Oxidized Low-Density Lipoprotein and Oxidative Stress in the Development of Glomerulosclerosis

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Key Words

Angiotensin II • Dyslipidemia • Mesangial matrix • NADPH oxidase • Reactive aldehydes • TGF- β signaling

Abstract

Glomerulosclerosis frequently complicates most renal diseases and is characterized by mesangial matrix accumulation. Oxidized low-density lipoprotein (Ox-LDL) could induce oxidative stress and profibrotic gene expression in mesangial cells. This article will review our current understanding of the pathogenetic mechanisms of lipid-mediated glomerulosclerosis, emphasizing the fibrogenic signaling cascades triggered by Ox-LDL. In addition, therapeutic strategies to prevent the development of Ox-LDL-mediated glomerulosclerosis will be discussed.

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Introduction

Plasma low-density lipoprotein (LDL) and total cholesterol are markedly elevated in nephrotic syndrome, and lipoprotein abnormalities are common features of the nephrotic syndrome and uremia. One of the deleterious effects of hypercholesterolemia and dyslipidemia on the kidney is enhanced oxidative stress [1–3]. LDL can be

oxidized in patients with chronic kidney disease [4–6], and the oxidized LDL (Ox-LDL) seems to participate in the development of glomerulosclerosis and interstitial fibrosis [7, 8].

In cultured mesangial cells, LDL and Ox-LDL induce the expression of inflammatory elements, such as platelet-derived growth factor [9, 10], monocyte chemoattractant protein-1 [11], macrophage colony-stimulating factor [11], interleukin-6 [10, 12], and tumor necrosis factor- α (TNF- α) [10]. LDL also stimulates angiotensin II (Ang II) production in mesangial cells [13] (table 1).

Both the glomerulosclerosis and interstitial fibrosis induced by lipid seem to be mediated by activation of transforming growth factor- β (TGF- β) [14, 15]. In cultured mesangial cells, LDL and Ox-LDL activate TGF- β 1 [16, 17], Smad3 [17–19], and extracellular signal-regulated kinase (ERK)1/2 [18, 20, 21]. They also increase the expression of connective tissue growth factor [22] and extracellular matrix (ECM), such as fibronectin [23], types I, III, and IV collagen [9, 16, 22], and plasminogen activator (PA) inhibitor-1 (PAI-1) [17–19, 21, 24] (table 1). In cultured glomerular epithelial cells, Ox-LDL stimulates the expression of TGF- β 1 and fibronectin [25].

The mechanisms whereby Ox-LDL might induce mesangial matrix accumulation that could culminate in the development of glomerulosclerosis in humans are not clear. In this regard, this review will focus on the fibro-

genic signaling cascades triggered by Ox-LDL that could lead to oxidative stress and increased mesangial matrix production. In addition, the *in vivo* effects of Ox-LDL and therapeutic strategies to prevent the development of Ox-LDL-mediated glomerulosclerosis will be discussed.

Nature of Oxidative Stress

Oxidative stress refers to conditions involving chronically elevated reactive oxygen species (ROS) levels. The excess production of ROS or impaired local antioxidant capacity leads to oxidative stress, which results in the oxidation of proteins, lipids, carbohydrates, and DNA.

Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) seem to be primary ROS generated in the body. O_2^- is mainly produced by NADPH oxidase in phagocytes participating in bacterial killing [26]. Non-phagocytes including mesangial cells also express the NADPH oxidase complex [27]. Expression of NADPH oxidase is increased in the arteries of atherosclerotic monkeys, but returns toward normal after regression of atherosclerosis [28]. In non-phagocytic cells, the major effects of ROS include regulation of cell growth, activation of numerous signaling molecules as second messengers, and inactivation of nitric oxide (NO) [29]. NO plays a key role in the maintenance of vascular homeostasis and has an antifibrotic effect on mesangial cells [30]. Evidence exists for the presence of increased oxidative stress in chronic kidney disease [4–6, 31] and hypertension [29, 31].

Lipid Peroxidation of LDL

LDL is an emulsion of cholesteryl ester stabilized by surface phospholipid, unesterified cholesterol and apolipoprotein B (apoB). Polyunsaturated fatty acids (PUFAs) in cholesterol esters, phospholipids, and triglycerides are particularly sensitive to free radical-initiated oxidation. A key feature of lipid peroxidation is the breakdown of these PUFAs to yield reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal [32]. The oxidation of LDL, either by incubation with $CuSO_4$ or cells in culture, causes selective loss of lysine and histidine residues in apoB forming apoB-bound aldehydes [33].

