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Review

Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): A critical player in the development of atherosclerosis and related disorders

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Abstract

LOX-1, a lectin-like 52-kD receptor for oxidized low-density lipoproteins (ox-LDL), is present primarily on endothelial cells. This receptor is upregulated by ox-LDL itself and by angiotensin II, endothelin, cytokines, and shear stress, all participants in atherosclerosis. This receptor is upregulated in the arteries of hypertensive, dyslipidemic, and diabetic animals. Upregulation of LOX-1 has been identified in atherosclerotic arteries of several animal species and humans, not only on the endothelial lining, but also in the neovasculature of the atherosclerotic plaque, and this receptor is often co-localized with apoptotic cells. Recent studies show upregulation of LOX-1 in the ischemic-reperfused myocardium. LOX-1 inhibition is associated with attenuation of atherosclerosis and associated ischemic injury. LOX-1 may be a novel, exciting target for drug therapy.

Keywords: Lipids receptors; LOX-1; Oxidised LDL

1. Introduction

Atherosclerosis is the narrowing or occlusion of the arteries by a plaque, which consists of cholesterol, platelets, monocyte/macrophages, calcium, aggregating proteins and other substances. When this process occurs in the coronary arteries, it is referred to as atherosclerotic coronary artery disease (CAD). A rupture of the atherosclerotic plaque resulting in total occlusion of the artery and cessation of blood flow is the basis of acute coronary syndrome or heart attack. There are multiple conditions associated with elevated risk of atherosclerosis, including high plasma levels of low density lipoprotein–cholesterol (LDL–cholesterol), smoking, diabetes mellitus, hypertension, sedentary life style, diet and several other conditions [1,2]. Among these, high plasma level of LDL–cholesterol is perhaps the most important risk factor for atherosclerosis. In vitro studies have demonstrated that oxidatively modified LDL (ox-LDL) is more important than native-LDL for atherogenesis. This is based on powerful data that ox-LDL modifies vascular endothelial cells, smooth muscle cells, monocyte/macrophages and fibroblasts into pro-atherogenic phenotypes.

2. Ox-LDL and cell surface receptors

It has been recognized that vessel wall components in vitro and in vivo internalize ox-LDL through receptor-mediated pathways. For smooth muscle cells and monocyte/macrophages as well as fibroblasts, this receptor-mediated

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pathway involves a family of scavenger receptors (SRs), such as class A macrophage scavenger receptor type I/II (SR-AI/II), class B macrophage scavenger receptor type I (SR-BI), CD36, macrophage receptor with a collagenous structure (MARCO) and macrosialin (CD68) [3]. However, these SRs are undetectable or are expressed in very small amount in vascular endothelial cells [4], which are the first line of cells that is affected by ox-LDL.

Since endothelial dysfunction is a very early step in atherogenesis, binding (and subsequent endocytosis) of ox-LDL to endothelial cells is an area of intense investigation.

3. Identification of a novel Ox-LDL receptor LOX-1

In 1997, Sawamura and his colleagues cloned a novel ox-LDL receptor, lectin-like ox-LDL receptor-1 (LOX-1), on bovine aortic endothelial cells [5]. They showed that LOX-1 is responsible for the binding, internalization and degradation of ox-LDL in endothelial cells [5]. Subsequently, our group identified LOX-1 in human coronary artery endothelial cells, and showed that coronary artery endothelial cells express LOX-1 with a Bmax of ~30 ng/mg protein and a K_D of 1.7×10^{-8} mol/L, as determined by radioligand-binding studies [6,7]. Other studies have since shown that besides endothelial cells, macrophages, platelets and smooth muscle cells also express a small amount of LOX-1 [8–10]. In a recent in vitro study, Chen et al. [11] from our laboratory found the expression of LOX-1, although in very small amounts, in cardiac fibroblasts. In addition to in vitro evidence, a number of in vivo studies have also confirmed the presence of LOX-1 in the blood vessels of humans, rabbits, rats and mice [12–15].

