

The Expression and Down Stream Effect of Lectin Like-oxidized Low Density Lipoprotein 1 (LOX-1) in Hyperglycemic State

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ABSTRACT

The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses. Low density lipoprotein (LDL), which may be modified by oxidation, glycation, aggregation, association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to the endothelium and vascular smooth muscle cells (VSMC).

The major cell types involved in atherogenesis, macrophages and VSMC, are activated by pro-inflammatory stimuli including modified LDL. Modified LDL induces inflammatory responses in macrophages, migration and proliferation of VSMC, and triggers foam cell formation. Scavenger receptors, including LOX-1, play a key role in foam cell formation by mediating the uptake of modified LDL. LOX-1 expression is detected in endothelial cells of early atherosclerosis lesions of human carotid arteries. Advanced lesions showed LOX-1 expression not only in endothelial cells but also in macrophages and more frequently in VSMC, and may be involved in foam cell transformation in macrophages and VSMC.

The metabolic abnormalities that characterize diabetes, particularly hyperglycemia, free fatty acids, and insulin resistance, provoke molecular mechanisms that alter the function and structure of blood vessels. These include increased oxidative stress, intracellular signal transduction disturbances, and activation of the receptor for advanced glycation end products (R-AGE). Data showed that LOX-1 expression is enhanced by proatherogenic factors relevant to human diabetes, including high glucose, oxLDL, advanced glycation end products, and C-reactive protein. LOX-1 expression increased also through oxygen species (ROS), endothelin-1 (ET-1), tumor necrosis factor- α (TNF- α), shear stress, activation of protein kinase-C (PKC), angiotensin-II (ANG-II), and through inflammatory pathways.

Key words: hyperglycemia, LOX-1.

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INTRODUCTION

The prevalence, incidence, and mortality from all forms of cardiovascular disease are increased in patients with diabetes. Among the cardiovascular risk factors documented in diabetes, hyperglycemia appears as an independent risk factor for diabetic macrovascular complications.¹ Some studies have explicitly examined the association between glycemic control, carotid intima-media thickness, and risk factors for cardiovascular disease. Impairment of endothelium and muscle function in hyperglycemic condition contributes to abnormalities of vasodilation.² Endothelial dysfunction is a key, early, and potentially reversible event in atherogenesis that is commonly present in human diabetes,^{2,3} and plays a key role in the pathogenesis of diabetic vasculopathies.⁴ Several mechanisms may cause or contribute to endothelial dysfunction in diabetes. These include hyperglycemia, oxidative stress, oxidized LDL (oxLDL), insulin resistance, advanced glycation end products (AGEs), activation of protein kinase C (PKC), and dislipidemia.^{5,6,7,8,9}

Scavenger receptors (SRs) on macrophages were first described as alternative receptors to the LDL receptors in the uptake of excessive cholesterol and lipid, which leads to the development of foam cells. The members of scavenger receptors are classified into: Class A, B, C, D, E, and F.¹⁰ Class A consists of SR-AI, SR-AII, SR-AIII, and the macrophage receptor with collagenous structure (MARCO). The ligands of SR-AI and II are acetylated LDL (AcLDL), AGEs, lipopolysaccharide (LPS), whole bacteria, polyinosinic acid, methylated BSA (M-BSA), and polyguanosinic acid.¹¹ The ligands of MARCO are AcLDL, LPS and whole bacteria. Class B consists of SR-Bi and CD36. High density lipoprotein (HDL), modified lipoproteins, anionic phospholipids, fatty acid, collagen, thrombospadin, malaria-infected red blood cells, and apoptotic cells are the ligand of SR-B. Class C contains only the

Drosophila SR-C, shows affinity to AcLDL, while class D, by amino acid analysis, this protein is identified as macrosialin, the murine homologue of human CD68 showed affinity to phosphatidylserine liposome, malonaldehyde, BSA, and OxLDL. Class E, consists of LOX-1 only that showed high affinity saturable binding of oxLDL but apparently did not recognize AcLDL. Class F SRs expressed by endothelial cells (SREC), bound and degraded AcLDL. Both of LOX-1 and SREC, can account for the observed high uptake of modified lipoproteins in vivo by liver sinusoidal endothelial cells.¹²

