Left Ventricular Expression of Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 in Failing Rat Hearts

Tomohide Takaya, MA*;12; Hiromichi Wada, MD*;11; Tatsuya Morimoto, MD*;24; Yoichi Sunagawa, MA*;11; Teruhisa Kawamura, MD*; Rieko Takanabe-Mori, MA*; Akira Shimatsu, MD*;2; Yoshiko Fujita, PhD2; Yuko Sato, PhD2; Masatoshi Fujita, MD11; Takeshi Kimura, MD1; Tatsuya Sawamura, MD1; Koji Hasegawa, MD*

Background: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a multiple ligand receptor induced by oxidative stress. However, its role in chronic heart failure remains unknown.

Methods and Results: The left ventricular (LV) expression of LOX-1 was examined in a salt-sensitive Dahl rat model of hypertension. Compared with controls, LOX-1 mRNA levels increased by 4.7-fold in the LV with hypertrophy, and by 32-fold in the LV with decreased systolic function. LV LOX-1 mRNA levels strongly correlated with the decrease in LV ejection fraction (EF) (r=−0.772), and with increases in the LV mRNA levels of B-type natriuretic peptide (r=0.814), monocyte chemoattractant protein-1 (r=0.943), transforming growth factor-β (r=0.936), and a macrophage marker, F4/80 (r=0.560). Serum levels of soluble LOX-1 were significantly elevated in patients with LV systolic dysfunction and hypertrophy, and significantly correlated with the decrease in EF (r=−0.495).

Conclusions: Marked increase in the LV expression of LOX-1 in failing hearts may contribute to increased serum levels, and might be involved in chronic inflammation during the development of heart failure. (Circ J 2010; 74: 723–729)

Key Words: Heart failure; Hypertension; Inflammation; LOX-1; Receptors

The lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1) was originally identified as an endothelial receptor for oxLDL. LOX-1 expression in vascular cells is relatively low in the normal state, but can be induced by various stimuli such as oxLDL, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), angiotensin II, and endothelin-1 (ET-1) in vitro. LOX-1 upregulation is involved in oxLDL-induced apoptosis through the intracellular production of reactive oxygen species. Endothelial expression of LOX-1 in vivo is increased in hypertension (HT), diabetes mellitus (DM), hyperlipidemia, hypercholesterolemia, and atherosclerosis. OxLDL-induced LOX-1 regulates the expression of monocyte chemoattractant protein-1 (MCP-1), a cytokine that mediates macrophage infiltration, and is considered to be involved in the pathogenesis of atherosclerosis at an early stage. The membrane proximal extracellular domain of LOX-1 can be proteolytically cleaved and released as soluble forms. Levels of soluble LOX-1 (sLOX-1) in sera are increased in acute coronary syndrome, type 2 DM, and obesity.

LOX-1 expression in cultured cardiomyocytes is also very low in the basal state, and can be induced by norepinephrine and ET-1, neurohormonal factors that are activated in heart failure (HF). The cardiac LOX-1 pathway is activated by oxidative stress in vitro and by ischemia–reperfusion injury in vivo. Although the activation of LOX-1 induces apoptosis in cardiomyocytes, the administration of anti-LOX-1 antibody is able to suppress their apoptosis in vitro and reduces the extent of myocardial infarction (MI) in vivo.

Left ventricular (LV) expression of LOX-1 is also increased in salt-sensitive Dahl (DS) rats with hypertensive HF compared with control normotensive salt-resistant Dahl (DR) rats. The administration of eplerenone, an aldosterone blocker, reduces LOX-1 activation and recovers the cardiac function of DS rats.
correlation between LV expression of LOX-1 and progression of HF using Dahl rats. Furthermore, we found that serum sLOX-1 levels are increased in patients with chronic HF and LV hypertrophy (LVH).

**Methods**

**Dahl Rats**

Male Dahl rats were fed a low-salt diet (0.3% NaCl) until the age of 6 weeks, after which, to induce HT, they were fed a high-salt diet (8% NaCl). All animal experiments conformed with the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, and the protocol was approved by the Ethics Committee of the Graduate School of Medicine, Kyoto University.

**Physiological Analysis**

Blood pressure (BP) was measured in the Dahl rats by the tail-cuff method. Cardiac functions were noninvasively evaluated by echocardiography, as previously described. In brief, images were recorded using a 10- to 12-MHz phased-array transducer (model 21380A with HP SONOS 5500 imaging system; Agilent Technologies). LV end-diastolic and end-systolic dimensions (LVEDD and LVESD) were measured with M-mode tracings from the short-axis view of the LV at the papillary muscle level. All measurements were performed in a blinded fashion according to the guidelines of the American Society for Echocardiography and averaged over 3 consecutive cardiac cycles. After physiological studies, surviving rats were euthanased, and their hearts were removed.