Advanced glycation end product modifications are present on both the apoB and the phospholipid components of LDL in patients with diabetes or end-stage renal disease, which contribute directly to oxidative modifica-

Table 1. Effects of LDL and Ox-LDL on cultured mesangial cells

Effects	References
Proinflammatory effects	
Increase PDGF	9, 10
Increase MCP-1, macrophage CSF	11
Increase IL-6, TNF- α	10, 12
Increase reactive oxygen species	13
Profibrogenic effects	
Increase TGF- β	16, 17, 21
Activate Smad3	17–19
Activate ERK	18, 20, 21
Increase CTGF	22
Increase fibronectin and collagen	9, 16, 22, 23
Increase PAI-1	17–19, 21, 24
Increase tPA and uPA	24

LDL = Low-density lipoprotein; Ox-LDL = oxidized LDL; PDGF = platelet-derived growth factor; MCP-1 = monocyte chemoattractant protein-1; CSF = colony-stimulating factor; IL-6 = interleukin-6; TNF- α = tumor necrosis factor- α ; TGF- β = transforming growth factor- β ; ERK = extracellular signal-regulated kinase; CTGF = connective tissue growth factor; PAI-1 = plasminogen activator inhibitor-1; tPA = tissue-type plasminogen activator; uPA = urokinase plasminogen activator.

tion of LDL [34]. Molecular events promoted by Ox-LDL could be amplified by coexisting glycoxidation [35].

LDL, Ang II and Oxidative Stress

Mounting evidence indicates that LDL communicates with the renin-angiotensin system in the development of atherosclerosis [36]. Ang II enhances O_2^- generation through the activation of membrane-bound NADPH oxidases in vascular smooth muscle cells (VSMCs) [29] and mesangial cells [13, 37, 38]. LDL increases Ang II production, which in turn results in enhanced O_2^- production in mesangial cells [13]. The LDL, which generates O_2^- , has already been oxidized, as LDL is rapidly oxidized after its incubation with mesangial cells in serum-free culture media [9]. Ox-LDL decreases the NO synthesis in mesangial cells [39]. Reactive aldehydes within the Ox-LDL tend to trigger the formation of ROS or are oxidants themselves and potentiate oxidative stress in the cells [40].

PKC [37] and Rac1 [38] are involved in the activation of NADPH-oxidase in Ang II-treated mesangial cells. Rac1 also mediates the albumin-induced activation of NADPH oxidase in proximal tubule cells [41].

Fig. 1. Proposed model for the activation of fibrogenic signaling via NADPH oxidase by Ox-LDL. Ox-LDL-stimulated Ang II and TGF- β 1 activate PKC- or Ras-dependent NADPH oxidase via Rac protein in mesangial cells. This process leads to increased reactive oxygen species generation and ERK1/2 activation. The activated ERK1/2 leads to maximal Smad activation resulting in overexpression of TGF- β 1 target genes. Ox-LDL = Oxidized low-density lipoprotein; Ang II/R = angiotensin II/receptor; TGF- β /R = transforming growth factor- β /receptors; PKC = protein kinase C; ERK1/2 = extracellular signal-regulated kinase 1/2.

Fibrogenic Signaling via NADPH Oxidase Triggered by Ox-LDL

In contrast to rat mesangial cells having specific receptors for Ox-LDL, the receptors for Ox-LDL are almost negligible in human mesangial cells [42]. Reactive aldehydes within the Ox-LDL seem to be responsible for the effects of Ox-LDL on signaling/transcriptional regulation associated with oxidative stress [43]. They may have no cell surface receptors and react directly with matrix tissue or cells surface proteins [40].

LDL, Ox-LDL and/or MDA increase ECM synthesis in mesangial cells as shown in table 1. LDL also stimulates the mRNA expression of urokinase PA and tissue-type

PA and PAI-1 in mesangial cells [24]. Exogenous TGF- β increases the levels of PAI-1 and urokinase PA but does not affect the tissue-type PA production in mesangial cells [44].