Recently, a role has been proposed for LOX-1 in vascular cell dysfunction and monocyte adhesion.¹³ LOX-1 was initially identified in endothelial cells.¹⁴ Subsequent studies show that LOX-1 is also expressed in macrophage and VSMC.¹⁵ The finding that LOX-1 expression is increased in the vascular endothelium of diabetic rat suggests a role for this receptor in endothelial dysfunction associated with diabetes.^{16,17} The study by Li also demonstrates a new role of LOX-1 as a mediator of glucose-induced monocyte adhesion.¹⁸

Information concerning the role of LOX-1 in vascular disease is accumulating, and it is clear that pro-atherogenic conditions, such as hypertension, hyperlipidemia, and diabetes, induces LOX-1 expression.^{16,19,20} The pathophysiological stimuli relevant to atherosclerosis in diabetes that may contribute to this vascular abnormality includes oxLDL, tumor necrosis factor- α (TNF α), and AGEs, PAI-1, TF, and decreasing of NO,^{16,21,22} but the study of the mechanisms responsible for the upregulation and activation of vascular LOX-1 in diabetes are always renewing.

The present review will focus on the relationship of hyperglycemia and atherosclerotic vascular disease, highlighting a molecular mechanism in the role of hyperglycemia in LOX-1 expression and activation.

EVIDENCE OF LOX-1

LDL, which can be modified by oxidation, glycation, aggregation, association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to the endothelium and vascular smooth muscle cells. LOX-1 is a receptor for oxLDL. This receptor can support the binding, internalization, and degradation of oxLDL. When LDL particles become trapped in an artery, they can undergo progressive oxidation and become internalized by macrophages by means of the scavenger receptors on the surfaces of these cells.^{23,24,25} The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol

esters, resulting in the formation of foam cells. OxLDL is internalized by several scavenger receptors, such as SR-AI/II, SR-BI, CD36, macrosialin, and CD68.¹¹ LOX-1 can account for the observed high uptake of oxLDL in vivo. LOX-1 and CD36 are mainly responsible for oxLDL uptake in VSMC.²⁶

LOX-1, which is also called oxidized LDL receptor 1 (OLR-1),²⁷ is a membrane glycoprotein whose structure belongs to the C-type lectin family and does not share any structural homology with other known molecules that can act as a receptor for oxLDL. This membrane protein synthesized as 40-kDa precursor protein and is composed of four domains: extracellular lectin-like domain at the C-terminal, a connecting neck domain, a transmembrane domain, and an N-terminal cytoplasmic domain. The lectin domain which is also called carbohydrate recognition domain, is the functional domain for the oxLDL binding.²⁸

Evidence indicates a key role for LOX-1 in atherogenesis. The expression of LOX-1 by vascular cells is enhanced by pro-atherogenic factors,^{21,29} and LOX-1 is expressed in vivo in the aortas of animals with pro-atherogenic settings.²⁰ Two main LOX-1 ligands, oxLDL and AGE, are implicated in the pathogenesis of atherosclerosis,³⁰ and uptake of oxLDL by endothelial cells through LOX-1 induces endothelial dysfunction.¹⁹

Upregulation of LOX-1

LOX-1, initially identified in endothelial cells, is also expressed in macrophage and VSMC. LOX-1 expression is detected in the endothelial cells of early atherosclerotic lesions of human carotid arteries. Advanced lesions show LOX-1 expression not only in endothelial cells but also in macrophages, and more frequently in VSMC.³¹ The earliest type of atherosclerotic lesions, called fatty-streak, is a purely inflammatory lesion, consisting of only monocyte-derived macrophage and T lymphocytes.³² Recent investigations showed that inflammatory stimuli modulated LOX-1 expression in vitro. An investigation by Hofnagel showed that all studied cytokines upregulated LOX-1 expression in a dose and time-dependent manner.³³ Three-hour incubation with TNF α caused upregulation of LOX-1 mRNA and LOX-1 protein expression in VSMC, while mRNA expression was increased 2.4 fold after eight-hour incubation. The effect of TNF α alone on LOX-1 protein expression was blocked by an anti-TNF α antibody.³⁴ After three-hour incubation with interleukin (IL)-1 α and IL-1 β , there was significantly increased expression of LOX-1 mRNA, while the expression of LOX-1 protein was also increased. Simultaneous incubation with a combination

