Modulation of Angiotensin II–Mediated Hypertension and Cardiac Remodeling by Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Deletion


Abstract—Angiotensin II via type 1 receptor activation upregulates the expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), and LOX-1 activation, in turn, upregulates angiotensin II type 1 receptor expression. We postulated that interruption of this positive feedback loop might attenuate the genesis of angiotensin II–induced hypertension and subsequent cardiac remodeling. To examine this postulate, LOX-1 knockout and wild-type mice were infused with angiotensin II or norepinephrine (control for angiotensin II) for 4 weeks. Angiotensin II–, but not norepinephrine–, induced hypertension was attenuated in LOX-1 knockout mice. Angiotensin II–induced cardiac remodeling was also attenuated in LOX-1 knockout mice. Importantly, angiotensin II type 1 receptor expression was reduced, and the expression and activity of endothelial NO synthase were preserved in the tissues of LOX-1 knockout mice given angiotensin II. Reactive oxygen species generation, nicotinamide-adenine dinucleotide phosphate oxidase expression, and phosphorylation of p38 and p44/42 mitogen-activated protein kinases were also much less pronounced in the LOX-1 knockout mice given angiotensin II. These alterations in biochemical and structural abnormalities were associated with preservation of cardiac hemodynamics in the LOX-1 knockout mice. To confirm that fibroblast function is modulated in the absence of LOX-1, cardiac fibroblasts from wild-type and LOX-1 knockout mice were treated with angiotensin II. Indeed, LOX-1 knockout mice cardiac fibroblasts revealed an attenuated profibrotic response on treatment with angiotensin II. These observations provide strong evidence that LOX-1 is a key modulator of the development of angiotensin II–induced hypertension and subsequent cardiac remodeling. (Hypertension. 2008;52:556-562.)

Key Words: angiotensin ■ hypertension ■ cardiac remodeling ■ LOX-1 ■ oxidative stress

Cardiac remodeling is initially an adaptive response to several forms of cardiac stress states, such as hypertension. Sustained remodeling results in heart failure and is a powerful independent risk factor for cardiac morbidity and mortality.1 Therefore, identification of the molecular mechanisms involved in cardiac remodeling is an important challenge for the cardiovascular biologists.

The renin-angiotensin system and its effector hormone, angiotensin II (Ang II), have well-known endocrine properties that contribute to cardiac remodeling and heart failure. Previous studies have shown that Ang II via type 1 receptor (AT1R) activation stimulates the expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1).2 In turn, activation of LOX-1 upregulates AT1R expression.3 Activation of both AT1R and LOX-1 induces a state of oxidative stress.4 In addition, activation of both AT1R and LOX-1 enhances the growth of cardiac fibroblasts and promotes collagen synthesis.5,6

Although LOX-1 mRNA expression is minimal in normal arterial tissues, it is markedly upregulated in vascular tissues of spontaneously hypertensive animals, suggesting a correlation between LOX-1 and hypertension.7 This concept is supported by in vitro observations that Ang II upregulates LOX-1 expression,2 and angiotensin-converting enzyme inhibitors and AT1R blockers decrease LOX-1 expression.8 These findings suggest that LOX-1 overexpression may contribute to the pathological states induced by Ang II. We postulated that interruption of the positive feedback loop between Ang II and LOX-1 might reduce the genesis of Ang II–induced hypertension and subsequent cardiac remodeling.
LOX-1 KO mice. LOX-1 deletion selectively attenuated blood pressure response to Ang II, but not NE, infusion.

To address this issue, we used a mouse model of LOX-1 deficiency (hereafter called LOX-1 knockout or KO mice).

Our specific aims were to examine the following hypotheses: (1) LOX-1 blockade (use of LOX-1 KO mice) will attenuate Ang II–induced hypertension; (2) cardiac remodeling after Ang II infusion will be less in the LOX-1 KO mice; and (3) fibroblasts from LOX-1 KO mice will generate less collagen (versus wild-type mice) when exposed to Ang II.

Materials and Methods
LOX-1 KO and wild-type mice were infused with Ang II (50 ng/min) or norepinephrine (100 ng/min) for 4 weeks. Blood pressure, cardiac remodeling, and oxidative stress sensitive signaling were determined. For details, please refer to the online data supplement (http://hyper.ahajournals.org). Data are expressed as means±SEs. All of the data were analyzed by a 2-way ANOVA with a Bonferroni posthoc test. A *P*<0.05 was considered significant.