The contributory role of LOX-1 in atherogenesis is supported by several lines of evidence: (i) LOX-1 shows a strong activity in binding, internalizing, and proteolytically degrading ox-LDL [5]; (ii) Ox-LDL activates LOX-1 and induces endothelial dysfunction/apoptosis [16,17], a major change in vascular biology seen at the beginning of atherogenesis; (iii) Besides ox-LDL, other mediators of atherosclerosis such as angiotensin II (Ang II) cytokines, sheer stress, advanced glycation end-products (AGE) all upregulate LOX-1; (iv) LOX-1 is dynamically upregulated by pro-atherogenic conditions, such as diabetes, hypertension and dyslipidemia [12,13,18–21]; and (iv) LOX-1 is present in atheroma-derived cells and is seen in large amounts in human and animal atherosclerotic lesions in vivo [12,20].

We believe that LOX-1 is involved in multiple steps in atherogenesis and its complications. Fig. 1 depicts various sites in the atherosclerotic process where LOX-1 expression and activation has been shown to be a participant.

This review summarizes recent findings concerning the structure, function, and regulated expression of LOX-1 in relation to its potential roles in atherosclerosis and other pathological status.

4. Structure, organization and promoter analysis of human LOX-1 gene

Sequence and structural analysis of LOX-1 has revealed that this receptor does not resemble any known SR. In contrast, it shares significant identity to natural killer (NK) cell receptors, such as CD94 (KLRD1) and NKR-P1 (KLRB1), which are involved in target cell recognition and NK cell activation [22,23]. In keeping with these reports, Yamanaka et al. [23] identified that the LOX-1 gene localizes within natural killer-gene complex (NKC), which mainly encodes receptors associated with natural killer cells. Aoyama et al. [22] analyzed the human LOX-1 gene and found that it is a single-copy gene and is assigned to the

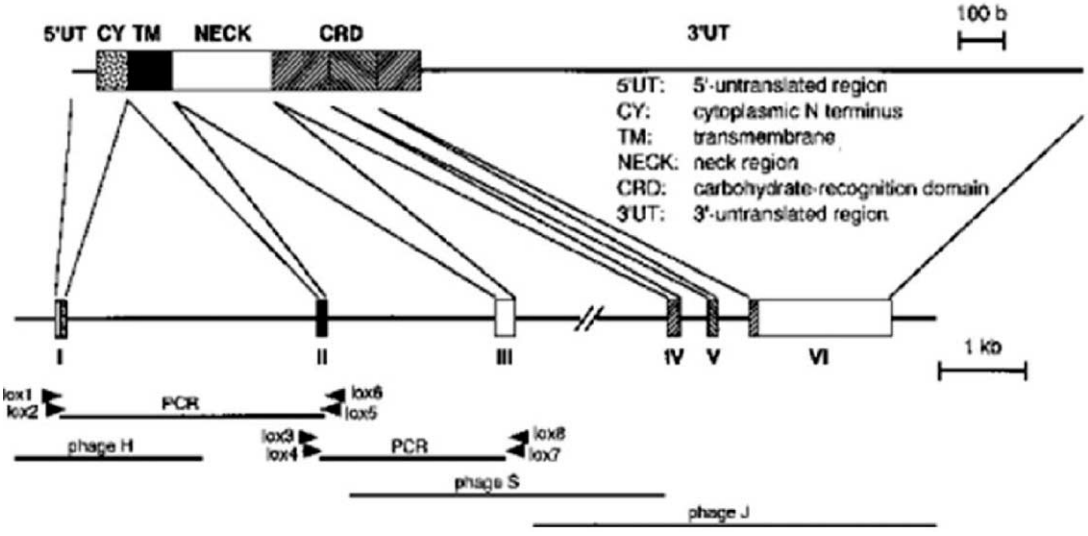

Fig. 1. LOX-1 is composed of 6 exons interrupted by 5 introns.

p12.3–p13.2 region on the short arm of human chromosome 12. Human LOX-1 (OLR1; low density lipoprotein, oxidized, receptor 1; OMIM #602601) gene spans more than 7000 base pairs (bp), and consists of 6 exons interrupted by 5 introns. Exons 1–5 ranges from 102 to 246 bp, whereas exon 6 is relatively long, being 1722 bp. Excluding exon 6, the average exon length is 148 bp, which is consistent with the reported average exon size. The intron size ranges from 324 bp to more than 6000 bp. All the junctions between exons and introns are in accordance with the rule that introns begin with GT dinucleotide and end with AG. Exon 1 encodes the 5′-untranslated region (UTR) and cytoplasmic domain, exon 2 encodes the remainder of the cytoplasmic domain and the transmembrane domain, exon 3 encodes the neck domain and exons 4–6 encode the lectin-like domain and the 3′-UTR. LOX-1 gene structure is shown in Fig. 1.