of interleukin (IL)-1 α , IL-1 β , and TNF- α at saturated concentrations of each of these cytokines increased LOX-1 mRNA and protein expression in an additive manner.³³ IL4 also upregulated LOX-1, and the activity of IL4 was controlled by TNF α and TGF β .³⁵ Upregulated LOX-1 expression in VSMC in advanced atherosclerotic lesions as a response to proinflammatory cytokines can be decreased by the anti-inflammatory effect of PPAR- γ .³³

Protein kinase-C (PKC) plays an important role in mediating biological responses. Studies on the regulation of LOX-1 expression in endothelial cell, macrophages, and VSMC revealed upregulation of LOX-1 after incubation with PMA, an activator of PKC.²¹ There was upregulation of LOX-1 mRNA and LOX-1 protein expression after three-hour incubation of the cells with PMA. This upregulation was also dose and time-dependent, revealing a peak after incubation with higher doses of PMA for sixteen hours.³³ Conversely, pretreatment with pan-specific PKC inhibitor, calphostin-C, PKC- α inhibitor, LY379196, or MAPK inhibitor PD98059, prior to exposure to high glucose, completely prevented the stimulatory effect of high glucose on LOX-1 mRNA expression in human aortic endothelial cells.¹⁷

Increased expression of LOX-1 in endothelial cells is most prominent at arterial bifurcations exposed to complex shear forces and circumferential strain,¹⁶ indicating that LOX-1 expression in endothelial cells might be upregulated by hemodynamic factors.³⁶ It has been previously shown that AGE also enhanced LOX-1 mRNA expression in cultured aortic endothelial cells and human macrophages.^{16,37}

Free radicals may also enhance LOX-1 expression. Pre-incubation of human macrophages with various antioxidants, including N-acetylcystein, vitamin E, vitamin C, and DMSO, totally prevented the stimulatory effect of high glucose on LOX-1 gene expression.³⁴ Peroxisome proliferators-activated receptor gamma (PPAR- α) ligand inhibits intracellular superoxide radical generation and subsequent expression of the redox-sensitive transcription factor such as NF κ B. This results in the downregulation of LOX-1 expression in response to a number of proinflammatory and proatherosclerotic stimuli such as oxLDL, angiotensin II, and TNF- α .³⁸ Similar results are also demonstrated by Chiba, that PPAR- γ ligand, but not PPAR- α ligands and fenofibric acid, inhibits TNF- α induced LOX-1 expression in bovine aortic endothelial cells.³⁹

OxLDL is a ligand for LOX-1. A study by Thum shows that oxLDL produces dose-dependent up to 4-fold upregulation in ICAM-1 and LOX-1 gene

expression.⁴⁰ Through phosphorylation of ERK, oxLDL upregulates LOX-1 expression in VSMC.⁴¹ Intracellular availability oxLDL activates LOX-1 and triggers expression of various adhesion molecules, exaggerated production of radical oxygen species (ROS) that will scavenge nitric oxide (NO), thereby reducing its availability and the synthesis of toxic peroxynitrite. The ROS produced by the ligation of oxLDL to LOX-1 could facilitate the oxidation of native LDL or partially oxLDL, which in turn can up-regulate LOX-1 expression.²¹ The increase of LOX-1 activity is also associated with an increased release of ROS and reduction in cellular concentration of NO.⁴²

