8-iso-Prostaglandin $F_{2\alpha}$ Increases Expression of LOX-1 in JAR Cells

Bente Halvorsen, Anne Cathrine Staff, Tore Henriksen, Tatsuya Sawamura, Trine Ranheim

Abstract—Lectinlike oxidized LDL receptor-1 (LOX-1), a cell-surface receptor for oxidized LDL (ox-LDL), is proposed to be involved in endothelial dysfunction and in the pathogenesis of atherosclerosis. Preeclampsia is a pregnancy complication diagnosed by hypertension and proteinuria, characterized by endothelial dysfunction, and supposedly caused by compounds from hypoxic uteroplacental tissues. A feature of preeclampsia is formation of foam cells in maternal arterial walls of gestational tissue ("acute atherosis"). Oxidative stress is believed to play a role in the pathophysiology of preeclampsia. 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is a marker of oxidative stress in vivo, is biologically active in vitro, and is elevated in preeclamptic plasma and gestational tissue. In the present article, we hypothesized that 8-iso-$PGF_{2\alpha}$ could induce the expression of LOX-1 in trophoblastic cells (JAR). We demonstrated augmented cellular uptake of ^{125}I-tyraminylcellobiose ox-LDL in JAR cells incubated with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) versus control cells. Ligand blots revealed an increased binding of ox-LDL to LOX-1 in JAR cells incubated with 8-iso-$PGF_{2\alpha}$ (10 μmol/L). Incubation with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) also resulted in augmented LOX-1 protein levels (Western blots) and mRNA levels (Northern blots). JAR cells transfected with 3 copies of a nuclear factor-κB binding site demonstrated dose-dependent activation of the reporter gene luciferase after incubation with 8-iso-$PGF_{2\alpha}$ (0 to 10 μmol/L). We also demonstrated increased accumulation of neutral fats in JAR cells incubated with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) and ox-LDL compared with controls by oil red O staining. We speculate a potential role of isoprostanes and LOX-1 in preeclampsia in the development of "acute atherosis" of gestational spiral arteries. **(*Hypertension*. 2001;37: 1184-1190.)**

Key Words: receptors, lipoprotein ■ isoprostanes ■ nuclear factor ■ trophoblast ■ preeclampsia

Lectinlike oxidized LDL receptor-1 (LOX-1)[1] has recently been identified as a cell-surface receptor for oxidized LDL (ox-LDL), a major atherogenic substance.[2] Ox-LDL is believed to get trapped in the macrophage through uptake by scavenger receptors on the surface, resulting in foam cell formation and development of atherosclerotic lesions.[3] Modified LDL is also internalized and degraded in the endothelium[4] and impairs endothelium-dependent vasorelaxation.[5] Besides the novel LOX-1, multiple molecules, including class A and B scavenger receptors, MARCO, CD36, and CD68 have also been identified to be ox-LDL receptors.[3] LOX-1 is expressed abundantly on vascular endothelial cells,[1] but is also expressed on macrophages in humans and mice.[6] Other cell types present in the atherosclerotic lesions, such as vascular smooth muscle cells and monocytes-macrophages, have also been demonstrated to express LOX-1.[7,8] This newly described receptor is proposed to be involved in endothelial dysfunction and foam cell formation in the pathogenesis of atherosclerosis.[9]

Preeclampsia complicates 3% to 7% of all human pregnancies, and is diagnosed by hypertension and proteinuria in the latter half of the pregnancy. Preeclampsia is a major cause of maternal and perinatal mortality and morbidity worldwide. The cause of the disease is still unknown, but dysfunction of maternal systemic endothelial cells is a key feature of the syndrome.[10] Altered placentation with a reduced invasion of cytotrophoblast cells from the outer cell mass of the blastocyst into the inner part of maternal uterine wall is another feature of preeclampsia.[11] This condition is probably the cause of incomplete transformation of maternal spiral arteries in the gestational endometrium (decidua),[12] which causes inadequate uteroplacental circulation and local ischemia. Among uteroplacental compounds that are proposed to mediate disturbance of the maternal endothelium are lipid products, in particular, lipid peroxides.[13]

