Angiotensin II Induces LOX-1, the Human Endothelial Receptor for Oxidized Low-Density Lipoprotein

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Background—Oxidatively modified LDL (oxLDL) plays an important role in the development of atherosclerosis. OxLDL effects, eg, foam cell formation, are mediated in part by the classic scavenger receptor, whereas other effects may involve the recently cloned endothelial oxLDL receptor, LOX-1 (lectinlike oxLDL receptor-1), which is distinct from macrophage scavenger receptors. Because the regulation of LOX-1 must still be defined, we investigated whether LOX-1 is regulated by the potentially proatherosclerotic stimulant angiotensin II (Ang II).

Methods and Results—Using competitive reverse transcription–polymerase chain reaction (RT-PCR), we quantified mRNA expression of LOX-1 in primary cultures of human umbilical vein endothelial cells (HUVECs). After treatment with Ang II for 3 hours (1 nmol/L to 1 μmol/L), LOX-1 mRNA was concentration-dependently induced (from 6.9±1.4 to 23.1±5.5 relative units [RU] by 1 μmol/L Ang II; *P*<0.05). The angiotensin II type 1 (AT1) receptor antagonist losartan prevented this induction. Incubation of HUVECs with Ang II (100 nmol/L, 3 hours) induced LOX-1 protein expression (212±21% of control level; *P*<0.01) and uptake of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled oxLDL (209±17% of control level; *P*<0.05) by an AT1-dependent pathway, reaching its maximum after 24 hours (680±89%; *P*<0.05). In internal mammary artery biopsy samples from patients with or without ACE inhibitor treatment before coronary artery bypass surgery, LOX-1 mRNA was downregulated by ACE inhibition (6.4±2.0 versus 19.3±5.9 RU; n=12 each; *P*<0.05).

Conclusions—We conclude that LOX-1 is regulated by Ang II in vitro and in vivo, that induction of LOX-1 is mediated by the AT1 receptor, and that repression of LOX-1 by long-term ACE inhibitor treatment may contribute to the antiatherosclerotic potential of this therapy. (Circulation. 1999;100:899-902.)

Key Words: angiotensin ■ atherosclerosis ■ coronary disease ■ endothelium ■ lipoproteins
provide further evidence that the in vitro findings take place in vivo, we analyzed the effect of long-term ACE inhibitor treatment on vascular expression of LOX-1 in patients with coronary heart disease.

**Methods**

**Cell Culture**

Cell culture reagents and chemicals were purchased from Sigma Chemical Co except when otherwise specified. Primary cultures of human umbilical vein endothelial cells (HUVECs) were isolated with collagenase IV and grown in medium M199 (Life Technologies) supplemented with 20% fetal serum. Confluent cell cultures were incubated with medium containing 0.5% fetal serum for 24 hours and subsequently treated with Ang II (1 nmol/L to 1 μmol/L) or with Ang II (100 nmol/L) and the angiotensin II type 1 (AT1) receptor antagonist losartan (1 μmol/L) and the angiotensin II type 1 (AT1) receptor antagonist losartan (1 μmol/L, Merck; Sharpe & Dohme).

**Patients**

Distal remnant specimens of the left internal mammary artery (arteria thoracica interna) obtained after informed consent from 24 patients undergoing elective CAGB surgery were used for this study. The use of human tissue was approved by the local ethics committee. Long-term ACE inhibitor treatment before surgery was evaluated in a retrospective manner. ACE inhibitor dosages prescribed by referring physicians were 31±5% of respective target dosages in recent heart failure megatriglycerides. Twelve consecutive patients without ACE inhibitor pretreatment were matched with 12 patients with ACE inhibitor treatment according to New York Heart Association functional classification (2.2±0.2 for both groups). The groups showed no significant differences in systolic (116±5.1 mm Hg without ACE inhibition versus 113±5.1 mm Hg with ACE inhibition; P=0.66) or diastolic blood pressure (59.9±3.0 versus 62.2±2.7 mm Hg, respectively; P=0.57). In addition, no differences in central venous pressure, heart rate, left ventricular ejection fraction, age, sex, weight, or concomitant therapy with calcium antagonists, β-blockers, diuretics, NO donors, anti-diabetics, or lipid-lowering drugs were found.

**Quantification of Human LOX-1 mRNA and Protein Expression**

Total RNA from HUVECs and internal mammary artery biopsy samples was isolated by guanidinium thiocyanate/cesium chloride centrifugation. LOX-1 mRNA expression was quantified by standard calibrated competitive reverse transcriptase–polymerase chain reaction (RT-PCR) by use of a linker primer, PCR-generated, internal-deleted, and in vitro-transcribed LOX-1 standard cRNA. Western analysis of proteins from HUVECs (50 μg/lane) with or without Ang II stimulation with a LOX-1 monoclonal antibody was performed as described previously.9

**Uptake of DiI-OxLDL in Human Endothelial Cells**

LDL was isolated by sequential ultracentrifugation from human plasma, and oxidative modification of LDL with cupric ion was performed as previously described.12 Oxidized LDL was labeled with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes), and uptake of DiI-oxLDL for 3 hours was quantified as described.8,12

**Statistical Analysis**

Data are shown as mean±SEM. Statistical analysis was performed with the ANOVA procedure followed by Dunnett’s method (multiple comparison) or Student t test (SigmaStat software, Jandel Corp). Differences were taken as statistically significant at P<0.05.