Results

Blood Pressure and Cardiac Hemodynamics in Response to Ang II or Norepinephrine
Basal systolic blood pressure was similar in the wild-type and LOX-1 KO mice and remained unchanged in all of the saline-treated mice for the duration of the study. On the other hand, systolic blood pressure exhibited a progressive increase during the infusion (with Ang II or norepinephrine) period, reaching a peak value on day 14 and remaining at plateau through day 28 in the wild-type mice (Figure 1). The rise in blood pressure was much less in the LOX-1 KO mice compared with that in wild-type mice (*P*<0.01), despite infusion with the same dose of Ang II. In contrast to the effect of Ang II, norepinephrine infusion caused a similar rise in blood pressure in wild-type and LOX-1 KO mice for the duration of the study. Thus, LOX-1 deletion resulted in a selective attenuation of blood pressure in response to Ang II.

At the end of Ang II or norepinephrine infusion, we measured left ventricular hemodynamics. As shown in Figure S1, heart rate, left ventricular systolic pressure, left ventricular end-diastolic pressure, and the first derivatives of the pressure over time were similar in all of the mice, indicating that the basal hemodynamics were comparable in wild-type and LOX-1 KO mice. Ang II or norepinephrine infusion had no significant effect on heart rate in wild-type and LOX-1 KO mice but induced a marked increase in left ventricular systolic pressure, left ventricular end-diastolic pressure, and first derivatives of the pressure over time compared with corresponding saline-treated control mice (*P*<0.01). It is of note that the increase in left ventricular systolic pressure, left ventricular end-diastolic pressure, and first derivatives of the pressure over time was much less in LOX-1 KO mice compared with that in the wild-type mice despite infusion of a similar dose of Ang II (*P*<0.05). Norepinephrine-induced hemodynamic changes remained similar in wild-type and LOX-1 KO mice.

Cardiac Remodeling After Sustained Hypertension and Effect of LOX-1 Deletion
Hearts from wild-type and LOX-1 KO mice were assessed with regard to their susceptibility to hypertrophic response to sustained hypertension. Chronic Ang II, as well as norepinephrine infusion, induced significant cardiac hypertrophy (expressed as a ratio of heart weight to body weight or to tibia length) in wild-type and LOX-1 KO mice (Figure 2A through 2C; *P*<0.01 versus corresponding saline-treated mice). The cardiomyocyte cross-sectional area also increased in response to sustained hypertension (Figure 2D and 2E). Ang II–induced cardiac hypertrophy was much less in the LOX-1 KO mice compared with that in wild-type mice (*P*<0.01), as evidenced from a smaller increase in the heart weight and cross-sectional area of cardiomyocytes.

On the other hand, there was no significant difference in cardiac hypertrophy in response to norepinephrine infusion between wild-type and LOX-1 KO mice. This is in keeping with a similar degree of hypertensive response to norepinephrine in both groups of animals.

Because the increase in cardiac mass and cardiomyocyte size is accompanied by induction of specific genes, atrial natriuretic peptide (ANP) and α-tubulin,9,10 we sought to measure their expression. As shown in Figure 3A and Figure S2, the expression of ANP and α-tubulin was increased in wild-type mice given Ang II or norepinephrine compared with saline-infused wild-type mice (*P*<0.01). Importantly, the LOX-1 KO mice demonstrated much less induction of ANP and α-tubulin despite Ang II infusion (*P*<0.01 versus Ang II–infused wild-type mice). This phenomenon was confirmed by immunohistochemical staining for α-tubulin (Figure 3B). Again, norepinephrine-induced induction of ANP and α-tubulin remained similar in wild-type and LOX-1 KO mice.

Sustained hypertension and resultant cardiac remodeling are characterized by abundant accumulation of matrix proteins in the extracellular space.11 We, therefore, determined the accumulation of collagen in multiple sections of hearts from different animal groups. Results of Masson trichrome
and Picrosirius red staining were similar. Representative examples are shown in Figure 3B, and the summary data from trichrome staining are shown in Figure 3C. In the wild-type mice, sustained hypertension after Ang II or norepinephrine infusion resulted in a significant increase in collagen accumulation (P < 0.01 versus saline-infused wild-type mice). In contrast, the LOX-1 KO mice exhibited much less increase in collagen accumulation despite Ang II infusion (P < 0.01 versus Ang II–infused wild-type mice). Norepinephrine-induced collagen accumulation remained similar in wild-type and LOX-1 KO mice (Figure 3B and 3C).