In preeclampsia, areas of lipid deposition in the maternal spiral arterial walls resemble early stages of atherosclerotic lesions, named "acute atherosis."[14] Maternal risk factors for

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the syndrome include risk factors for atherosclerosis in later life, such as obesity, lipid abnormalities, insulin resistance, black race, hypertension, and elevated serum homocysteine concentrations.[15] In particular, a predominance exists in preeclampsia of smaller, denser LDL particles that are more prone to oxidative modification than larger particles.[16] In addition, elevated levels of autoantibodies against ox-LDL occur in established preeclampsia.[17] Finally, hyperlipidemia of preeclampsia is present long before onset of the disease, and levels of triglycerides and free fatty acids are higher than in uncomplicated pregnancies.[18]

Isoprostanes are prostaglandin isomers formed by free radical peroxidation of arachidonic acid present in phospholipids. Isoprostanes are released to free forms by the action of phospholipase A_2.[19] Among the isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is of special interest; it is a marker of oxidative stress in vivo and also has potent biological activity in vitro. 8-iso-$PGF_{2\alpha}$ is a potent vasoconstrictor, mediates smooth muscle cell growth, activates platelets,[20] and induces derangement of endothelial cell barrier function.[21] Oxidative stress has been proposed to be involved in the pathophysiology of preeclampsia. Elevated formation of 8-iso-$PGF_{2\alpha}$ is associated with cardiovascular risk factors such as hypercholesterolemia and diabetes mellitus.[20] In women with preeclampsia, plasma level of free 8-iso-$PGF_{2\alpha}$ is elevated compared with in control pregnancies.[22] In previous studies, we have demonstrated elevated levels of lipid peroxides[23] and free 8-iso-$PGF_{2\alpha}$[24] in gestational endometrium (decidua) at delivery in preeclamptic pregnancies compared with controls.

In the present article, we investigated whether the receptor for ox-LDL, LOX-1, was expressed by trophoblastic cells, and we hypothesized that the expression as well as the functional activity of the receptor was induced by 8-iso-$PGF_{2\alpha}$. We further questioned whether the oxidative stress marker 8-iso-$PGF_{2\alpha}$ could influence the red-ox level in JAR cells, by examining the effect of 8-iso-$PGF_{2\alpha}$ on nuclear factor (NF)–κB activity in JAR cells transfected with NF-κB binding sites coupled to a reporter gene. Finally, we studied whether trophoblastic cells could accumulate neutral lipids after incubation with 8-iso-$PGF_{2\alpha}$ and ox-LDL.

Methods

Materials

Tissue-culture dishes and flasks were supplied by Costar. Reagents, when not otherwise specified, were purchased from Sigma Chemical Co. Polyvinyldifluoride (PVDF) filters were from NEN, and Hybond-N filters were from Amersham. Sodium dodecyl sulfate–polyacrylamide gel electrophoretic (SDS-PAGE) gels (5%) were obtained from Bio-Rad.

Cell Cultures

The human choriocarcinoma cell line JAR from American Type Tissue Collection (ATTC), was grown in RPMI-1640 supplemented with 10% heat-inactivated FBS, L-glutamine 2 mmol/L, penicillin 50 IU/mL, and streptomycin 50 μg/mL (Bio Whittaker). The murine macrophage cell line J774 A1 (J774) from ATTC was grown in DMEM with the same supplements as above except that gentamycin (60 μg/mL) was used as the only antibiotic.[25] The J774 cells were used as positive controls in the various experiments for expression of the macrophage type of LOX-1, which has previously been described.[8]

Isolation, Labeling, and Oxidation of LDL

LDL was isolated, labeled with ^{125}I-tyraminylcellobiose (^{125}I-TC), and oxidized with $CuSO_4$ as described previously.[25]

