Original Article

LOX-1, an Oxidized Low-Density Lipoprotein Receptor, Was Upregulated in the Kidneys of Chronic Renal Failure Rats

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LOX-1 is a novel receptor for oxidized low-density lipoprotein (LDL) isolated from vascular endothelial cells and has been suggested to be involved in the formation of atherosclerotic and hypertensive vascular lesions. We previously reported that salt loading caused glomerulosclerosis and upregulation of LOX-1 in the kidney of Dahl salt-sensitive hypertensive rats. In the present study, we investigated LOX-1 expression in the remnant kidney, an established rat model for chronic renal failure. Six weeks after 5/6 nephrectomy, the rats showed elevated blood pressure, impaired renal function and increased renal expression of type I collagen. The LOX-1 gene expression in the remnant kidney was markedly increased compared with that in control rats, and immunohistochemical analysis showed that LOX-1 was widely expressed in the interstitial cells, whereas there was almost no staining in the glomeruli or tubules. Moreover, reduction of blood pressure by the angiotensin II type 1 (AT1) receptor antagonist candesartan significantly suppressed the renal LOX-1 expression, and this suppression was accompanied by amelioration of renal injury. These results suggest that enhanced renal expression of LOX-1 might play some roles in the progression of chronic renal failure in rats. (Hypertens Res 2003; 26: 117–122)

Key Words: LOX-1, oxidized low-density lipoprotein, chronic renal failure, remnant kidney, angiotensin II type 1 receptor antagonist

Introduction

It is well established that progression to end-stage renal failure is relatively independent of initial insult and underlying renal disease. Lipid abnormality is commonly observed in various renal diseases and in particular low-density lipoprotein (LDL) and oxidized LDL (ox-LDL) have been suggested to play a role. Indeed, animal studies have shown that a high cholesterol diet deteriorates renal function (1) and cholesterol-lowering therapy has a renoprotective effect in renal injury models (2), although the significance of lipoprotein in human renal diseases has not yet been determined.

In vitro studies show that ox-LDL causes endothelial and mesangial activation/dysfunction, stimulating the production of various cytokines and growth factors (3). Ox-LDL exerts its effect on the cells through receptors, including scavenger receptors A and B and CD68, which are mainly expressed in macrophages (4). Recently, Sawamura et al. identified a novel receptor for ox-LDL (lectin-like oxidized receptor; LOX-1), a lectin-like oxidized LDL receptor, from bovine vascular endothelial cells (5). LOX-1 is normally expressed in vascular-rich organs such as the lungs, spleen, and kidneys (5, 6). Subsequent studies have shown that LOX-1 is expressed not only in endothelial cells but also, to a lesser extent, in macrophages, vascular smooth muscle cells and glomerular mesangial cells (7, 8). Interestingly, LOX-1 has been shown to be induced by a variety of stimuli, including shear stress...
(9, 10), transforming growth factor-β1 (TGF-β1) (11), inflammatory cytokines (10, 12), and angiotensin II (13), as well as by oxidative stress and ox-LDL itself (14, 15). In an in vivo study, increased expression of LOX-1 was observed in coronary arterial endothelial cells from an early stage of atherosclerosis (16). Also, we previously reported an elevated expression of LOX-1 in the aorta of hypertensive rats (17). Although these studies suggest that LOX-1 may be involved in the pathogenesis of atherosclerotic and hypertensive vascular diseases, the role of LOX-1 in kidney disease has not been determined.

Recently, we reported that LOX-1 expression was elevated in the kidney as well as in the aorta in Dahl salt-sensitive rats administered a high salt diet containing 8% NaCl (18). The LOX-1 upregulation was accompanied by impaired renal function and blood pressure elevation, which were all suppressed by anti-hypertensive treatment. However, the Dahl salt-sensitive rat is a hereditary model for salt-sensitive hypertension, and it is unknown whether LOX-1 expression is regulated in acquired, common renal diseases. In this study, therefore, we investigated renal expression of LOX-1 using 5/6 nephrectomized rats, the most established animal model for chronic renal failure. The results showed that the LOX-1 expression was significantly stimulated in the remnant kidney, especially in the interstitial cells, and was suppressed by administration of the angiotensin II type 1 (AT1) receptor antagonist candesartan in accompaniment with amelioration of renal damages. These findings suggest that LOX-1 upregulation might be involved in the progression of chronic renal failure.