**Measurement of Plasma B-Type Natriuretic Peptide (BNP)**

Blood samples were obtained from surviving rats for measurement of plasma BNP concentrations using a radioimmunoassay kit (Peninsula Lab).

**Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**

Total RNAs from the LVs were isolated, reverse transcribed, and subjected to quantitative real-time RT-PCR as previously described. Primer sequences of LOX-1, BNP, MCP-1, TGF-β, IL-1β, F4/80, and GAPDH have been described previously.

**Western Blotting**

Whole cell lysates from rat LVs were prepared and subjected to Western blotting as described previously, using mouse monoclonal anti-LOX-1 and mouse monoclonal anti-β-actin (Sigma) antibodies. Protein amounts were semi-automatically quantified by using Image J software (National Institutes of Health).

**Histological Analysis**

The excised hearts were cut into 2 transverse slices at the mid-level of the papillary muscles. The specimens were fixed in 10% formalin, embedded in paraffin, sliced into 4-μm-thick sections, and stained using mouse monoclonal anti-LOX-1 antibody.

**Human Subjects**

A cross-sectional study was carried out during a specified period between July and September 2007. Patients with chronic congestive HF and LVH (CHF-LVH) and apparently healthy subjects with normal cardiac function without LVH (controls) were recruited in the Outpatient Department of Cardiovascular Disease of Kyoto Medical Center. CHF was defined according to the ACC/AHA Guideline. LVH was defined as LV mass index (LVMi) >116 g/m² in men and >104 g/m² in women on echocardiographic examination. Chronic HF was defined as the patient being in a stable New York Heart Association functional class for at least 3 months. Most of the controls attended for further examination of risk factors after periodical health checkup. The echocardiographic criteria for CHF-LVH were defined as the presence of LVH, ejection fraction (EF) <60%, and LVEDD >50 mm, and those for controls were LVMi <100 g/m², EF >60%, and LVEDD <50 mm. Exclusion criteria were: (1) infection or illness with pyrexia; (2) recent (<3 month) acute coronary syndrome, MI, or stroke; (3) chronic, systemic illness, including renal failure, hepatic impairment, cancer, and inflammatory connective tissue disease; inflammatory bowel disease. BP was measured twice with an automatic electronic sphygmonanometer (BP-103i II; Nippon Colin, Komaki, Japan).

The study protocol was approved by the Institutional Ethics Committee of Kyoto Medical Center.
Measurement of sLOX-1
Patients’ blood samples were taken from the antecubital vein in the morning after a 12-h fast. Blood was immediately centrifuged and the serum obtained was divided into aliquots. Serum sLOX-1 concentrations were measured by ELISA. The analyses were performed by an investigator who was unaware of the source of each sample.

Statistical Analysis
Results are presented as means±SE. Statistical comparisons were performed using ANOVA with Scheffe’s test. Linear regression analysis with Pearson’s coefficients was performed to investigate correlations. The Mann-Whitney U test was used for comparisons of human sLOX-1. P<0.05 was taken to indicate significance.

Results
Development of HF in Dahl Rats
Cardiac function of the Dahl rats was assessed before and after (at 11 and 18 weeks) they were fed a high-salt diet from the age of 6 weeks. As shown in Table 1, BP was significantly higher than in the DS compared with the DR rats at 11 and 18 weeks. Accordingly, DS rats exhibited LVH: increased LV weight-to-body weight ratio (LVW/BW) and LV posterior wall thickness (LVPWT) compared with DR rats at 11 and 18 weeks. The LVEF of DS rats was preserved at 11 weeks but significantly reduced at 18 weeks. These data demonstrate that DS rats showed progressive LVH at 11 weeks, followed by systolic dysfunction at 18 weeks. The LVW/BW ratio was significantly higher in the DS (5.14±0.30) than in the DR (3.71±0.04) rats at 18 weeks. The increased lung weights and plasma BNP levels in the DS compared with the DR rats suggest that the LV end-diastolic pressure increased at 18 weeks. LV dilatation would subsequently occur after 18 weeks in the DS rats. However, LV dilatation was not observed in this series of experiments, because DS rats rapidly die after 18 weeks and the time period of LV dilatation is very short.