Cellular Uptake of ^{125}I-TC Ox-LDL

We measured cellular uptake of ^{125}I-TC ox-LDL in JAR cells seeded in 24-well plate (1×10^5 cells/well) and grown overnight (o/n) in growth medium. Fresh growth medium was then added with either vehicle (0.09% ethanol) or 10 μmol/L 8-iso-$PGF_{2\alpha}$ and incubated for 6 hours at 37°C. Thereafter, RPMI-1640 was added to ^{125}I-TC ox-LDL (10 μg/mL; 100 cpm/ng), 2% BSA, 2 mmol/L $CaCl_2$, and either vehicle or 10 μmol/L 8-iso-$PGF_{2\alpha}$ and incubated for $\leq$24 hours at 37°C. Thereafter, the cells were chilled on ice and harvested, and cell-associated radioactivity was determined and results calculated as described elsewhere.[25] The ^{125}I-TC ox-LDL cannot escape the endosomal-lysosomal compartments, and the cell-associated radioactivity represents both bound and internalized as well as degraded forms of ^{125}I-TC ox-LDL. "Cell-associated radioactivity" is used synonymously with "cellular uptake" of ^{125}I-TC ox-LDL in the present article.

Isolation and Preparation of Membrane Proteins From JAR Cells

JAR cells were incubated in growth medium supplemented with either 0.09% ethanol (vehicle and control cells), 100 nmol/L 8-iso-$PGF_{2\alpha}$, or 10 μmol/L 8-iso-PGF_2 for 6 or 24 hours. After they were incubated, cells were washed in ice-cold buffer A (150 mmol/L NaCl, 50 mmol/L Tris-HCl, and 2.5 mmol/L $CaCl_2$; pH 7.5) that contained a protease inhibitor cocktail (Boehringer Mannheim). JAR cell membranes were isolated and solubilized, and the protein concentration was determined as described previously.[26]

Visualization of Ox-LDL Binding Proteins by Immunolabeling on Blot

Solubilized membrane proteins (50 μg per lane) from the JAR and J774 cells were separated by nonreducing SDS-PAGE (5%) and blotted onto PVDF membranes, as described elsewhere.[26] The membranes were blocked for 2 hours at room temperature with 5% skimmed milk in TBS that contained 0.2% Tween (vol:vol) (TBST) after incubation with ox-LDL (10 μg/mL) o/n at 4°C. After several washes in TBST with 0.5% skimmed milk, blots were incubated at room temperature for 1 hour with anti–human apolipoprotein B-100 antibody (ICN Biomedicals) in TBST with 0.5% milk. After blots washed, proteins were detected by enhanced chemiluminescence with horseradish peroxidase–labeled anti-mouse IgG (Vector Laboratories).

Western Blot Analysis

JAR cells were incubated in growth medium supplemented with either 0.09% ethanol (control cells) or 8-iso-$PGF_{2\alpha}$ (10 μmol/L) for 6 and 24 hours. Western blotting was performed as described previously,[27] which separated 20 μg of cell protein from each experiment by SDS-PAGE (10%) and transferred it to PVDF membranes. Filters were incubated with anti–bovine LOX-1 antibody[28] from mouse ascites, which cross reacts with human LOX-1. Proteins were detected by enhanced chemiluminescence with horseradish peroxidase–labeled anti-mouse IgG.

Northern Blot Analysis

Triazol (Gibco) was used according to manufacturer directions for total RNA isolation from JAR cells incubated either 6 or 24 hours in growth medium that contained either 0.09% ethanol (control) or 8-iso-$PGF_{2\alpha}$ (10 μmol/L). Northern blotting was performed as previously described with the use of Hybond-N membranes.[25] A human LOX-1 cDNA probe was used[1] and calibrated against the signals from yeast 18S ribosomal RNA.

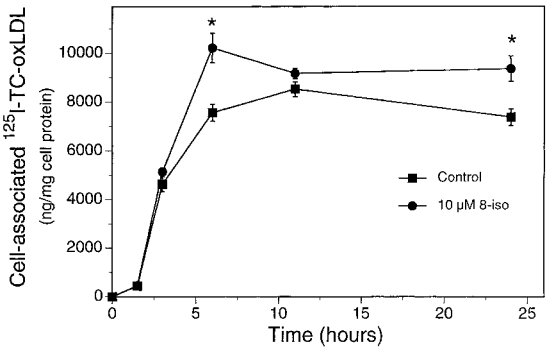

Figure 1. Cell-associated ^{125}I-TC ox-LDL in JAR cells preincubated for 6 hours in either 0.09% ethanol or 10 μmol/L 8-iso-$PGF_{2\alpha}$ and incubated ≤24 hours with additives and ^{125}I-TC ox-LDL (10 μg/mL; 100 cpm/ng). Results are mean±SEM of 4 separate cell cultures; *$P<0.05$.

