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Invited critical review

LOX-1 in atherosclerotic disease

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ABSTRACT

Oxidized low-density lipoprotein (LDL) exhibits various biological activities and accumulates in atheromas. LOX-1 (lectin-like oxidized LDL receptor) is the receptor that mediates oxidized LDL activity in vascular endothelial cells. Activation of LOX-1 results in oxidized LDL-induced endothelial dysfunction and hyperlipidemia-induced vascular lipid deposition. We hypothesized that LOX-1 is a candidate risk factor beyond LDL cholesterol (LDLC) and developed a novel assay to quantify LOX-1 ligand containing apoB (LAB). In men from the United States, serum LAB showed a significant positive association with carotid intima-media thickness, independent of LDLC. LAB and the LOX index (obtained by multiplying LAB by sLOX-1) were significantly associated with the incidence of coronary artery disease and ischemic stroke after adjusting for confounding factors, including non-HDL cholesterol. sLOX-1 is thought to be a better biomarker for early diagnosis of acute coronary syndrome than traditional biomarkers, including troponin T. LAB was associated with various atherosclerotic risk factors such as smoking, obesity, diabetes, diastolic hypertension, hypertriglyceridemia, and metabolic syndrome. Measurement of the soluble form of LOX-1 (sLOX-1) and LAB seems to be useful for evaluating the state and risk of atherosclerosis and atherosclerosis-related diseases. Further prospective studies using large populations and randomized clinical trials on sLOX-1, LAB, and the LOX index are needed.

Contents

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1. Introduction

1.1. Oxidized LDL hypothesis and pertinent questions

The relationship between cholesterol and atherosclerosis is well established, as demonstrated by the considerable success of statins. However, a large amount of cholesterol is present in a healthy individual, and it is an essential cell component as well as an indispensable source of hormones. Therefore, clarification of the specific mechanisms of cholesterol activity is needed to determine its role in atherosclerosis.

In the 1980s, Steinberg's group proposed the hypothesis that native LDL gains atherogenic properties by "oxidative modification" [1]. Oxidized LDL exhibits various biological activities and accumulates in atheromas. Furthermore, antioxidants such as vitamins E and C effectively suppress the progression of atherosclerosis in animal models [2].

However, clinical trials have consistently shown that these antioxidant vitamins fail to reduce cardiovascular events. Is the oxidized LDL hypothesis applicable to animals but not to humans? As pointed out by Libby et al. [2], despite a large body of literature on the possibly pro-atherogenic properties of oxidized LDL, its pathophysiologic and therapeutic relevance in humans remain unclear.

It is true that modification of LDL enhances its atherogenic properties. The enhancement is so remarkable in the case of in vitro oxidation of LDL that researchers in the field of atherosclerosis have devoted considerable attention to oxidation specifically, as opposed to modification more broadly. Seen from this viewpoint, the ineffectiveness of antioxidant vitamins in human atherosclerosis does not contradict the "modified LDL" hypothesis. Furthermore, the importance of modified LDL in human atherosclerosis remains undeniable.

1.2. Understanding atherothrombotic diseases as receptor-centric rather than oxidized LDL-centric

Several receptors for oxidized LDL have been identified, such as SR-A and CD36, both of which function primarily in macrophages [3,4], and LOX-1 (lectin-like oxidized LDL receptor), which is largely localized to endothelial cells [5]. These and other multi-ligand receptors are called "scavenger receptors."

A recently proposed working definition of scavenger receptors is as follows [6]: "Scavenger receptors are cell surface receptors that typically bind multiple ligands and promote the removal of non-self or altered-self targets. They often function by mechanisms that include endocytosis, phagocytosis, adhesion, and signaling that ultimately lead to the elimination of degraded or harmful substances." Thus scavenger receptors are a functionally defined molecular family that includes multiple and structurally unrelated classes of molecular subfamilies.

When we think about lipoprotein ligands of scavenger receptors, it is important to note the term "altered-self targets." It clearly indicates the importance of native LDL modifications, which are not limited to oxidation. In fact, the first demonstration of scavenger receptor activity was performed with acetylated LDL.