LV Expression of LOX-1 in Dahl Rats
Real-time RT-PCR analysis indicated that LV mRNA levels of LOX-1 in the DS rats progressively increased at 11 and 18 weeks, while those in the DR rats did not change (Figure 1A). LOX-1 expression revealed 4.7- and 32-fold increases in the DS compared with the DR rats at 11 and 18 weeks, respectively. Compatible with the mRNA levels, the amount of LOX-1 protein in the LV was greater in the DS rats than in the DR rats at 18 weeks (Figure 1B). DS rats showed a 5.8±3.3-fold increase in the levels of LOX-1 protein compared with the DR rats at 18 weeks. Sections of LV from these rats at 18 weeks were stained using anti-LOX-1 antibody (Figure 1C). LOX-1 immunoreactivity was observed in vessel walls and very faintly in the cardiomyocytes of DR rats. However in the DS rats, LOX-1 was strongly and clearly detected in cardiomyocytes as well as vessel walls. These
results clearly indicate that the expression of LOX-1 in LV cardiomyocytes was upregulated during the development of LVH and HF in DS rats.

LV Expression of Cytokines Involved in HF
Levels of the mRNA of BNP, MCP-1, TGF-β1, IL-1β, and F4/80 in the LV were also quantified by real-time RT-PCR (Figure 2). Those of BNP, which reflect the extent of LV wall stress, were increased in the DS rats during the development of HF and were significantly higher than those of the DR rats at 18 weeks (Figure 2A). Those of MCP-1 in the DS rats showed 2.1- and 10.2-fold increases at 11 and 18 weeks, respectively, compared with the DR rats (Figure 2B). Those of TGF-β1 (Figure 2C) and IL-1β (Figure 2D) showed 3.1- and 2.9-fold increases, respectively in the DS compared with the DR rats, at 18 weeks. Compatible with the increased expression of these cytokines in the DS rats, the LV mRNA level of F4/80, a marker of macrophages, showed 1.9- and 3.7-fold increases at 11 and 18 weeks, respectively, in the DS compared with the DR rats (Figure 2E).

Correlation Between LOX-1 Expression and Parameters of HF
As shown in Table 2, LV mRNA levels of LOX-1 positively correlated with the levels of HT (systolic and diastolic BP) and LVH (LVW/BW and LVPWT). LOX-1 expression was also associated with deterioration of systolic function (increase in LVESD and decrease in EF, Figure 3A). In addition, LOX-1 strongly indicated positive correlations with the plasma and mRNA levels of BNP (Figure 3B). Thus, LOX-1 expression was closely associated with the extent of HF in Dahl rats. Importantly, the LV mRNA levels of LOX-1 were most closely correlated with those of MCP-1 (Figure 3C). The levels also strongly correlated with those of TGF-β1 (Figure 3D) and IL-1β (Figure 3E). In addition to these cytokines, the LV mRNA levels of LOX-1 significantly correlated with those of F4/80 (Figure 3F).
LV Expression of LOX-1 in HF

Figure 3. Correlations between the LV mRNA levels of LOX-1 and the parameters of heart failure. Abbreviations see in Tables 1 and 2.

<table>
<thead>
<tr>
<th>Table 3. Clinical and Echocardiographic Measurements of Human Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>n (M/F)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
</tr>
<tr>
<td>EF (%)</td>
</tr>
<tr>
<td>LV mass (g)</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
</tr>
<tr>
<td>History of hypertension, n (%)</td>
</tr>
<tr>
<td>Etiology of CHF, n (%)</td>
</tr>
<tr>
<td>Ischemic</td>
</tr>
<tr>
<td>Non-ischemic</td>
</tr>
<tr>
<td>Idiopathic dilated cardiomyopathy</td>
</tr>
<tr>
<td>NYHA functional class, n (%)</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
</tbody>
</table>

Data are means±SE. Controls, no organic cardiac diseases; CHF-LVH, chronic heart failure with LV hypertrophy; NS, not significant; NYHA, New York Heart Association. Other abbreviations see in Table 1.
Serum Levels of sLOX-1 in Chronic HF Patients

