome, before TnT and hs-PCR and decrease in the first 24 hours. Levels of sLOX-1 don't correlate with the increase of TnT suggesting that it can't be considered a myocardial necrosis marker. Furthermore, time-dependent changes, during the acute phase of ACS, indicated that elevated levels of sLOX-1 were detectable at an earlier stage after the onset of ACS than those of Troponin-T, indicating that sLOX1 reflects atherosclerotic plaque vulnerability or rupture before ischemic cardiac damage becomes apparent.

Tina E. Brinkley et al. Are the first, with their study, to demonstrate a relation between the polymorphism of OLR-1 gene e serum level of sLOX-1. The allele 3'UTR-T and the genotype LOX-1 501 G are associated with lower level of sLOX-1; this are only preliminary results but it is basic for future research the demonstration of a relationship between OLR-1 polymorphism, serum level of sLOX-1 and the risk of developing a coronary disease [36]. However it should be noted that the study in question was performed on a healthy population, whose average values of sLOX-1 were significantly lower than values reported by Hayashida in its population of patients with ACS; becomes important in the near future to rely on more detailed studies to evaluate the function of LOX-1 and the role of its serum equivalent on a healthy population. It remains crucial for the future of the current studies demonstrate a significant influence of OLR-1 polymorphism also on serum LOX-1.

Kume et al. in their last work also demonstrated that sLOX-1 is a prognostic biomarker for ACS with high sensitivity and specificity. ACS patients, who underwent emergency percutaneous coronary intervention, were prospectively followed with optimal medical treatment. Circulating sLOX-1 levels were significantly higher in the re-ACS/death group than in the event free survival group, with a median of 6.60 vs 2.54 ng/ml ($P=0.0024$). Furthermore there was a significantly more prevalent and earlier incidence of re ACS/death in patients with sLOX-1 levels in the upper quartile or tertile than in the others [37].

Higher level in the acute stage of ACS may reflect a greater burden of vulnerable atherosclerotic plaque, because atherosclerotic plaques other than culprit lesions are also found to be ruptured or destabilized. Moreover, it should be determined, in the future whether circulating sLOX-1 levels are associated with morphological atherosclerotic plaque vulnerability, which can be evaluated in humans by updated vascular imaging modalities.

Recently, it has been observed that activation of LOX-1 in humans can be evaluated by use of the LOX index, obtained by multiplying the circulating concentration of LOX-1 ligands containing apolipoprotein B (LAB) times that sLOX-1 [LOX index = LAB x sLOX-1]; higher LOX index values are associated with an increased risk of CHD. Low LOX index values may be protective against ischemic stroke [38].

Conclusion

Coronary artery disease is multifactorial with a not completely known pathogenesis. One of the correlations, until now unknown, between risk factors and pathogenesis could be LOX-1 and its genetic polymorphism that could explain the clinical differences among patients. Actual knowledge can't explain completely this differences but a continuous work out is necessary to produce cheaper genetic test for SNPs and to identify how they correlate to the severity of ACS, to the response to pharmacological treatment and to evaluate how risk factors act between different patients.

The finding of a soluble isoform of the oxidized LDL receptor opens up new scenarios, until now not assumed, using this molecule as an early and specific marker of myocardial damage, but the data of the first studies need further confirmation in order to define a specific field of usage of these markers and their real importance in the clinical setting.

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