Collagen type I, derived from its precursor procollagen I, is considered a major determinant of myocardial stiffness. Osteopontin has been shown to interact with fibronectin and plays an important role in left ventricular remodeling. Therefore, we determined the expression of procollagen I, osteopontin, and fibronectin in mice hearts. As shown in Figure 3A, the expression of procollagen I, osteopontin, and fibronectin increased significantly after infusion of Ang II or norepinephrine in the wild-type mice (P < 0.01 versus saline-infused wild-type mice). However, LOX-1 KO mice given Ang II infusion exhibited much less increase in the expression of procollagen I, osteopontin, and fibronectin in the LOX-1 KO mice (P < 0.01 versus wild-type mice). The expression of procollagen I, osteopontin, and fibronectin induced by norepinephrine infusion was similar in wild-type
and LOX-1 KO mice. We also performed immunohistochemical staining for osteopontin in heart sections, and the data were consistent with the results by Western analysis (Figure 3B).

Expression of AT1R and Endothelial NO Synthase Induced by Ang II Infusion and the Effect of LOX-1 Deletion

Most of the cardiovascular actions of Ang II have been attributed to AT1R activation, whereas the hypertensive response to norepinephrine is related to α1-adrenoceptor activation.13,14 We, therefore, determined the expression of AT1R and α1-adrenoceptors. As shown in Figure 4A and Figure S3, AT1R expression increased in the aortic and cardiac tissues after infusion of Ang II in the wild-type mice (P<0.01 versus saline-infused wild-type mice). However, Ang II-infused LOX-1 KO mice exhibited much less increase in AT1R expression (P<0.01 versus wild-type mice). Interestingly, the expression of α1-adrenoceptors induced by norepinephrine infusion remained similar in aortic and cardiac tissues from wild-type and LOX-1 KO mice (Figure 4B).

Our recent study has shown that endothelium-dependent relaxation of aorta, as well as endothelial NO synthase (eNOS) expression, is preserved in the LOX-1 KO mice fed a high-cholesterol diet.15 In the present study, we measured the expression of eNOS and phosphorylated S1177-eNOS in the aorta and found that wild-type mice given Ang II had low levels of eNOS and phosphorylated S1177-eNOS (P<0.01 versus control mice). Importantly, LOX-1 deletion preserved the expression of eNOS and phosphorylated S1177-eNOS despite Ang II infusion (P<0.01; Figure 4C). It is of note that the downregulation of eNOS and phosphorylated eNOS expression induced by norepinephrine infusion remained similar in aortas from wild-type and LOX-1 KO mice (Figure 4C).

Oxidative Stress Induced by Ang II and Norepinephrine and the Effect of LOX-1 Deletion

Oxidative stress and resultant release of reactive oxygen species (ROS) have been linked to the development of cardiac remodeling and progression to heart failure.16 Ang II also upregulates LOX-1 expression.2 Therefore, we determined nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (p47phox and p22phox subunits) expression, ROS production, phosphorylation of oxidative stress–sensitive mitogen-activated protein kinases (MAPKs; p38 and p44/42 MAPK isoforms), and LOX-1 expression. In keeping with previous in vitro studies,17 Ang II infusion induced oxidative stress (dichlorofluorescein fluorescence, 8-isoprostane in hearts, serum malondialdehyde, and p47phox

Figure 3. Markers of cardiac hypertrophy (ANP and α-tubulin) and fibrosis (collagen, procollagen-I, osteopontin, and fibronectin) in mice given Ang II and NE. A, Representative Western blot. B, Immunostaining of α-tubulin, collagen, and osteopontin. C, Summary of data on trichrome staining. These markers of cardiac remodeling were less pronounced in LOX-1 KO mice given Ang II. Data are representative of 4 experiments.
Treatment with Ang II for 24 hours also increased procollagen I expression in cardiac fibroblasts from wild-type mice, but to a much smaller extent in fibroblasts from LOX-1 KO mice (Figure S6). These data are consistent with the data on collagen staining in the hearts from wild-type and LOX-1 KO mice. LOX-1 expression was also quite marked in cardiac fibroblasts isolated from wild-type mice. As expected, fibroblasts isolated from LOX-1 KO mice showed the absence of LOX-1 (Figure S6).