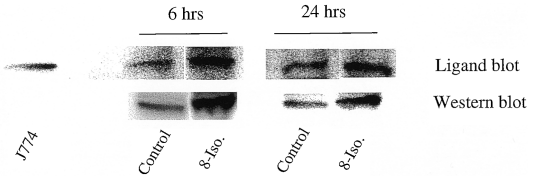

Figure 2. Visualization of proteins from JAR cells after incubation with control (0.09% ethanol; vehicle) or 10 μmol/L 8-iso-$PGF_{2\alpha}$ for 6 or 24 hours. Left, J774 cells. Top, ox-LDL–binding proteins visualized by immunolabeling of ligand blot with an anti–apolipoprotein B-100 antibody (50 μg of solubilized cell membrane protein per lane). Three separate experiments were performed, each in duplicate. A representative blot is presented. Bottom, representative Western blot from 1 of 3 separate experiments; each experiment was performed in duplicate. Cell proteins (20 μg) were separated by reducing SDS-PAGE, electrotransference, and incubation with monoclonal antibody against LOX-1 followed by horseradish peroxidase–labeled anti-mouse IgG and detected by enhanced chemiluminescence.

DNA Transfection

JAR cells were plated at 1×10^5 cells per well in 6-well plates and transfected 2 days after plating. Transfections were performed in the absence of serum with 3 μg of DNA by use of 9 μL of TransFast reagent (Promega Corp). The 3x-κB-luciferase (3x-κB-luc) plasmid was kindly provided by Dr T. Wirth.[29] This promoter construct contains 3 copies of a binding site for NF-κB. All transfections were overlaid with growth medium after 1 hour. Cells were incubated 1 day after transfection in growth medium supplemented with 8-iso-$PGF_{2\alpha}$ (100 nmol/L, 1 μmol/L, or 10 μmol/L) for ≤24 hours. Control cells were incubated in growth medium supplemented with 0.09% ethanol. After incubation, the cells were harvested for luciferase assays (Promega Corporation), and the luminescence was measured by a luminometer (Turner Designs, TD-20/20).

Oil Red O Staining

JAR cells were seeded on Chamber slides (Laboratory-Tek) (5×10^5 cells per slide) and grown o/n in 3 mL of growth medium. Cells were then added either to fresh growth medium alone, growth medium with 0.9% ethanol and ox-LDL (20 μg/mL), or growth medium with 10 μmol/L 8-iso-$PGF_{2\alpha}$ and ox-LDL (20 μg/mL). Additives were freshly renewed after 24 hours. After a total of 48 hours of incubation, the slides were washed, fixed, and stained with oil red O as described previously[30] and examined by light microscopy.

Statistical Analyses

Results are presented as means±SEM, and the differences between control and treatment groups were tested using the Mann-Whitney *U* test. $P<0.05$ was considered statistically significant.

Results

Cellular Uptake of ^{125}I-TC Ox-LDL

Figure 1 demonstrates increased cell-associated ^{125}I-TC ox-LDL in JAR cells incubated with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) for 6 and 24 hours (35% elevation, $P=0.02$, and 27% elevation, $P=0.04$, respectively) compared with control cells. The time course shows a high uptake of radiolabeled ligand (8 to 10 μg ^{125}I-TC ox-LDL per milligram cell protein) early in the time course that reached a plateau after 6 hours of incubation, which suggests saturated kinetics of the uptake of ^{125}I-TC ox-LDL in JAR cells.

To rule out the possibility that 8-iso-$PGF_{2\alpha}$ could affect the binding of ox-LDL to LOX-1, we performed a binding assay similar to the uptake assay but omitted the preincubation step. JAR cells were kept for 2 hours at 4°C in ^{125}I-TC ox-LDL to which RPMI was added, 2% BSA, 2 mmol/L $CaCl_2$, and either vehicle (0.09% ethanol) or 10 μmol/L 8-iso-$PGF_{2\alpha}$. No statistical significant difference was seen in cell-associated ^{125}I-TC ox-LDL between control and 8-iso-$PGF_{2\alpha}$-treated cells ($P=0.5$; n=4), which showed that 8-iso-$PGF_{2\alpha}$ does not interfere with the ligand-binding activity of LOX-1.