The clinical and echocardiographic measurements in the patients with CHF-LVH and the apparently healthy subjects with normal cardiac function (control) are shown in Table 3. LVEDD, LV mass, and LVMi were significantly larger and EF significantly lower in the CHF-LVH patients than in the control group. However, there were no significant differences in age, body mass index, BP, and heart rate between the 2 groups. Interestingly, serum levels of sLOX-1 were significantly increased in the CHF-LVH group compared with the controls (Figure 4). In simple regression analysis, there was a weak, but non-significant correlation between serum sLOX-1 levels and LVMI (r=0.437, P=0.07). However, there was a significant negative correlation between serum sLOX-1 levels and EF (r=-0.495, P=0.037). Since previous reports have shown that sLOX-1 levels are increased in patients with DM and those with HT, we compared the sLOX-1 levels in CHF-LVH patients with and without DM or HT. There was no significant difference in the sLOX-1 levels of CHF-LVH patients with and without DM or HT (DM 892±316 pg/ml vs non-DM 1,023±311 pg/ml, P=0.8; HT 845±275 pg/ml vs non-HT 1,086±366 pg/ml, P=0.6). To evaluate whether the etiology of chronic HF affects sLOX-1 levels, we compared sLOX-1 levels in patients with CHF-LVH caused by ischemic heart disease (IHD) with those in patients with CHF-LVH caused by dilated cardiomyopathy (DCM). There was no significant difference (P=0.5): IHD, 1,083±348 pg/ml; DCM, 769±178 pg/ml.

Discussion

In the present study, we showed that levels of mRNA and LOX-1 protein were markedly upregulated in the LV of DS rats with HF, which was compatible with the results of a previous report. We have found that LV mRNA levels of LOX-1 closely correlated with decreased EF and increases in the plasma and mRNA levels of BNP. These findings suggest that LV expression of LOX-1 serves as a novel biomarker of HF in hypertensive heart disease. We have also shown that the serum levels of sLOX-1 were significantly increased in chronic HF patients with LVH and that they correlated with the decrease in EF. Thus, a marked increase in the LV expression of LOX-1 in the failing heart may significantly contribute to increased serum levels of sLOX-1. However, the origin of increased serum sLOX-1 levels during hypertensive heart disease should be examined in further studies, because HT enhances LOX-1 expression not only by the heart, but also by the vascular endothelium.

LV mRNA levels of LOX-1 showed a very strong positive correlation with those of MCP-1, an important chemotactic factor for macrophages. LOX-1 expression also closely correlated with those of TGF-β1 and IL-1β, proinflammatory cytokines produced by macrophages. Furthermore, LV expression of LOX-1 positively correlated with that of F4/80, a marker of macrophages, suggesting that increased LOX-1 expression is involved in macrophage infiltration and inflammation. In the heart, MCP-1 expression and the number of interstitial macrophages in the LV are significantly increased in models of hypertensive heart disease with HF and of post-MI HF. The number of macrophages in the LV myocardium shows a 4-fold increase in DS compared with DR rats at 11 and 18 weeks. Our results for the LV mRNA levels of F4/80, a marker of macrophages, are compatible with those of the previous report.

MCP-1 is considered to be downstream of LOX-1 because the antiseNSE of LOX-1 inhibits MCP-1 expression in endothelial cells. Inhibition of MCP-1 in a mouse MI model reduced macrophage infiltration and the levels of cytokines such as TGF-β and IL-1β in the heart. It has also been reported that anti-LOX-1 antibody reduces IL-1β expression in vascular cells. Our results indicated that LOX-1 upregulation in the LV of DS rats compared with DR rats was most prominent at the stage of systolic HF. Furthermore, LV expression of LOX-1 showed a close relationship with that of inflammatory cytokines, as well as MCP-1 and F4/80, which are markers of increased macrophage infiltration. These findings suggest that LOX-1 upregulation and MCP-1 expression are involved in macrophage infiltration, and that the migrating macrophages then produce proinflammatory cytokines in the heart. TGF-β and IL-1β are well-known inducers of LOX-1, so it is possible that increased LOX-1, macrophage infiltration, and the release of inflammatory cytokines may form a feed-back loop that progresses to fibrosis and apoptosis during the progression of HF. At present, it is unknown whether activation of LOX-1 is a cause or result of HF. However, our results, together with those of previous reports, suggest that upregulation of LOX-1 in HF is a very important key event, leading to inflammation of the heart. The development of a specific antagonist is awaited to clarify the precise role of LOX-1, and to investigate the therapeutic potential of the antagonist for chronic HF.

Acknowledgments

We thank Mika Kiriyma, Noboru Chiba, Shuichi Ura, Akira Yamada, and Yuko Iida for their technical assistance and Akemi Wada for secretarial assistance. This work was supported by grants-in-aid for scientific research awarded to K. Hasegawa from Ministry of Education, Culture, Sports, Science and Technology of Japan and from Research on Publicly Essential Drugs and Medical Devices, The Japan Health Sciences Foundation, and awarded to H. Wada from Suzuken Memorial Foundation, Japan Research Foundation for Clinical Pharmacology, and the Smoking Research Foundation.

References

LV Expression of LOX-1 in HF


33. Hunt SA, Abraham WT, Ch ~~