Aoyama et al. [22] and Yamanaka et al. [23] also studied the 5′ promoter/enhancer region (about 2500 bp) of the human LOX-1 gene, and found TATA and CAAT boxes in the proximal part of the 5′-flanking region (located at nucleotide (nt)-29 bp and -99 bp upstream of the transcription initiation site, respectively). Computer-based analysis identified a wide variety of potential transcription factor binding sites [22,23]. These studies [22,23] also suggest a proximal fragment of 180 bp (from nt-150 to +30 bp) that is sufficient for basal promoter activity.

We examined the transcriptional mechanism for ox-LDL-induced human LOX-1 gene expression by "promoter bashing" and identified that the promoter region between nt-1494 and -1599 is required for ox-LDL-induced human LOX-1 promoter activation. Finer mutational analysis and electrophoretic mobility shift assays strongly suggested that the Oct-1 binding site within this region plays an important role in human LOX-1 promoter trans-activation in response to ox-LDL (unpublished data).

We also examined the transcriptional mechanism for Ang II-induced human LOX-1 promoter activation using the same methodology. As will be discussed later, Ang II is a powerful stimulus for LOX-1 gene upregulation. Interestingly, we found that the promoter region between nt-2131 and -2247, which includes an active NF-κB binding site, is required for Ang II-induced human LOX-1 promoter trans-activation (unpublished data).

5. Structure and function of LOX-1 protein

As with all NK cell receptors encoded in the NKC, LOX-1 is a type II membrane protein (~50 kDa). It belongs structurally to the C-type lectin family, which binds carbohydrates in a Ca^{2+}-dependent manner, and is comprised of four domains: a short N-terminal cytoplasmic domain, a single transmembrane domain, a connecting neck domain, and a lectin-like domain at the C-terminus. LOX-1 structure is shown in Fig. 2. Sawamura et al. [5] found that the human LOX-1 protein contains 273 amino acids, and is 72% identical to its bovine counterpart. Mouse and rat homologues have also been identified. They share similar characteristics to human and bovine LOX-1, except for possessing a longer neck domain which is encoded by the unique triple-repeat sequences [24]. Recently, rabbit and porcine counterparts of LOX-1 have been identified and characterized by their close similarity to human LOX-1, both in sequence and in domain structure organization [12,25]. The extracellular domain of LOX-1 is post-translationally modified by N-linked glycosylation. Murase et al. [26] have shown that LOX-1 can be cleaved by unknown proteases at the extracellular juxtamembrane region to release the soluble form of LOX-1. The importance of soluble forms of LOX-1 is as yet not known, but its levels may be increased in patients with atherosclerosis risk factors.

To understand how LOX-1 recognizes and responds to ox-LDL, it is important to characterize the structure-function relationship of LOX-1. Although there are many observations within the last decade, the function of these domains is still not clear. Notably, the C-terminal lectin-like domain is highly conserved among species, especially at the position of the six cysteine residues. This is consistent with its function as a ligand-binding domain and an initiator of internalization and phagocytosis, as suggested by Chen et al. [27]. These authors also found that the positively charged amino acids within the lectin-like domain cooperatively recognize ox-LDL which exhibits strong negative charge since the lipid peroxidation products were generated and linked to its apolipoprotein B (apo-B) moiety [25]. Subsequently, Xie et al. [28] found that the C-terminal lectin-like domain alone is sufficient for the binding of ox-LDL. Chen et al. [29] confirmed the ox-LDL-binding function of the lectin-like domain of LOX-1, and found that the correct cell-surface localization is dependent on a positively charged motif present in the cytosolic juxtamembrane region of LOX-1. Although these authors [28,29] showed that the neck domain has no specific role in ligand-binding activity of LOX-1, in a recent study, Ohki et al. [30] analyzed the crystal structure of the extracellular domain of LOX-1, and found that a short region within neck domain, connecting the ligand-binding domain and the transmem-

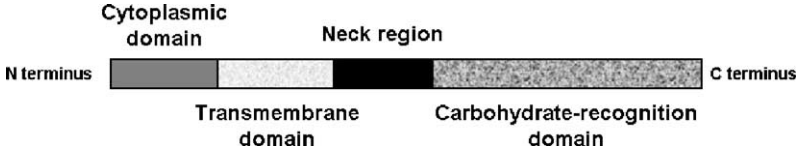

Fig. 2. LOX-1 is composed of four domains.