Immunolabeling of Ligand Blot

Figure 2, top, demonstrates a major immunoreactive band at 50 kDa, which indirectly shows LOX-1. The figure shows 1.7-fold elevated expression of binding protein for ox-LDL at 50 kDa in JAR cells after 6 hours of incubation with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) versus control cells ($P=0.01$; SEM, 0.5). A 2.3-fold elevated expression occurred after 24 hours' incubation with the same test compound ($P=0.01$; SEM, 0.7). On the other hand, incubation for 6 or 24 hours with 100 nmol/L 8-iso-$PGF_{2\alpha}$ did not result in significantly altered expression of immunoreactive protein (1.0-fold and 1.1-fold elevated compared with control, respectively; data not shown). The immunoblots also demonstrated a distinct band at a lower molecular size of 40 kDa from the membrane proteins of JAR cells (not shown), which was comparable to the major band of the untreated J774 cells. J774 cells were used as positive controls for macrophage-type LOX-1 at 40 kDa.[8] In addition, a immunoreactive band occurred at 50 kDa from the membrane proteins of J774 cells, which was comparable to endothelial-type LOX-1 (Figure 2, top).

Western Blot

Figure 2, bottom, demonstrates immunoreaction to the LOX-1 protein at 50 kDa in JAR cells, which corresponds to the endothelial size of the receptor. JAR cells subjected to 8-iso-$PGF_{2\alpha}$ (10 μmol/L) for 6 hours demonstrate a 1.4-fold elevated protein level of LOX-1 of 50 kDa versus controls ($P=0.04$; SEM, 0.07). The 24-hour incubation of JAR cells with 8-iso-$PGF_{2\alpha}$ (10 μmol/L) resulted in unaltered 50-kDa protein expression of LOX-1 (1.0 relative to control cells, $P=0.5$; SEM, 0.06). Weaker immunoreactivity existed at a lower molecular size of 40 kDa, which probably corresponded to the macrophage type of the receptor (not shown).

Expression of mRNA

Northern blots demonstrated that JAR trophoblasts exhibit the 2.8-kb LOX-1 transcript. A statistically significant 1.4-

Effects of 6 and 24 Hours of Incubation of 8-iso-PGF$_{2\alpha}$ on mRNA Signal Density of LOX-1 in JAR Cells

Incubation, h	8-iso-PGF$_{2\alpha}$, μmol/L	Relative mRNA Expression	*P*
6	0	100	1.0
6	10	142±10	0.02
24	0	100	1.0
24	10	109±20	0.4

Relative mRNA signal density of LOX-1 in JAR cells treated ≤24 hours with either vehicle (0.9% ethanol for control cells) or 10 μmol/L 8-iso-PGF$_{2\alpha}$. Results are percentage of control, and represents mean±SEM of 4 different experiments, each consisting of cells pooled from 2 separate culture wells.

fold elevated mRNA–level signal density of LOX-1 exists relative to 18S ribosomal RNA in JAR cells subjected to 10 μmol/L 8-iso-PGF$_{2\alpha}$ for 6 hours compared with control cells (Table). The 1.1-fold elevated mRNA–level relative signal density for LOX-1 in JAR cells subjected to 10 μmol/L 8-iso-PGF$_{2\alpha}$ for 24 hours compared with control cells was not statistically significant.

NF-κB–Transfected JAR Cells

Figure 3 demonstrates dose-dependent induction of luciferase activity in the transfected JAR cells incubated with 8-iso-PGF$_{2\alpha}$ relative to control cells, which ranged from 1.6-fold (0.1 μmol/L 8-iso-PGF$_{2\alpha}$) to 12.8-fold (10 μmol/L 8-iso-PGF$_{2\alpha}$) induction (all $P<0.03$).