Ox-LDL activates the PAI-1 transcription through autocrine activation of TGF- β /Smad signaling in mesangial cells [17, 19]. TGF- β signals through sequential activation of two cell surface receptor serine-threonine kinases [45]. Smad proteins are activated by these receptors, transducing signals to the target genes. In the nucleus, Smad3 and Smad4 bind preferentially to specific DNA sequences such as CAGA boxes that are present in several TGF- β -inducible promoters including α 2(I) collagen, TGF- β 1 and PAI-1 genes [19, 46]. Smad3 activated by Ox-LDL directly binds to two of the three CAGA boxes in the PAI-1 promoter [19]. Smad3, which does not bind directly to the TGF- β -inducible promoters, might cooperate and interact with general transcription factors to activate transcription [23, 47].

Increased oxidative stress has been shown to be a sufficient condition for increased mesangial expression of TGF- β 1 [48, 49], and ROS activate latent TGF- β [50]. TGF- β 1 protein itself can induce ROS generation in mesangial cells [49].

All of the major mitogen-activated protein kinases in the vasculature are activated by Ang II [51] and H₂O₂ [49]. TGF- β activates ERK in mesangial cells and ERK is involved in activation of Smad2 and Smad3 [52]. In fact, TGF- β -induced ERK activity leads to phosphorylation of the linker segments of Smad2 and Smad3 that results in maximal Smad activation [52, 53].

Ox-LDL activates ERK in mesangial cells through the induction of TGF- β signaling pathway, Ras activation, or Rac 1-mediated ROS production [18, 20, 21]. Ox-LDL-induced Ras/ERK activation leads to Smad3 activation and subsequent overexpression of TGF- β 1 target genes [21]. Ras or Rac1 leads to an increase in intracellular ROS formation in fibroblasts, in which Rac1 functions downstream of Ras [54].

To sum up, these important facts suggest that Ang II and TGF- β 1 stimulated by Ox-LDL (or its oxidation products) activate PKC- or Ras-dependent NADPH oxidase via Rac protein in mesangial cells. This process leads to increased ROS generation resulting in ERK1/2 activation. The activated ERK1/2 leads to maximal Smad activation resulting in overexpression of TGF- β 1 target genes in mesangial cells, leading to increased mesangial matrix production (fig. 1).

Table 2. Targets for prevention of oxidized LDL-induced glomerulosclerosis

Target	Therapeutic approach	References
Oxidant	Vitamins C and E, probucol	1, 2, 4, 31, 62, 70, 71
	α -lipoic acid, polyphenols	72, 73, 76
	Histidine and carnosine	74
	Statins	4
	Melatonin	75
PKC	Vitamin E	77
	Ruboxistaurin, LY333531	78, 79
RAS	AT1 R blocker, ACEI	13, 36, 82–87
	Angiotensin-(1–7)	88
	TNF- α antagonist	89
NADPH oxidase	Vitamins C and E	2
	Statins	90
	Fasudil	91
	Estrogen	92
Ras/ERK	Statins	21
TGF- β signaling	Estrogen (see table 3)	93

PKC = Protein kinase C; RAS = renin-angiotensin system; AT1 R = angiotensin II type I receptor; ACEI = angiotensin-converting enzyme inhibitor; TNF- α = tumor necrosis factor- α ; ERK = extracellular signal-regulated kinase.

Clinical Significance of Ox-LDL

In uremic patients, plasma concentrations of lipid peroxidation products and Ox-LDL are increased [4]. In fact, they are increased in nephrotic or nephritic patients early on before the onset of renal failure [5, 6]. Dyslipidemia in chronic kidney disease is characterized by reduced clearance of the apoB-containing lipoproteins but misleadingly masked by normal plasma cholesterol levels [55, 56]. Hypercholesterolemia observed in nephrotic syndrome is thought to be due to increased LDL synthesis and impaired LDL clearance [57]. The delayed catabolism in both the nephrotic syndrome and uremia may contribute to increased susceptibility of LDL to oxidation *in vitro*, as long-lived lipoproteins are more subject to oxidation [58].

In a number of human glomerular diseases, apoB-containing lipoproteins accumulate within the glomeruli and tubulointerstitium [59]. When the LDL is trapped for a prolonged period of time in the mesangial matrix or tubulointerstitium, it may be oxidized due to depletion of antioxidants found in plasma and extracellular fluid. Furthermore, due to infiltration by neutrophils and monocyte/macrophages in the inflamed glomeruli or in-

terstitium, the conditions for LDL oxidation would be favorable. Indeed, Ox-LDL or lipid peroxidation products are demonstrated in the glomeruli and interstitium in renal biopsies [60, 61].