brane domain, forms a homodimer which is linked by an interchain disulfide bond and is indispensable for the ligand-binding activity of LOX-1. Park et al. [31] analyzed the 1.4 angstrom crystal structure of the extracellular lectin-like domain of human LOX-1, and revealed a heart-shaped homodimer of LOX-1 and a central hydrophobic tunnel that extends through the entire molecule. The hydrophobic tunnel within the LOX-1 homodimer appears large enough to accommodate and recognize a cholesterol molecule, a fatty acid chain, or possibly a six-to seven-residue non-polar peptide, suggesting that it could participate in the recognition of extracellular ligands containing these structures [31]. Besides ox-LDL, LOX-1 exhibits binding activity for multiple ligands. So far, four groups of substances have been identified as LOX-1 ligands, including (i) modified lipoproteins (ox-LDL, acetylated LDL and hypochlorite-modified high density lipoprotein) [32,33]; (ii) polyanionic chemicals (polyinosinic acid and carrageenan) [24]; (iii) anionic phospholipids (phosphatidylserine and phosphatidylinositol) [33,34]; and (iv) cellular ligands (apoptotic/aged cells, activated platelets and bacteria) [34–36]. The potential binding of these ligands to LOX-1 imply its versatile physiological functions. Unfortunately, the function of N-terminal cytoplasmic domain of LOX-1 is not clear. Whether it plays a critical role in the signal transduction pathways activated by ox-LDL and other ligands needs to be studied.

6. LOX-1 polymorphisms and myocardial infarction in humans

Tatsuguchi et al. [37] identified a single nucleotide polymorphism in the OLR1 gene, a 501G-C transversion, resulting in a lys167-to-asn (K167N) substitution. In 102 patients with a history of myocardial infarction (MI), the authors found a significantly higher frequency (38.2%) of the 501G-C polymorphism compared to 102 controls (17.6%). The odds ratio for the risk of MI associated with the 501G-C change was 2.89. In a study of 589 white and 122 black women who underwent angiography for suspected ischemia, Chen et al. [38] found that the frequency of the 3 UTR T-allele was significantly higher in whites than in blacks ($p<0.0001$). Among white women, the frequency of the T-allele was 67.9%, 75.0%, and 79.2% in individuals with less than 20%, 20–49%, and greater than 49% stenosis, respectively (chi square trend=6.23, $p=0.013$). The T-allele carriers had significantly higher IgG anti-ox-LDL levels than those with the CC genotype ($p=0.032$); and electrophoretic mobility shift assay data indicated that the 3′ UTR binds regulatory proteins and that the C-allele has a higher affinity for binding than the T-allele. We identified a polymorphism in the 3 UTR of the OLR1 gene, 188C-T, which was significantly associated with myocardial infarction in a group of 150 patients. Genotypes with the T-allele were found in 91.3% of patients compared to 73.8% of controls, yielding an odds ratio of 3.74. In particular, we have identified seven different SNPs, six of them located within introns 4, 5, and 3′ UTR comprised in a linkage disequilibrium block strongly associated with the elevated risk to develop MI [39]. Recently, we have demonstrated that these SNPs regulate the expression of LOXIN, an isoform of LOX-1 which lacks exon 5 [40].

7. Regulation of LOX-1 gene expression and underlying mechanisms

In the in vitro setting, the basal expression of LOX-1 in endothelial cells is very low. However, it can be rapidly induced by pro-inflammatory, pro-oxidant and mechanical stimuli such as ox-LDL [16], Ang II [7], cytokine tumor necrosis factor-α (TNF-α) [18] and shear stress [19]. Oxidant species are also potent inducers of LOX-1 [41]. All these are abundantly present in the regions of atherosclerosis and other forms of vascular injury. The most widely acknowledged stimuli for LOX-1 expression are shown in Table 1.