Oil Red O Staining

Staining of neutral lipids on the Chamber slides with oil red O revealed that JAR cells incubated with ox-LDL accumulate significant amounts of oil red O–positive material compared with control cells without ox-LDL added (Figure 4B and A, respectively). In addition, cells grown with both 8-iso-PGF$_{2\alpha}$ (10 μmol/L) and ox-LDL show a more intensive red staining versus cells exposed to only ox-LDL and vehicle (Figure 4C and B, respectively).

Discussion

In the present study, we report the expression and 8-iso-PGF$_{2\alpha}$-induced upregulation of the endothelial scavenger receptor LOX-1 in the trophoblastic cell-line JAR, by ligand, Western, and Northern blots. In addition, we report dose-dependent activation of the reporter gene luciferase after incubation with 8-iso-PGF$_{2\alpha}$ (0 to 10 μmol/L) in JAR cells transfected with 3 copies of an NF-κB binding site. We also show that incubation with 8-iso-PGF$_{2\alpha}$ (10 μmol/L) augments cellular uptake of ^{125}I-TC ox-LDL in addition to enhancing the lipid staining of JAR cells incubated with ox-LDL.

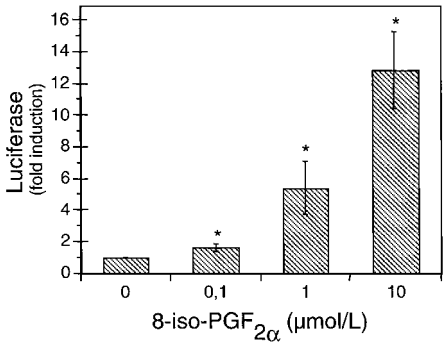

Figure 3. Transcriptional induction by 8-iso-PGF$_{2\alpha}$ in JAR cells transfected with 3x-κB-luc that contained vector. Cells were incubated 1 day after transfection in RPMI-1640 growth medium that contained 8-iso-PGF$_{2\alpha}$ (0, 0.1, 1, or 10 μmol/L) for 24 hours and harvested for luciferase assays. Results are given as -fold inductions of luciferase activity, and each bar graph represents mean±SEM of 10 to 13 separate cell cultures; *$P<0.05$.

The plasma level of 8-iso-PGF$_{2\alpha}$ is elevated in pregnant compared with nonpregnant women,[31] but even the elevated plasma level found in preeclampsia is usually much lower than used in our study.[22] On the other hand, the local level in gestational decidual tissue is likely to be much higher than plasma levels as a result of areas of oxidative stress and successively atherosclerosis-like foam cell lesions in preeclampsia. Analogously, very high tissue levels of 8-iso-PGF$_{2\alpha}$ in atherosclerotic arteries compared with control vessels and plasma levels have been demonstrated previously.[32] Therefore, the high concentrations of 8-iso-PGF$_{2\alpha}$ used in our study could be considered physiological for purposes of studying the local effects on trophoblastic cells in preeclamptic decidua. In a manner analogous to 8-iso-PGF$_{2\alpha}$, lysophosphatidylcholine (lysoPC) is accumulated at high concentrations in atherosclerotic plaques, whereas the concentration in plasma is low.[33] LysoPC constitutes 40% of the total lipids of ox-LDL,[34] whereas the measured amount of ox-LDL is negligible in plasma.[35] 8-iso-PGF$_{2\alpha}$ is associated with the LDL particle in plasma. In addition, 8-iso-PGF$_{2\alpha}$ is generated during oxidation of LDL.[36] The high amounts of 8-iso-PGF$_{2\alpha}$ and lysoPC in atherosclerotic plaques could stem from local oxidation of LDL.