Iwanaga demonstrates that LOX-1 expression can be induced in cardiac myocytes by stimulation with norepinephrine and endothelin-1, important neurohormonal factors activated in heart failure.⁴³ There is increasing evidence that angiotensin-II (AngII) modulates the effects of oxLDL on endothelial cell function, thereby promoting atherosclerosis. In endothelial cells, AngII upregulates LOX-1 via the angiotensin-1 receptor (AT1R). Angiotensin receptor blockers (ARB) and angiotensin converting enzymes (ACE) inhibitors decrease LOX-1 expression in the aorta of hypercholesterolemic rabbits²⁰ and arteries of patients with coronary artery disease.⁴⁴

Linoleic acid (LA) is increased in all LDL subfractions in patients with type-2 diabetes. LA led to significant increase in LOX-1 gene expression through oxidative stress-sensitive and PKC-dependent pathways. This effect seems to be exerted at the transcriptional level and to involve the activation of NF κ B.⁴⁵

Some evidence has demonstrated that LOX-1 expression are enhanced through several pathways, involves proatherogenic factors, oxLDL, AGE, free radicals, PKC, TNF- α , ET-1, Ang-II, NE, IL-1, and shear force.

The Downstream Effect of Activated LOX-1

Accumulation of cholesterol-loaded foam cells in the arterial intima is a hallmark and key event of early atherogenesis. LOX-1 is highly expressed in macrophages present in human atherosclerotic lesion.³¹ A study by Li demonstrates that increased LOX-1 surface expression in glucose-treated macrophages is associated with enhanced uptake of oxLDL by these cells, and this suggests the role of LOX-1 in mediating foam cell formation.³⁴

OxLDL activates LOX-1, exaggerating the production of radical oxygen species (ROS) that will scavenge nitric oxide (NO), thereby reducing its availability and thus the synthesis of toxic peroxynitrite.²¹ A similar result was found by Cominacini, whereby increased

LOX-1 activity was also associated with an increased release of ROS and reduction in cellular concentration of NO.⁴² Activation of LOX-1 induces myocardial cell apoptosis and this apoptosis requires the oxidative stress-sensitive p38 mitogen activated protein kinase (MAPK) pathway.⁴⁶ OxLDL will also upregulate the expression and activity of matrix metalloproteinases 1 and 3 (MMP-1 and 3) in human coronary artery endothelial cells, through LOX-1 activation¹⁸.

LOX-1 facilitates the uptake of oxLDL and mediates several of the biological effects of oxLDL in endothelial cells.⁴⁷ Activation of this receptor by ligand oxLDL, induces expression of adhesion molecules. LOX-1 is shown to induce expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) on the vascular endothelial cells.⁴⁸ Activation of this pathway also stimulates ROS production, mitogen activator protein kinases (MAPKs), and NFkB.⁴⁹

LOX-1 expression is well co-localized with Bax expression in the rupture-prone areas of human atherosclerotic plaque in vivo. LOX-1 may play an important role in oxLDL-induced apoptosis in VSMCs. LOX-1 induces expression of a proapoptotic factor Bax, down-regulates an antiapoptotic factor Bcl-2, and increases the Bax to Bcl-2 ratio. These molecular mechanisms may be involved in the destabilization and rupture of atherosclerotic plaque⁴¹. LOX-1 is not the only thing that has a role in VSMC apoptosis. For example, oxLDL induces apoptosis in human coronary artery endothelial cells via LOX-1 in concert with upregulation of this receptor, and activates NFkB that plays an important role as a signal transduction in this process.⁵⁰ Thus, vascular cell apoptosis mediated by oxLDL and its receptor may be crucial for atherosclerotic plaque rupture in advanced stages, as well as endothelial dysfunction in early stages.

Chen (2004) explored the involvement of caspases and the related molecules, such as Bcl2 and cIAP2, and reported that activation of caspase-9 and subsequent activation of caspase-3 were responsible for oxLDL-induced apoptosis of cultured vascular endothelial cells through its receptor LOX-1.⁵¹ In addition, release of mitochondrial apoptotic proteins, such as cytochrome-c and Smac, was associated with oxLDL-induced caspase activation and apoptosis.