In the in vivo settings, basal LOX-1 expression is also low, but can be enhanced by several pathological conditions, including hypertension [13], diabetes mellitus [21,42] and hyperlipidemia [12] as well as others which are also presented in Table 1. Since most of these pathological conditions (inflammation, hyperlipidemia, hypertension and diabetes) are directly or indirectly associated with atherogenesis [43],

Table 1
In vitro and in vivo stimuli for lox-1 gene upregulation

In vitro stimuli for LOX-1 upregulation
Angiotensin II (Ang II)
C-reactive protein (CRP)
Endothelin-1 (ET-1)
Glucose
Histamine
Homocysteine
Human cytomegalovirus (HCMV)
Interferon-γ (IFN-γ)
Interleukin-1β (IL-1β)
Oxidant species
Oxidized-low density lipoprotein (ox-LDL)
Phorbol ester
Shear stress
Transforming growth factor-β (TGF-β)
Tumor necrosis factor-α (TNF-α)
In vivo conditions for LOX-1 upregulation
Atherosclerosis
Diabetes mellitus
Hyperlipidemia
Hypertension
Ischemia reperfusion injury
Transplantation

their presence could have additive or synergistic effects on the regulation of LOX-1 gene expression (Fig. 3).

7.1. Renin-angiotensin system, dyslipidemia and LOX-1 expression

As suggested earlier, renin-angiotensin system (RAS) activation via angiotensin converting enzyme (ACE) and Ang II type 1 (AT1) receptor expression is a powerful mediator of atherosclerosis, since the expression of both ACE and AT1 receptors is enhanced in developing lesions, and inhibiting RAS reduces atherogenesis [44]. Li et al. first reported from our laboratory that ox-LDL induces the upregulation of the AT1 receptors [45] and ACE [46]. On the other hand Ang II via AT1 receptor activation upregulates the expression of LOX-1 in a transcriptional fashion and enhances the uptake of ox-LDL in human coronary artery endothelial cells [7]. Both ox-LDL and Ang II activate similar intracellular pathways leading to cell activation and injury, precursors of atherosclerosis and myocardial ischemia. This has led to the concept of a positive cross-talk between dyslipidemia and RAS as depicted in Fig. 4.

To examine the concept of cross-talk between RAS and hyperlipidemia, Chen et al. [14] conducted a study in apo-E knockout mice, a model of spontaneous hypercholesterolemia, and found that the stimulated LOX-1 expression in these mice is reduced by feeding mice with rosuvastatin, an HMG CoA reductase inhibitor, which lowers LDL-cholesterol and also reduces the formation and uptake of ox-LDL [44]. Interestingly candesartan, an Ang II type 1 receptor blocker, which does not lower LDL-cholesterol, also reduced LOX-1 expression. More importantly, when the two reagents were given concurrently, LOX-1 expression was ablated. The two agents given concomitantly had a synergistic inhibitory effect on the development of atherosclerosis and other markers of inflammation such as CD40/40L and MMPs [47]. This study provided solid evidence of a cross-talk between hypercholesterolemia and RAS with regard to inflammation, oxidative stress and a key role of LOX-1 in this process.

7.2. Oxidative stress and LOX-1 expression

A number of studies have shown that oxidant species, such as H2O2 upregulate LOX-1 expression in endothelial cells, smooth muscle cells and fibroblasts [41]. Muscoli et al. [48] recently showed that balloon catheter-induced rat carotid injury is associated with oxidant stress and LOX-1 expression, and M40401, a potent anti-oxidant, reduced carotid injury and downregulated LOX-1 expression simultaneously.

Hayashida et al. [49] showed that the peroxisome proliferator-activated receptor-α (PPAR-α) is one of the key regulators for LOX-1 upregulation, while Chiba et al. [50] suggested that PPAR-γ activators can inhibit TNF-α-induced LOX-1 expression in cultured bovine aortic endothelial cells. We showed that the PPAR-γ ligand pioglitazone can inhibit ox-LDL and Ang II-induced oxidative stress and LOX-1 upregulation in coronary artery endothelial cells and fibroblasts, and that NF-κB plays a powerful role in the effects of pioglitazone [51]. Li et al. [43] identified that glucose-induced LOX-1 upregulation is mediated through activating protein-1 (AP-1). Related studies showed that the activation of these redox-sensitive transcription factors are mediated via different signaling pathways include p38 mitogen-activated protein kinase (MAPK) [52], p44/42 MAPK [46], protein kinase C (PKC) [53], protein kinase B (PKB) [54] and protein tyrosine kinase (PTK) [55].