To further study the specific involvement of LOX-1 in the uptake of ^{125}I-TC ox-LDL in JAR cells, we used an antibody against the ligand-binding domain of LOX-1 receptor (JTX-92).[28] After 6 hours of preincubation in the presence or absence of 10 μmol/L 8-iso-PGF$_{2\alpha}$, we performed an uptake study as described in "Methods" above. After 6 hours of incubation at 37°C with or without 8 μg/mL of the blocking antibody, JAR cells were harvested and the degraded radiolabeled material was determined after TCA precipitation as reported elsewhere.[37] We showed that the level of degraded ^{125}I-TC ox-LDL was 25% higher in JAR cells grown with 10 μmol/L 8-iso-PGF$_{2\alpha}$ versus control cells ($P=0.02$). These results are comparable to the augmented uptake demonstrated in Figure 1. When 8 μg/mL of the blocking antibody was added to the JAR cells incubated with 10 μmol/L 8-iso-PGF$_{2\alpha}$, a 46% reduction occurred in the level of degraded ^{125}I-TC ox-LDL compared with JAR cells grown in 10 μmol/L 8-iso-PGF$_{2\alpha}$ without antibody ($P=0.034$; n=3). These data suggest that 40% to 50% of the cellular uptake of ox-LDL in the JAR cells could be LOX-1–mediated. This is in line with results of other groups that have reported that LOX-1 contributes to 50% to 70% of ox-LDL uptake in endothelial cells.[34]

NF-κB is a red-ox sensitive transcription factor,[38] and 8-iso-PGF$_{2\alpha}$ represents a marker of oxidative stress. We showed that 8-iso-PGF$_{2\alpha}$ produced dose-dependent activation of the reporter gene luciferase in the NF-κB–transfected JAR cells (Figure 3). We thereby demonstrated in the JAR cells a

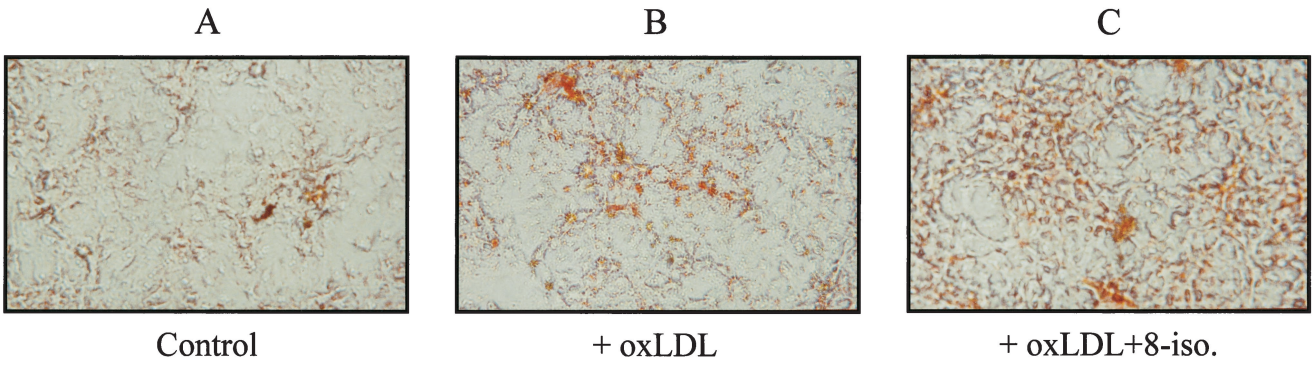

Figure 4. Oil red O staining of JAR cells grown on Chamber slides for 2×24 hours at 37°C in fresh growth medium (A), growth medium plus 0.09% ethanol (vehicle) plus ox-LDL (20 μg/mL) (B), or growth medium plus 10 μmol/L 8-iso-$PGF_{2\alpha}$ plus ox-LDL (20 μg/mL) (C). Magnification ×40.

link between the oxidative stress marker 8-iso-$PGF_{2\alpha}$ and the red-ox–regulated system of NF-κB. The presence of an NF-κB site on the LOX-1 promoter[39] may suggest that 8-iso-$PGF_{2\alpha}$ could regulate the LOX-1 gene through this site in JAR cells and explains the upregulation of LOX-1 mRNA (Table) and protein (50 kDa) (Figure 2, bottom) in trophoblastic JAR cells and augmented ligand binding (Figure 2, top) and elevated uptake of modified LDL (Figure 1) demonstrated in the present article. Interestingly, recent research has demonstrated that ox-LDL binding to LOX-1 in endothelial cells induces activation of NF-κB through increased production of intracellular reactive oxygen species.[40] Also, Li and Mehta[41] show upregulation of LOX-1 by its ligand ox-LDL and indicate that NF-κB activation plays an important role in this process.