The results of gene knockout of SR-A, CD36, and LOX-1 in mice suggest that these oxidized LDL receptors promote atherosclerosis, at least in murine models [7–11], while conflicting results were reported regarding SR-A and CD36 [12,13].

2. What is LOX-1?

Along with cholesterol metabolism, endothelial dysfunction has been implicated in atherogenesis [14]. Endothelial cells in a monolayer at the innermost surface of blood vessels regulate vessel function. Identification of nitric oxide (a vasorelaxant substance), endothelin-1 (a vasoconstrictive peptide), adhesion molecules, and chemokines in endothelial cells has facilitated our understanding of the importance of endothelial function.

Dysregulation of endothelial function initiates pathological changes in vascular function that may be proinflammatory, prothrombotic, or proatherogenic. Oxidized LDL causes such dysregulation [15] via the LOX-1 receptor [5]. Activation of LOX-1 impairs the release of nitric oxide by inactivating it via generated superoxides and by phosphorylating endothelial nitric oxide synthase [16,17]. LOX-1 also induces the expression of chemokines and leukocyte adhesion molecules [9] by activating the small GTPases rho and rac, p38 MAP kinase, protein kinase C beta II, and NF-kB, among others [18–20].

In vivo, LOX-1 expression is up-regulated in the context of hypertension, diabetes, and hyperlipidemia, and down-regulated by AT1 receptor blockers and statins [21–26]. Overexpression of LOX-1 driven by the endothelin-1 promoter was shown to cause lipid deposition in the coronary arteries of ApoE knockout mice [9]. LOX-1 gene knockout mice showed delayed progression of atherosclerosis and resistance to oxidized LDL-induced impairment of endothelium-dependent vasorelaxation [10]. Furthermore, administration of anti-LOX-1 antibody to spontaneously hypertensive stroke-prone rats suppressed high-fat diet-induced acute lipid deposition in mesenteric arteries, preventing vascular hyperpermeability [27]. Thus, it was demonstrated that LOX-1 mediates hyperlipidemia-induced vascular lipid deposition in vivo as well as oxidized LDL-induced endothelial dysfunction.

In addition to the chronic role of LOX-1 in atherosclerosis, roles of LOX-1 in acute phase of diseases have been suggested, namely administration of anti-LOX-1 antibody or LOX-1 gene deletion suppressed ischemia-reperfusion injury in the heart and brain, and decreased intimal thickening after balloon injury of arteries in animal models [28–30]. Furthermore, LOX-1 was found to function as a proinflammatory adhesion molecule in leukocytes and platelets [31].

As for the LOX-1 ligand in human plasma, an electronegative lipoprotein fraction, L5, showed oxidized LDL-like activity in inducing endothelial cell apoptosis and platelet aggregation in a LOX-1-dependent manner [32–34]. In addition to lipoprotein ligand, C-reactive protein (CRP), the functions of which are gaining attention as an important marker of the risk for ischemic heart disease [38], was found to bind to LOX-1 and induce Clq-dependent complement system activation and endothelial dysfunction [35–37].

3. LOX-1 ligand containing apoB (LAB)

3.1. LOX-1 in humans

With growing attention on the significance of endothelial function in atherosclerosis and coronary artery disease (CAD) in humans, various clinical tests for endothelial function have been employed, such as measurement of flow-mediated dilatation by ultrasound and assessment of reactive hyperemia by pulse amplitude tonometry [39,40]. In complementary to clinical/functional in vivo testing, biochemical testing is a valuable tool, i.e. for investigation on large-size populations.

Accordingly, we developed a novel assay to quantify LAB regardless of its modification status which may reflect the state of endothelial function (Fig. 1) [41]. Instead of antibodies against oxidized LDL, we utilized recombinant LOX-1 protein to bind the lipoprotein ligand of LOX-1. Bound lipoproteins were then detected by anti-apoB antibody. Although this procedure may seem to be a simple receptor binding assay, it solves two problems with the experimental procedures previously used in oxidized LDL assays:

(1) LDL may be modified in a number of ways, and it is not always clear which modification should be assessed. Evaluation of a specific modified LDL epitope might be of significance under some conditions but not others.
(2) We cannot determine the biological activity of modified LDL by measuring a single epitope, since different LDL modifications result in different receptor binding potencies.