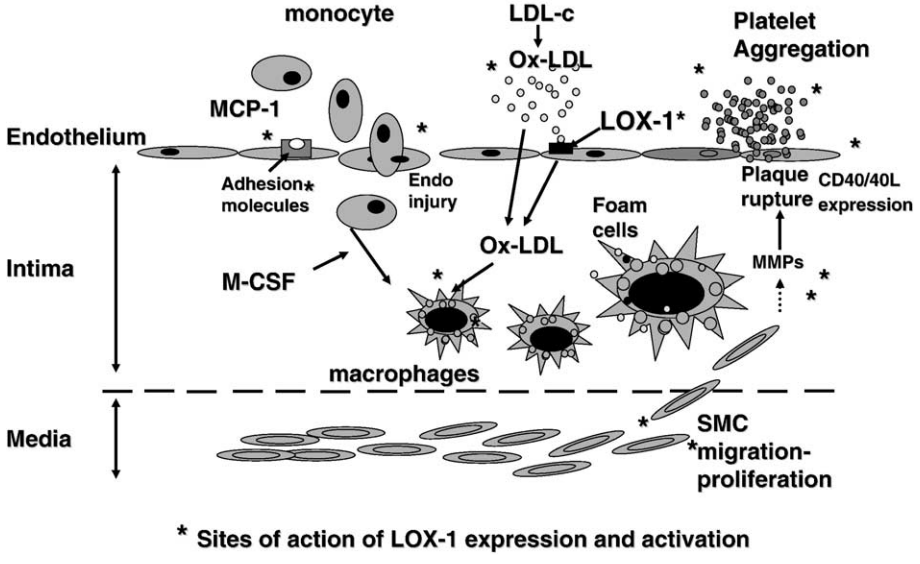

Fig. 3. These hypothesized steps have been reviewed in several recent papers. LOX-1 expression and activation have been demonstrated from the beginning of atherosclerosis (such as endothelial activation and apoptosis) to the culmination into an acute event (such as plaque rupture).

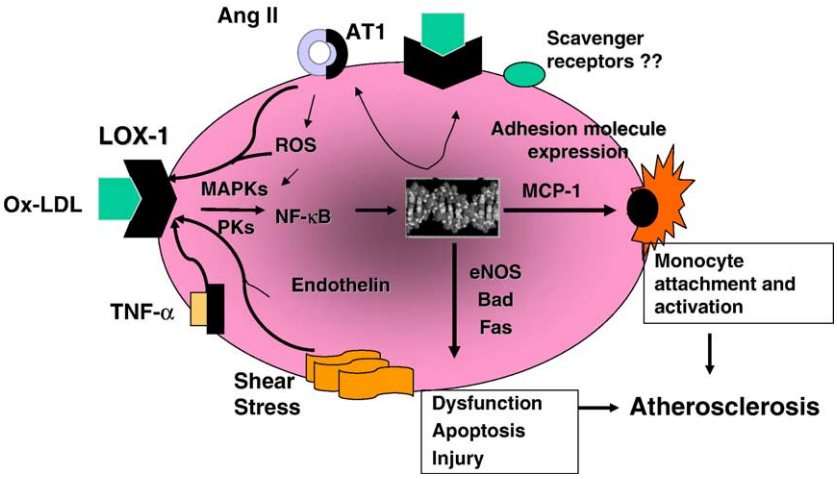

Fig. 4. Important interactions between two major determinants of atherosclerosis, angiotensin II and dyslipidemia. Ox-LDL via LOX-1 upregulates Ang II type 1 receptor expression and Ang II transcriptionally upregulates the expression of LOX-1. Shear stress, endothelin and TNF-α also upregulate LOX-1. Activation of both AT1 and LOX-1 receptors via redox signaling leads to cell dysfunction culminating in apoptosis and cell injury, monocyte adhesion and activation and eventually atherosclerosis.