In plasma, 8-iso-$PGF_{2\alpha}$ is associated with the LDL particle, and 8-iso-$PGF_{2\alpha}$ is found to be generated during oxidation of LDL.[36] Interestingly, increased levels of autoantibodies against ox-LDL epitopes are found in preeclamptic women versus those in normal pregnancies, which suggests an increased oxidative condition.[17] Moreover, in preeclampsia, the level of plasma lipid peroxides is elevated and antioxidant capacity is reduced versus in uneventful pregnancies.[42] In addition to the lipid peroxide species generated by ox-LDL, 8-iso-$PGF_{2\alpha}$ may be involved in the transcription activation of NF-κB described in endothelial cells and macrophages.[43,44] Recently, ox-LDL was found to increase LOX-1 expression both in bovine aortic endothelial[28] and in human coronary artery endothelial[41] cells. Li et al[45] demonstrated that angiotensin (Ang) II upregulates LOX-1 receptor and ox-LDL uptake in human coronary artery endothelial cells (HCAECs) and that Ang II enhances ox-LDL–mediated injury to these cells, which suggests a plausible link between atherogenesis and hypertension.[9] Interestingly, a study recently has found a connection between free 8-iso-$PGF_{2\alpha}$ plasma levels and low-dose Ang II infusion.[46] In preeclampsia, heightened pressor sensitivity to infused Ang II occurs, although plasma Ang II concentrations are reduced; the mechanisms for this are unknown.[47]

LOX-1 mRNA has been demonstrated in highly vascularized organs such as the placenta, lung, kidney, and vasculatures.[1,48] Expression is low in the brain, heart, adrenals, and other organs, and LOX-1 has been suggested to be expressed specifically in endothelial cells and macrophages and to play an important role in atherosclerotic lesion formation.[39] The present study demonstrates that LOX-1 is also expressed in trophoblastic cells; as far as we know, the present article represents the first demonstration of accumulation of neutral lipids (such as cholesterol and cholesteryl esters) in trophoblastic cells (Figure 4). Recent research has demonstrated that smooth muscle cells in vitro can gain a macrophage-like phenotype by expressing the scavenger receptor CD36, and thus possibly could present an origin of foam cells other than monocytes.[49] Our findings supports the notion that cells other than macrophages can be converted to foam cells. We speculate that this augmented LOX-1 expression could contribute to the formation of foam cells in the spiral arterial wall in preeclampsia (acute atherosis).

The biological function of upregulation of LOX-1 receptor in JAR trophoblastic cells and possibly trophoblasts in gestational tissues in response to 8-iso-$PGF_{2\alpha}$ remains to be seen. LOX-1 has been proposed to be involved in atherosclerosis through the actions of ox-LDL on the endothelium,[39] such as induction of adhesion molecules[50] and growth factors.[51] Moreover, LOX-1 recently has been demonstrated to work as an adhesion molecule for platelets, and could therefore initiate and promote atherosclerosis by binding both ox-LDL and platelets.[52] One hypothesis is that more-adhesive trophoblasts could be less prone to invade the maternal spiral arteries, which could result in less remodeling of the spiral arteries, with the more narrow, tortuous, and thick-walled arteries that are seen in preeclampsia. This would fit with our previously published data that showed that 8-iso-$PGF_{2\alpha}$ reduces invasion of JAR cells, possibly by reducing the activity of matrix metalloproteinase-2 and matrix metalloproteinase-9.[53]

We chose the choriocarcinoma cell line JAR for the present study because it provides a large number of uniform cells and shares many of the characteristics of early placental trophoblasts.[53] On the other hand, this malignant cell line also differs from normal trophoblast cells in the regulation of, for example, cell invasion. In vivo, interaction with, for example, maternal host cells, is complicated; therefore, one cannot directly extrapolate from the present in vitro study.

In conclusion, 8-iso-$PGF_{2\alpha}$ induces expression of LOX-1 at the mRNA and protein levels in addition to functional capacity of the receptor in JAR cells. This establishes a link

between a marker of oxidative stress and a receptor for ox-LDL. We speculate a potential role of LOX-1 in pre-eclampsia, both in formation of the foam cells in the spiral arterial walls (acute atherosis) and in reduced trophoblast invasion.

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