**Methods**

**Animal Model**

Male Sprague-Dawley rats weighing 200–220g were purchased from Tokyo Laboratory Animal Center (Tokyo, Japan). Rats were anesthetized by intraperitoneal administration of sodium pentobarbital. In experimental animals (n = 23), the right kidney and two-thirds of the left kidney were surgically removed (Nx rats, n = 23). Control animals (n = 13) underwent the same anesthetization and laparotomy without ablation of the kidneys. In some 5/6 nephrectomized rats (n = 10), the AT1 receptor antagonist candesartan was administered at a dose of 10 mg/kg/day by gavage. The rats were fed a normal rat chow (0.5% NaCl), and 6 weeks later urine was collected using metabolic cages. Blood pressure was measured by the tail-cuff method. The rats were sacrificed by decapitation, and the blood samples were collected and centrifuged to separate the serum. All animal procedures were performed as described previously (17).

**Northern Blot Analysis**

For RNA extraction, isolated kidneys were immediately frozen in liquid nitrogen and stored at - 80°C until use. Total RNA was extracted using the acid guanidinium thiocyanate/phenol/chloroform method. Northern blotting was performed as described previously (17). Briefly, 10 µg of total RNA was electrophoresed with 1.2% agarose gels containing formaldehyde, transferred onto a nylon membrane and fixed by incubating at 80°C for 2 h. The blots were hybridized with a fragment of rat LOX-1 cDNA labeled with [α-32P] d CTP by a random primer labeling method. After hybridization at 42°C for 24 h, the membrane was washed.
twice with 2× SSC (0.15 mol/l sodium chloride, 15 mmol/l sodium citrate, pH 7.0, for 1× SSC) and 0.1% sodium dodecyl sulfate (SDS) at room temperature for 10 min and then washed with 0.2× SSC and 0.1% SDS at 60 °C for 1 h. The filter was exposed to the imaging plate and densitometric scanning was performed using a PhosphoImager SI system (Molecular Dynamics, Amersham Pharmacia Biotech, Buckinghamshire, UK).

**Statistical Analysis**

All data are expressed as the mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by post hoc test using the StatView program (Abacus Concepts, Berkley, USA). Values of \( p < 0.05 \) were considered to indicate statistical significance.
Results

Functional Parameters and Renal Histology of 5/6 Nephrectomized Rats with or without Candesartan Administration

As shown in Table 1, body weight did not differ between sham-operated and 5/6 nephrectomized rats. Systolic blood pressure, serum urea nitrogen, serum creatinine and urinary protein were significantly elevated in 5/6 nephrectomized rats (n = 13) compared with sham-operated rats (n = 13). Serum total cholesterol was also elevated. Candesartan normalized blood pressure and all other parameters, with the exception of serum total cholesterol, in 5/6 nephrectomized rats.

Light microscopic examination revealed glomerular and interstitial changes in 5/6 nephrectomized rats, as previously reported by others. It was of note that 5/6 nephrectomized rats showed marked interstitial changes with infiltration of mononuclear cells and glomerular hypertrophy with increased mesangial matrix (Fig. 1B and D). These histological changes were ameliorated by administration of candesartan (Fig. 1C).

LOX-1 and Type I Collagen Gene Expression in the Kidney of 5/6 Nephrectomized Rats

LOX-1 mRNA expression in the whole kidney was examined by Northern blotting. In 5/6 nephrectomized rats, the LOX-1 mRNA level was significantly elevated compared with that of sham-operated rats, and this elevation was suppressed by administration of candesartan (Fig. 2). The densitometric analysis showed the LOX-1 mRNA levels were 1.5-fold and 4.5-fold higher in 5/6 nephrectomized rats with or without candesartan, respectively, compared with those in sham-operated rats. The mRNA expression of type I collagen, a marker of renal fibrosis, was also significantly elevated in 5/6 nephrectomized rats, and this elevation was decreased by administration of candesartan.

We further examined the correlations between LOX-1 mRNA level and systolic blood pressure or renal functional parameters. The correlation coefficients between LOX-1 mRNA level and systolic blood pressure, urinary protein and serum creatinine were 0.624, 0.876 and 0.472, respectively. All correlations were statistically significant.