8. LOX-1 dependent intracellular signaling pathways

In vascular endothelial cells, LOX-1 activation has been suggested to induce several intracellular signaling pathways, including protein kinases and transcription factors, regulating the expression of genes related to atherosclerosis. These ox-LDL-induced signaling pathways include p38 (MAPK) [52], p44/42 MAPK [46], protein kinase C (PKC) [53], protein kinase B (PKB) [54], protein tyrosine kinase (PTK) [55], transcription factor NF-κB [45] and AP-1 [56]. The fact that their activation is LOX-1 dependent comes from the observations that their activation is attenuated by pretreating cells with specific LOX-1 antibody of LOX-1 mRNA antisense. Also, it was shown in a recent study that in endothelial cells another LOX-1 dependent signaling pathway is programmed cell death, also called apoptosis [57]. In this study, Chen et al. identified that ox-LDL induces caspase-9 dependent apoptotic pathway, and that this process is LOX-1 dependent, since LOX-1 mRNA antisense significantly blocks ox-LDL-induced apoptosis.

9. Role of LOX-1 in pathological states

9.1. Atherosclerosis

Ox-LDL causes activation followed by dysfunction of endothelium, which appears in the early stages of atherogenesis. Ox-LDL stimulates expression of chemokines and adhesion molecules such as monocyte chemotactic protein-1 (MCP-1), E- and P-selectins, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells [17,56,58]. These molecules facilitate the adhesion of monocytes to endothelium, initiating atherosclerosis. Ox-LDL also triggers the CD40/CD40L signaling pathway that activates the inflammatory reaction and atherosclerosis-associated features in endothelial cells [53]. Besides stimulating the inflammatory cascade, ox-LDL can cause the release of matrix metalloproteinases (MMPs) without significant effect on tissue inhibitors of metalloproteinase (TIMPs) [59]. This may well be the basis of the rupture of soft plaque in acute coronary syndromes. Ox-LDL has also been shown to induce the production of superoxide anion in bovine aortic endothelial cells [60], while impairing endothelial nitric oxide synthase (eNOS) activation, and consequently the release of nitric oxide (NO) falls [61]. These effects result in increased intracellular oxidative stress, which is a major stimulus for endothelial dysfunction.

Notably, LOX-1 plays an important role in these pro-atherogenic effects of ox-LDL. LOX-1 also plays a critical role in the expression of two smooth muscle/fibroblast-directed growth factors, the A and B chains of PDGF and heparin-binding EGF-like protein (HB–EGF), in cultured human endothelial cells [62]. The expression and secretion of these growth factors are associated with the migration and proliferation of smooth muscle cells and fibroblasts, leading to the progression of atherosclerosis.

In vitro studies have demonstrated that ox-LDL causes apoptosis and necrosis in human coronary endothelial cells [16]. Recent studies from our laboratory show that ox-LDL leads to endothelial apoptosis via Bcl-2 and caspase-9 dependent manner [57]. This pro-apoptotic effect of ox-LDL can be blocked by LOX-1 antibody [63] as well as LOX-1 antisense [57]. Caspase-3 independent pathway involving apoptosis-inducing factor, also participates in ox-LDL-mediated endothelial apoptosis [64]. This effect can also be blocked by LOX-1 antibody [64].

Besides endothelial cells, LOX-1 is also expressed on smooth muscle cells and monocyte/macrophages [65]. Although the expression level is relatively low, LOX-1 functions as a SR, which binds and internalizes ox-LDL,

resulting in the transformation of smooth muscle cells and monocyte/macrophages into foam cells. In addition, LOX-1 has also been identified on platelets [8], and plays a role in platelet activation and thrombus formation.

The role of LOX-1 in atherosclerosis is also suggested by animal and human studies, showing that LOX-1 expression is increased in atherosclerotic aorta from hyperlipidemic animals, including rabbits [12], mice [14] and rats [66], and humans [20]. LOX-1 is mainly expressed on endothelial cells, smooth muscle cells and monocyte/macrophages as well as in the microvasculature infiltrating advanced atherosclerotic plaque [15]. LOX-1 appears to localize in cells undergoing apoptosis [15]. In recent studies, we demonstrated increased levels of LOXIN, a new functional splicing isoform of ORL1 gene, in macrophages which reduce the levels of apoptosis, suggesting that this isoform protects cells from ox-LDL-induced apoptosis and provides the basis for the rationale to develop a therapeutic approach directed at LOX-1/LOXIN [40].

9.2. Hypertension

Nagase et al. [13] reported that LOX-1 mRNA expression is minimal in the aorta from normal rats, but is markedly upregulated in spontaneously hypertensive rats, suggesting a correlation between LOX-1 and hypertension. This concept is supported by the in vitro observations that Ang II upregulates LOX-1 gene expression in endothelial cells [7] and ACE inhibitors markedly decrease LOX-1 gene expression [67]. All these findings suggest that LOX-1 may contribute to the pathogenesis of hypertension which is induced by the activation of RAS. We postulate that the activation of RAS induces LOX-1 expression and activation, which in turn participates, at lest in part, in the genesis of the diminished endothelium-dependent vasorelaxation and induction of hypertension.