The result of several studies showed that LOX-1 activation was responsible for the increase of oxLDL metabolism, ROS generation, upregulated of MMP and adhesion molecules expression, as well as cells apoptosis.

HYPERGLYCEMIA AND LOX-1 UPREGULATION

Cellular Mechanism of Diabetes Complications

Several mechanisms may contribute to the development of diabetic complications. Increased intracellular glucose leads to the formation of AGEs via the nonenzymatic glycosylation intra and extracellular protein. Nonenzymatic glycosylation results from the interaction of glucose with amino group of protein. Intracellular hyperglycemia increases glucose metabolism via the sorbinil pathway. When intracellular glucose increases, some glucose is converted to sorbitol by the enzyme aldose reductase. Increased sorbitol concentration alters redox potential, increases cellular osmolarity, generates active oxygen species, and leads to cellular dysfunction. Hyperglycemia increases the formation of diacylglycerol (DAG), leading to the activation of PKC α , β , γ , δ , ϵ , ζ , η , θ , ι , κ , λ , μ , ν , ξ , \omicron , π , ρ , σ , τ , υ , ϕ , χ , ψ , ω , which can be prevented by aldose reductase inhibitors tolrestat or sorbinil 53. Hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter the function by glycosylation of protein such as endothelial nitric oxide synthase (eNOS), or changes in gene expression of TGF- β or plasminogen activator inhibitor-1 (PAI-1).⁵² The other mechanisms that contribute to the development of diabetic complication including insulin resistance leads to the formation free fatty acid, hyperlipidemia, an increase in oxLDL, oxidative stress, hyperglycemia itself,^{5,9} and an increase in intracellular calcium ions.^{54,55}

Under physiological conditions, glucose primarily undergoes glycolysis and oxidative phosphorylation. Under pathologic conditions of hyperglycemia, excessive glucose levels can swamp the glycolytic process and inhibit glyceraldehydes catabolism, which causes glucose, fructose-1,6-biphosphate, and glyceraldehyde-3-phosphate to be shunted to other pathways, including: enolization and α -ketoaldehyde formation, PKC activation, dicarbonyl formation and glycation, sorbinil metabolism, hexosamine metabolism, and oxidative phosphorylation. All these six biochemical pathways along with glucose metabolism can form ROS.⁵⁶

Upregulation of PKC can promote monocytes to secrete increased amounts of interleukin-6 (IL-6)⁵⁷, and increase VSMC-transforming growth factor- β 1 (TGF- β 1) and transforming growth factor- β receptor1 (TGF- β R1) expression, but not TGF- β R2 expression.⁵⁸

It has been established that the endothelium plays a vital role in the regulation of reactivity of vascular tissues by releasing the endothelium-derived factors that

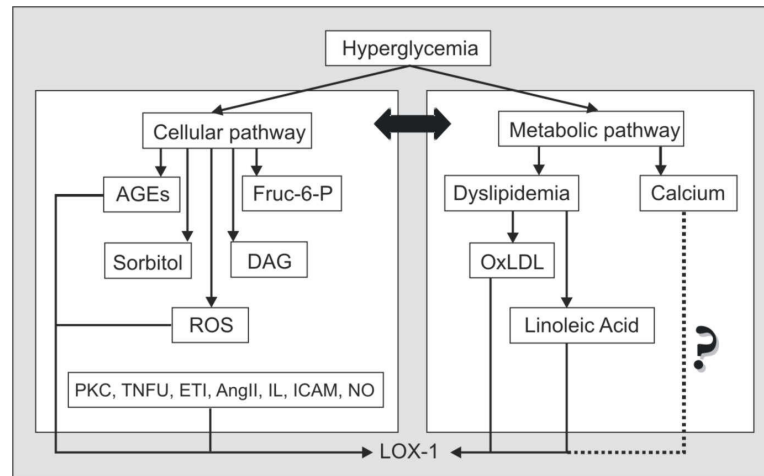


Figure 1. The role of hyperglycemia in LOX-1 expression. Through a cellular pathway, hyperglycemia will increase PKC, TNF- α , ET1, Ang II, IL, ICAM expression, and decrease the availability of intracellular NO. Hyperglycemia also promotes oxLDL, linoleic acid production, and increases intracellular calcium ions. All of these conditions will increase LOX-1 expression.