Localization of LOX-1 Protein in the Kidney

To determine the localization of LOX-1 within the kidney, immunohistochemical analysis was performed using a specific polyclonal anti-LOX-1 antibody. The results showed that LOX-1 was widely expressed in the interstitial cells in 5/6 nephrectomized rats, whereas there was a little staining in the glomeruli and almost no staining in the tubules (Fig. 3B and D). LOX-1 staining was negative in control rats (Fig. 3A). Together with the results of the Northern blotting, this indicated that the basal expression of LOX-1 was very low. The LOX-1 protein expression in the 5/6 nephrectomized rats was diminished by administration of candesartan (Fig. 3C).

Discussion

We previously reported that LOX-1 was upregulated in the kidney of Dahl salt-sensitive rats, a hereditary animal model for salt-dependent hypertension (17). In this study we used 5/6 nephrectomized rats, an established chronic renal failure model, and demonstrated that renal ablation induced LOX-1 in the remnant kidney. The renal LOX-1 expression was suppressed by blockade of angiotensin II action, concomitant with a reduction of systolic blood pressure and amelioration of renal damages. Among the parameters examined, the level of renal LOX-1 mRNA was most highly correlated with urinary protein. Immunohistochemical analysis showed that LOX-1 protein was expressed mainly in the interstitial cells, whereas a little staining in the glomeruli and almost no stain-
ing in the tubules. These results suggest that LOX-1 might play a role in the pathogenesis of progressive renal disease.

LOX-1 is a novel ox-LDL receptor recently isolated from endothelial cells (7). Subsequent studies have shown that LOX-1 is induced by a variety of stimuli, such as inflammatory cytokines, TGF-β, shear stress, and angiotensin II, as well as by oxidative stress or ox-LDL itself, in cultured cells (9–15). Moreover, LOX-1 has been shown to act as a functional receptor that mediates the cytotoxic effect of ox-LDL, leading to endothelial activation and dysfunction such as up-regulation of monocyte chemotactic protein-1 (MCP-1) and adhesion molecules (19), induction of apoptosis (20) and inhibition of nitric oxide production (21). LOX-1 has also been shown to mediate both the increase in intracellular oxidative stress and the resulting activation of transcription factor NF-κB following exposure to ox-LDL (22). Thus, it is plausible to speculate that LOX-1 overexpression may enhance the cellular dysfunction caused by ox-LDL, leading to damage in the remnant kidney and the vasculature of other organs although further studies will be needed to elucidate the precise role of LOX-1.

In the present study, LOX-1 expression in the remnant kidney was suppressed by administration of an AT1 antagonist, candesartan, and this suppression was accompanied by a reduction in blood pressure. It is thus possible that LOX-1 upregulation was the direct effect of blood pressure elevation, or that it occurred through angiotensin II. In fact, LOX-1 expression in the kidneys of 5/6 nephrectomized rats appeared to be closely related to systemic blood pressure level, much as in our previous study using Dahl salt-sensitive rats (18). Moreover, it has been shown that angiotensin II directly stimulates LOX-1 expression in cultured vascular endothelial cells (13), and we previously showed that LOX-1 was induced in rat aorta after infusion of angiotensin II in rats (14). Renal activation of the renin-angiotensin system has been suggested to be pathogenically involved in a variety of renal diseases, including diabetic nephropathy and the nephritis in nephritic-model rats (23, 24). In a recent study by Mackie et al., 5/6 nephrectomized rats showed higher serum and renal angiotensin II levels than control rats (25). Other candidate factors that may be responsible for LOX-1 upregulation include cytokines such as TNF-α (12) and TGF-β (11) or oxidative stress (14).

The present results in 5/6 nephrectomized rats were similar to those previously reported for Dahl salt-sensitive rats, but the distribution of LOX-1 was a little different. In Dahl salt-sensitive rats, LOX-1 expression was observed in both glomerular and interstitial cells, whereas in the remnant kidney LOX-1 was localized mostly in the interstitium. Thus, the distribution of LOX-1 appears to be more highly correlated with the interstitial changes. Considering the relatively low blood pressure in 5/6 nephrectomized rats
(158 mmHg) compared with Dahl salt-sensitive rats (255 mmHg) (18), it may be that the blood pressure level was not high enough to stimulate LOX-1 expression of the glomerular cells in 5/6 nephrectomized rats. Although it is unknown which cell types express LOX-1, candidates include capillary endothelial cells, fibroblasts, or infiltrating inflammatory cells such as macrophages.

In summary, we showed that LOX-1 was upregulated in the kidney of chronic renal failure rats, and was suppressed by an AT1 receptor antagonist. These results suggest that LOX-1 might play a role in the progression of chronic renal damages.

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References