9.3. Thrombosis and myocardial ischemia reperfusion injury

Thrombosis is usually the event that leads to myocardial ischemia and stroke, and platelets are the usual initiators in this process [68]. Platelets have been shown to internalize ox-LDL, resulting in diminished eNOS activity in platelets and enhanced platelet aggregation [69]. Chen et al. [8] demonstrated that platelets express a modest amount of LOX-1, and Kakatani et al. [70] found LOX-1 antibody decreases arterial thrombus formation in the rats, suggesting a contributory role of LOX-1 in ox-LDL-mediated platelet activation and thrombosis.

There is a body of evidence pointing to the detrimental role of ox-LDL in myocardial ischemia-reperfusion injury. Firstly, ox-LDL is present in the atherosclerotic tissues of rupture-prone segments [71]; secondly, the degree of myocardial ischemia is characterized by the oxidative state, which facilitates the oxidation of native-LDL [71]; and thirdly, an in vitro study showed that perfusion with ox-LDL significantly decreases myocardial contraction in the isolated rat heart [72]. We observed a marked upregulation of LOX-1 in the myocardium of Sprague Dawley rats subjected to coronary artery occlusion for 1 h and reperfusion for 1 h. Most importantly, treatment of rats with a specific antibody to LOX-1 before ischemia reduced LOX-1 expression and prevented myocardial injury, apoptosis and dysfunction [63]. All these studies imply a very important role of LOX-1 during myocardial ischemia, in terms of determination of cardiac function and infarct size.

9.4. Diabetes mellitus

Diabetes is characterized by a state of oxidative stress, endothelial dysfunction and increased expression of adhesion molecules in the inflammatory cells [73]. In an in vitro study, Li et al. [43,74] reported that LOX-1 expression is increased by glucose both in macrophages and in endothelial cells. Kita et al. [42] found that LOX-1 expression is increased in the aortas of diabetic rats, compared with that of the non-diabetic rats. As such, it is not surprising that diabetics are prone to develop atherosclerosis. Interestingly, Chui et al. [75] demonstrated a critical role for LOX-1 in the regulation of adipocyte lipid metabolism and, potentially, insulin sensitivity, by PPARγ ligands.

9.5. Inflammation and immune response

Adhesion of bacteria to vascular endothelial cells as well as mucosal cells and epithelial cells appears to be one of the initial steps in the process of bacterial infection. It has been reported previously that LOX-1 supports the adhesion of Gram-positive and Gram-negative bacteria to vascular endothelial cells [36], suggesting a role of LOX-1 in bacterial inflammation. Honjo et al. [76] found that LOX-1 is involved in endotoxin-induced inflammation and leukocyte recruitment and infiltration in vivo. In a recent study, Jeannin et al. [77] identified that pneumonia activates macrophages and dendritic cells via LOX-1. Kielian et al. found that LOX-1 mRNA expression is elevated in both *S. aureus*-stimulated glia in vitro as well as brain abscesses in vivo (data not published). These data are not surprising in light of early in vitro studies demonstrating upregulation of LOX-1 by inflammatory cytokines such as TNF-α [18] and transforming growth factor-β (TGF-β) [78], while in vivo studies have shown the localization of these cytokines to be spatially correlated with LOX-1 expression in advanced atherosclerotic lesions [79]. These studies, taken together, suggest that LOX-1 plays a role in inflammation and immune response.

10. Conclusion

Identification of LOX-1 and a definition of its biologic role in pathophysiologic states provide a new clue to the

reason for the uptake of ox-LDL by vessel wall components. Internalization of ox-LDL leads to a cascade of events that may induce a variety of diseases characterized by endothelial dysfunction, activation and injury. Activation of endothelial cells by ox-LDL through LOX-1 may be a key event in hypertension, diabetes mellitus and hyperlipidemia, the most important risk factors for atherosclerosis. As such, therapies targeting LOX-1 or LOXIN may be effective strategies for treating atherosclerotic and hypertensive as well as diabetic patients.

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