Hypercholesterolemia aggravates glomerular and interstitial macrophage accumulation in a variety of experimental renal diseases [8, 14, 62]. Macrophages have a large number of scavenger receptors (SRs), such as SR-A, CD36 (SR-B) and lectin-like Ox-LDL receptor-1 (SR-E) [63–65]. Because the expression of SRs is not downregulated by intracellular cholesterol [66], macrophages expressing SRs can internalize substantial quantities of cholesterol ester from Ox-LDL present in the diseased glomeruli and interstitium leading to foam cell formation. Once converted into foam cells, internalized lipid peroxide products can activate the cells to produce inflammatory cytokines, chemokines, and growth factors [64], inducing injury and death to surrounding cells resulting in renal fibrosis [67]. In support of this hypothesis, mice deficient in SR-A or CD36 have been reported to show decreased atherosclerosis [68, 69].

Altogether, the risk for glomerulosclerosis might be magnified by oxidative stress in the presence of Ox-LDL (or its oxidation products) in the previously injured glomeruli via foam cell formation and mesangial matrix accumulation.

Measures to Inhibit Oxidative Modification of LDL and Ox-LDL-Induced Renal Fibrosis

Antioxidants

Dietary supplementation of vitamin E and probucol reduces renal injury in chronic nephrotic rats [62, 70]. Vitamins C and E in experimental hypercholesterolemia decrease LDL oxidizability [1] and interstitial fibrosis [2]. In patients with end-stage renal disease, vitamin E supplementation results in the amelioration of oxidative stress [4, 31] and myocardial infarction [71]. Statins reduce the plasma levels of Ox-LDL in dialysis patients [4].

Green tea and concentrated red grape juice (sources of polyphenols), α -lipoic acid, histidine and carnosine, as well as melatonin have antioxidant activity in experimental animals [72–75] and hemodialysis patients [76] (table 2).

PKC Inhibitors

LDL-induced synthesis of α 1(IV) collagen and PAI-1 mRNAs was inhibited by a PKC inhibitor, GF-109203X,

Table 3. Modulation of TGF- β signaling in oxidized LDL-induced renal fibrosis

Target	Therapeutic approach	References
TGF- β /R action	Anti-TGF- β antibody	17, 96, 97
	Antisense TGF- β 1 ODN	98
	TGF- β RNA interference	99
	Decorin	100
	Soluble TGF- β type II receptor	101
	Inhibitor of TGF- β receptor kinases	102, 103
Smad signaling	Smad7	104
	BMP-7	105, 106
	Hepatocyte growth factor	107
	TGIF, SnoN	108, 109

TGF- β /R = Transforming growth factor- β /receptor; ODN = oligodeoxynucleotide; BMP-7 = bone morphogenic protein-7; TGIF = transforming growth-interacting factor.

in cultured mesangial cells [16, 24]. Vitamin E reduces PKC-signaled increases in TGF- β in mesangial cells [77]. Inhibition of PKC- β with LY333531 or ruboxistaurin reduced renal injury and TGF- β expression in experimental diabetic nephropathy [78]. In patients with diabetic nephropathy, ruboxistaurin has given supportive results in a pilot study [79] but no effects in other long-term studies [80]. Recently, activation of PKC- ϵ in the diabetic state seems to ameliorate the renal fibrosis by inhibiting the TGF- β 1 signaling [81].

Inhibitors of Renin-Angiotensin System

Losartan inhibits the LDL-induced increased O₂⁻ generation in mesangial cells [13]. Long-term blockade of the renin-angiotensin system seems to have a beneficial effect on uremic dyslipidemia [82]. Losartan reduces oxidative stress, renal fibrosis and TGF- β expression in rats with NO deficiency, subtotal nephrectomy, or unilateral ureteral obstruction [83–85]. Olmesartan also prevents renal damage in Imai rats with spontaneous glomerulosclerosis [86]. Clinically, the combination of angiotensin-converting enzyme inhibitors and angiotensin receptor blocker reduces proteinuria more than either agent alone in patients with kidney disease [87].

Angiotensin-(1–7), a vasodilatory compound formed from Ang II, alleviates oxidative stress and renal vascular dysfunction in diabetic hypertensive rats [88].

Hypertension also increased T-lymphocyte production of TNF- α , a promoter of fibrosis in the kidney, and treatment with the TNF- α antagonist etanercept pre-

vented the Ang II-induced hypertension and increase in vascular O₂⁻ [89] (table 2).