**Discussion**

We show that LOX-1 deletion attenuated Ang II–induced hypertension and cardiac remodeling. Most importantly, our in vitro studies confirmed that fibroblasts from LOX-1 KO mice generated much less collagen (versus wild-type mice) when exposed to Ang II.

Ang II via AT1R activation upregulates LOX-1 through redox-sensitive pathways, and activation of LOX-1 upregulates AT1R expression. We, accordingly, postulated that Ang II and LOX-1 operate in a positive feedback fashion with the common theme being generation of ROS and activation of MAPKs. To examine the relevance of this postulate in the in vivo state, we measured blood pressure response to Ang II in wild-type mice and studied the role of LOX-1 abrogation by using the LOX-1 KO mice. In these LOX-1 KO mice, endothelium-dependent relaxation, as well as eNOS generation, is preserved when the animals are fed a high-cholesterol diet. In the present study, we observed that, whereas the resting blood pressure was similar in the LOX-1 KO and wild-type mice, blood pressure rise in response to Ang II was markedly attenuated in the LOX-1 KO mice. Interestingly, AT1R expression increased in the wild-type mice given Ang II infusion, as observed by others as well. The expression and activity of eNOS both decreased in the wild-type mice given Ang II infusion exhibited a much smaller increase in the expression of AT1R and a marked upregulation of eNOS expression/activity compared with the wild-type mice. These findings confirm that the Ang II-AT1R-LOX-1 loop is an important regulator of blood pressure.

The blood pressure increase in response to norepinephrine was not affected by LOX-1 abrogation. It is of note that norepinephrine infusion caused only a minimal change in LOX-1 expression (versus a large increase in Ang II–infused mice). Norepinephrine infusion, as expected, increased α1-adrenoceptor expression, and LOX-1 deletion did not affect α1-adrenoceptor upregulation. LOX-1 deletion also had no effect on the downregulation of eNOS expression/activity induced by norepinephrine infusion. As such, it is not surprising that the norepinephrine-α1-adrenoceptor-hypertension pathway was not affected by LOX-1 deletion. Many studies have shown that Ang II causes ROS generation by activating NADPH oxidases, which, in turn, activate p38 and/or p44/42 MAPKs and redox-sensitive transcription factors. This process influences downstream signals, resulting in cardiomyocyte hypertrophy and procollagen synthesis. Activation of this cascade has been thought to lead from wild-type mice, was less in fibroblasts from LOX-1 KO mice despite their exposure to Ang II (Figure S5).

**Studies in Cultured Cardiac Fibroblasts**

Fibroblasts form a significant component of cardiac mass, and their growth and activity (collagen formation) contribute to cardiac remodeling. To mimic the in vivo state, we treated mouse cardiac fibroblasts with Ang II (1 μmol/L) for 24 hours. Prolonged exposure of cardiac fibroblasts from wild-type mice to Ang II induced dichlorofluorescein fluorescence and NADPH oxidase (p47phox and p22phox subunits) expression, but these changes were much less pronounced in fibroblasts from LOX-1 KO mice despite their treatment with Ang II (P<0.01). In addition, phosphorylation of p38 and p44/42 MAPK, which was quite marked in the fibroblasts and p22phox subunits, Figure 5A through 5D) in the wild-type mice, but much less so in the LOX-1 KO mice. Norepinephrine infusion also increased oxidative stress in the wild-type mice, but it was not affected by LOX-1 deletion.

As shown in Figure 5D and Figure S4, protein levels of p38 and p44/42 MAPKs were unchanged in response to Ang II and norepinephrine infusion, but their phosphorylation increased significantly during Ang II infusion (P<0.01 versus saline-infused wild-type mice). Importantly, Ang II–infused LOX-1 KO mice exhibited much less phosphorylation of MAPKs (P<0.01 versus Ang II–infused wild-type mice). However, the increased phosphorylation of MAPKs during norepinephrine infusion was not affected by LOX-1 deletion.

In keeping with previous in vitro studies, Ang II infusion enhanced LOX-1 expression in the wild-type mice (Figure 5D). As expected, LOX-1 was not detectable in LOX-1 KO mice hearts. Importantly, LOX-1 expression was only minimally upregulated by norepinephrine infusion.