PKC = protein kinase-C, TNF = tumor necrosing factor, ET = endothelin, Ang = angiotensin, No = nitric oxide, oxLDL = oxidized low density lipoprotein, LOX = lectin like oxidized LDL receptor.

act on adjacent VSMC. One such factor produced by endothelial cells is the potent vasoconstrictive peptide endothelin-1 (ET-1). Recently, several reports have shown that insulin stimulates ET-1 secretion from endothelial cells and enhances ET-1 binding to its receptors.⁵⁹ It has also been shown that plasma ET-1 levels are elevated in type-2 diabetes patients with microvascular complications.⁶⁰

One potential key determinant that may induce activation and injury to the endothelium in the diabetic state including selected unsaturated fatty acids, such as linoleic acid (LA), and oxLDL,⁶¹ and may contribute to the upregulation of LOX-1.

The Role of Hyperglycemia on Expression and Activation of LOX-1

Chronic hyperglycemia has been shown to be responsible for multiple micro and macrovascular complications as a result of hyperglycemic damage through major biochemical processes, including AGE production, the polyol pathway, the hexoamine pathway, activation of PKC,⁵² and increase of intracellular calcium ion. Hyperglycemia increases free radical production, associated with the elevation of C-reactive protein (CRP), PAI-1, FFA, IL-6, and TNF- α expression.^{62,63} All biochemical changes can increase LOX-1 expression and activity.

The finding that LOX-1 expression is increased in the vascular endothelium of diabetic rats (Chen, 2001) suggests a role for this receptor in diabetic complications. Data show that LOX-1 expression is enhanced

by proatherogenic factors relevant to human diabetes, including oxLDL,^{31,40} AGEs,^{16,37} C-reactive protein,⁶⁴ and high glucose levels.¹⁷ Macrophages of patients with type 2 diabetes also demonstrated a significant increase in LOX-1 mRNA levels compared to those isolated from healthy control.³⁴ Linoleic acid (LA) is increased in all LDL subfractions in patients with type-2 diabetes. LA leads to a significant increase in LOX-1 gene expression through oxidative stress-sensitive and PKC-dependent pathways. This effect seems to be exerted at the transcriptional level and to involve the activation of NF κ B.⁴⁵ Hyperglycemia increases LOX-1 expression through metabolic and cellular pathways. The role of hyperglycemia on LOX-1 expression can be seen in figure-1.

Glucose has been established to enhance LOX-1 expression through several pathways. Intracellular calcium ion acts as a second messenger that will affect PKC activity and some other intracellular proteins. The increase of this ion may affect the expression and activity of LOX-1, but the role of intracellular calcium ion on LOX-1 expression and activation has not yet been established.

CONCLUSION

It can be concluded that under high glucose conditions, several cells, including endothelium, macrophages, and VSMC, can increase LOX-1 expression and activation through several mechanisms.

Proinflammatory factors like IL, TNF- α , CRP such as

PKC, oxLDL, LA, AGEs, free radicals, shears stress, ET-1, Ang-II, and NE and other metabolic pathway that involve linoleic acid and intracellular calcium ion may be involved in the up-regulation of LOX-1 in hyperglycemic state.

Increases of LOX-1 activity are associated with an increased release of ROS and reduction in cellular concentration of NO, an induction of cell apoptosis, and an upregulation in the expression and activity of matrix metalloproteinases (MMP). LOX-1 facilitates the uptake of oxLDL and induces expression of adhesion molecules. Activation of this pathway also stimulates ROS production, mitogen activator protein kinases (MAPKs), and NFκB.

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