Inhibitors of NADPH Oxidase

LDL-induced O₂⁻ overproduction was suppressed by diphenylene iodonium, a potent NADPH oxidase inhibitor, in mesangial cells [13]. Vitamins C and E decrease the expression of NADPH oxidase subunits in hypercholesterolemic pigs [2]. Statins decrease the activity of NADPH oxidase in VSMC by diminishing the geranylgeranylation of Rac1 [90]. Fasudil, a Rho-kinase inhibitor, attenuates the renal mRNA expression of NADPH oxidase subunits, TGF- β and collagen in experimental hypertensive glomerulosclerosis [91] (table 2).

Estrogen also decreases Rac1 expression and NADPH oxidase activity in VSMC [92]. It prevents the progression of experimental diabetic nephropathy by inhibiting the effects of TGF- β [93] (table 2). Estrogen deficiency accelerates while estrogen replacement retards glomerulosclerosis in young sclerosis-prone oligosyndactyly mutant mice [94]. Exceptionally, estrogen contributes to severity of hyperlipidemia and renal injury in Nagase rats with hereditary analbuminemia [95].

Ras/ERK Inhibitors

Blockade of ERK signaling by PD98059 or UO126, mitogen-activated ERK-activating kinase 1/2 inhibitors, attenuates the Ox-LDL-induced increase in PAI-1 expression in mesangial cells [18]. Lovastatin abrogates the Ox-LDL-induced Ras and ERK activity resulting in the downregulation of TGF- β 1 and PAI-1 genes in mesangial cells [21] (table 2).

TGF- β Signaling Antagonists

Inhibitors of TGF- β /Receptor Action

Incubation with anti-TGF- β or SB-431542, an inhibitor of TGF- β type I receptor, decreases Ox-LDL-induced nuclear Smad3 expression and PAI-1 synthesis in mesangial cells [17]. Monoclonal antibody to TGF- β (1D11) reduces renal fibrosis in different animal models [96, 97]. Antisense TGF- β oligonucleotides [98] and RNA interference targeting TGF- β 1 [99] also reduce expression of renal matrix components in animal models. Blockade of TGF- β activation by transfer of decorin gene suppresses ECM accumulation in experimental glomerulonephritis [100].

Soluble TGF- β type II receptor decreases the renal cortical fibrosis in experimental diabetic nephropathy [101]. Inhibition of TGF- β type I receptor kinase (ALK5) also ameliorates renal fibrosis in experimental nephro-

sis [102]. Recently, oral administration of GW788388, an inhibitor of TGF- β type I and II receptor kinases, reduces the renal fibrosis in diabetic mice [103] (table 3).

Inhibitors of Downstream Pathways of TGF- β Signaling

Overexpression of Smad7, an inhibitory factor in TGF- β signaling, reduces renal fibrosis in rat remnant kidney [104].

Bone morphogenic protein (BMP)-7 counteracts the fibrogenic action of TGF- β . Supplementation with exogenous BMP-7 suppresses renal fibrosis in experimental renal disease [105]. Maintenance of BMP-7 also reduces renal fibrosis in BMP-7 transgenic mice with diabetic nephropathy [106].

Hepatocyte growth factor (HGF) shows efficacy in ameliorating renal fibrosis in different animal models [107]. HGF antagonizes TGF- β /Smad signaling in kidney cells by blocking the nuclear translocation of activated Smad [107] and activation of Smad transcriptional co-repressors, TGIF [108] and SnoN [109] (table 3).

Although supplementation of exogenous BMP-7, HGF and Smad antagonists would eradicate the fibrogenic actions of TGF- β 1, this has not been tested in humans yet.

Conclusions

Clinical evidence to support a relationship between Ox-LDL and oxidative stress and progressive, proteinuric kidney disease continues to grow. Experimental data suggest that Ox-LDL (or its oxidation products)-stimulated TGF- β 1 and Ang II activate NADPH oxidase in mesangial cells resulting in activation of ERK1/2, which leads to maximal Smad activation and subsequent overexpression of TGF- β 1 target genes. Despite a paucity of studies to test this hypothesis in humans, research on the fibrogenic signaling cascades triggered by Ox-LDL will further our comprehension of the mesangial matrix accumulation and provide new therapeutic strategies to prevent the development of glomerulosclerosis in patients with chronic kidney disease and dyslipidemia.

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