**Results**

**Induction of LOX-1 Expression and OxLDL Uptake by Ang II in HUVECs**

In HUVECs, Ang II maximally induces LOX-1 mRNA after 3 hours (Figure 1A; control: 6.9±1.4 relative units [RU]; 1 nmol/L Ang II: 8.0±2.6 RU; 10 nmol/L Ang II: 19.8±3.0 RU [P<0.05 versus control]; 100 nmol/L Ang II: 21.5±3.7 RU [P<0.05 versus control]; and 1 μmol/L Ang II: 23.1±5.5 RU [P<0.05 versus control]). This induction of LOX-1 mRNA was completely prevented by the AT1 antagonist losartan (1 μmol/L) (control: 6.9±1.4 RU; 100 nmol/L Ang II: 21.5±3.7 RU [P<0.05 versus control]; 100 nmol/L Ang II plus 1 μmol/L losartan: 6.6±1.0 RU) (Figure 1B). In addition, incubation of HUVECs with Ang II (100 nmol/L, 3 hours) induced LOX-1 protein expression (212±21% of control level; n=6; P<0.01) (Figure 1C).

To verify this induction of LOX-1 at a functional level, HUVECs were incubated with Ang II (100 nmol/L), and uptake of DiI-labeled oxLDL was quantified (Figure 1D). Ang II stimulated uptake of oxLDL in human endothelial cells after 3 hours (209±17% of control level; P<0.05) by an AT1-dependent pathway (100±3% with losartan), reaching its maximum after 24 hours (680±89%; P<0.05). The level of oxLDL uptake (~2-fold induction after 3 hours) was similar to induction of LOX-1 mRNA and protein expression by Ang II.

**Downregulation of LOX-1 mRNA by ACE Inhibitor Treatment**

Because Ang II induces LOX-1 mRNA in vitro, we analyzed LOX-1 mRNA expression in internal mammary artery biopsy samples of patients with coronary heart disease with or without ACE inhibitor treatment (Figure 2). Long-term ACE inhibitor treatment causes significant downregulation of LOX-1 mRNA in internal mammary artery (without ACE inhibitor, 19.3±5.9 RU; ACE inhibitor, 6.4±2.0 RU; P<0.05; n=12 in each group). No significant correlation of LOX-1 mRNA expression with other medications could be found.

**Discussion**

Activation of oxLDL uptake is thought to play a key role in the initiation and progression of atherosclerosis.1,2 A major risk factor in the development of atherosclerosis and coronary heart disease is hypertension. It is associated with an activated tissue renin-angiotensin system. Our data show Ang II–mediated increased expression of the endothelial oxLDL receptor LOX-1 and augmented oxLDL uptake in human endothelial cells. These findings suggest a new mechanism to explain how hypertension might promote early initiation and progression of atherosclerosis. Increased Ang II levels would lead to stimulated uptake of proatherogenic oxLDL in endothelial cells. This could result in endothelial dysfunction by impaired endothelium-dependent arterial relaxation,7 infiltration of vessel wall with monocytes/macrophages by increased endothelial expression of adhesion molecules,4 and secretion of growth factors for vascular smooth muscle cells, resulting in vascular hypertrophy.7 Other early proatherosclerotic effects of oxLDL would include delay of endothelial wound healing, eg, by inhibition of migration of aortic endothelial cells into endothelial lesions; cytotoxicity to vascular cells, eg, through induction of oxidative stress; and induction of apoptosis in endothelial cells.2,6,7 Therefore, Ang II–stimulated oxLDL uptake into endothelial cells would accelerate...
early proatherosclerotic processes, before macrophage participation in the progression of atherosclerosis.

Recently, expression of LOX-1 also has been demonstrated in mature human monocyte-derived macrophages. Increased Ang II levels might therefore promote foam cell formation by LOX-1–mediated oxLDL uptake into both endothelial cells and macrophages.

The induction of LOX-1 by Ang II can be completely prevented by AT1 receptor blockade. This finding suggests an antiatherosclerotic potential of pharmacological interventions.

Figure 1. Ang II induces LOX-1 expression and oxLDL uptake in human endothelial cells. A, HUVECs were incubated with Ang II for 3 hours. LOX-1 mRNA expression was quantified by competitive RT-PCR. Ang II at concentrations >10 nmol/L induces LOX-1 mRNA. B, Induction of LOX-1 by Ang II is mediated by AT1. HUVECs were incubated with Ang II (100 nmol/L, 3 hours) in presence or absence of AT1 antagonist losartan (1 μmol/L). Losartan completely prevented induction of LOX-1 mRNA by Ang II. C, Ang II (100 nmol/L, 3 hours) induces LOX-1 protein expression in HUVECs. D, Functional activation of LOX-1 receptor by Ang II. Ang II (100 nmol/L) stimulates uptake of DiI-labeled oxLDL in human endothelial cells after 3 hours by an AT1-dependent pathway, reaching its maximum after 24 hours. Values for each bar are mean ± SEM from ≥3 separate experiments. *P < 0.05, **P < 0.01 vs control (con) or indicated bar.
in the renin-angiotensin system. This view is supported by the downregulation of LOX-1 expression by long-term ACE inhibition in internal mammary arteries of patients with coronary heart disease. This effect seems to be specific for the renin-angiotensin system, because neither group of patients showed a significant difference in blood pressure or concomitant therapy. Therefore, this effect might represent an antiatherosclerotic mechanism contributing to the vasoprotective potential of ACE inhibitors and the improved survival of patients in recent ACE inhibitor megatrials.

Conclusions
The endothelial receptor for oxLDL is upregulated by the renin-angiotensin system in vitro and in vivo. The downregulation of Ang II–stimulated LOX-1 expression might represent a novel mechanism contributing to the antiatherosclerotic potential of long-term ACE inhibitor treatment or AT1 receptor blockade.

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References
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