**Figure 4.** Expression of AT1R, α1-adrenoceptor (α1R), and eNOS in mice given Ang II and norepinephrine (NE). LOX-1 deletion caused attenuation of AT1R expression and enhancement of eNOS expression/activity in response to Ang II. The expression of α1R and eNOS in response to NE was not affected by LOX-1 deletion.

**Figure 5.** Western blot analysis of protein expression levels in wild-type (WT) and LOX-1 KO cardiac fibroblasts. Aorta and heart tissue was stimulated with Ang II (1 μmol/L) for 24 hours. The expression of α1R and eNOS in response to Ang II was significantly increased in wild-type mice. It was markedly attenuated in the LOX-1 KO mice. Interestingly, the expression of LOX-1 was also quite marked in cardiac fibroblasts isolated from wild-type and LOX-1 KO mice. Treatment with Ang II for 24 hours also increased procollagen I expression in cardiac fibroblasts from wild-type mice, but to a much smaller extent in fibroblasts from LOX-1 KO mice (Figure S6). These data are consistent with the data on collagen staining in the hearts from wild-type and LOX-1 KO mice. LOX-1 expression was also quite marked in cardiac fibroblasts isolated from wild-type mice. As expected, fibroblasts isolated from LOX-1 KO mice showed the absence of LOX-1 (Figure S6).
to the development of cardiac remodeling in sustained hypertension. This concept is supported by the observations that the NADPH oxidase inhibitor apocynin reduces blood pressure elevation and prevents vascular remodeling in Ang II–infused mice. Furthermore, hydralazine, which decreases blood pressure but does not affect ROS and related pathways, has no effects on vascular remodeling induced by Ang II.

We observed that prolonged infusion of Ang II, as well as norepinephrine, increased heart weight in the wild-type mice. The increase in heart weight was a manifestation of cardiomyocyte hypertrophy, because cardiomyocyte cross-sectional area and α-tubulin and ANP expression in the heart were enhanced. In addition, there was clear evidence of fibroblast proliferation and collagen accumulation. It is worth noting that the cardiomyocyte hypertrophy and fibrosis were greater in Ang II–infused mice as compared with norepinephrine-infused mice, perhaps an indication of greater ROS generation in response to Ang II despite a similar degree of blood pressure elevation (Figure 1). Ang II–infused LOX-1 KO mice exhibited a very low level of ROS generation and downstream signaling, and, hence, showed no change in cardiomyocyte hypertrophy and fibrosis. Previous studies have demonstrated that LOX-1 is a key modulator of cardiac remodeling, which starts immediately after a brief period of ischemia reperfusion via ROS-dependent pathways, and that the signals of cardiac remodeling are attenuated in the LOX-1 KO mice.

We also found that the expression of osteopontin, as well as fibronectin, increased in the wild-type mice given Ang II and norepinephrine. Osteopontin has been shown to interact with fibronectin and plays an important role in matrix organization and stability. Previous studies also showed that Ang II increases osteopontin expression, and the osteopontin null mice given Ang II infusion have much less cardiac fibrosis and hypertrophy. Xie et al showed that the signals for Ang II–induced osteopontin expression also involve oxidative stress and resultant p42/44 MAPK activation. Gorin et al have similarly shown a relationship between NADPH oxidase activation and fibronectin generation both in vitro and in vivo. In keeping with these studies, we found that the expression of procollagen I, as well as osteopontin and fibronectin, was lower in the hearts of Ang II–treated LOX-1 KO mice that had low levels of oxidant stress and activation of MAPKs.

The data on cultured cardiac fibroblasts exposed to Ang II in vitro support the in vivo observations in mice given Ang II.

This study provides exciting in vivo evidence of a positive feedback loop between Ang II-ROS-LOX-1, which results in the syndrome of chronic sustained hypertension, and cardiac remodeling. The in vitro component using cardiac fibroblasts from LOX-1 KO and wild-type mice lends support to this concept. Our study does not allow us to dissociate the direct effect of LOX-1 deletion on cardiac hypertrophy from its blood pressure–attenuating effects. Therefore, the role of LOX-1 in the regulation of cardiac remodeling needs to be further investigated in other models of pressure or volume overload or in primary cultured cardiomyocytes.

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Disclosures

None.

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