Oxidized LDL and Lysophosphatidylcholine Stimulate Plasminogen Activator Inhibitor-1 Expression in Vascular Smooth Muscle Cells

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Abstract—Plasminogen activator inhibitor-1 (PAI-1) functions as an important regulator of fibrinolysis by inhibiting both tissue-type and urokinase-type plasminogen activator. PAI-1 is produced by smooth muscle cells (SMCs) in atherosclerotic arteries, but the mechanisms responsible for induction of PAI-1 in SMCs are less well understood. In cultured human aortic SMCs, PAI-1 mRNA expression and protein secretion were increased after incubation with oxidized low-density lipoprotein (LDL) and the lipid peroxidation product lysophosphatidylcholine, whereas the effects of native LDL on PAI-1 production and release were more variable and did not reach statistical significance. The effect of LDL on arterial expression of PAI-1 in vivo was also studied in an animal model. Intravenous injection of human LDL in Sprague-Dawley rats resulted in accumulation of apolipoprotein B in the aorta within 12 hours as assessed by immunohistochemical testing. Epitopes specific for oxidized LDL began to develop in the aorta 12 hours after injection of LDL and peaked at 24 hours; this peak was accompanied by intense expression of PAI-1 immunoreactivity in the media. Also, increased aortic expression of PAI-1 mRNA after LDL injection was detected by using in situ hybridization. The transcription factor activator protein-1, which is known to bind to the promoter of the PAI-1 gene, was activated in the aortic wall 24 hours after LDL injection as assessed by electrophoretic mobility shift assay. Pretreatment of LDL with the antioxidant probucol decreased expression of oxidized LDL and PAI-1 immunoreactivity and activator protein-1 induction in the aorta but did not affect expression of apolipoprotein B immunoreactivity. These findings demonstrate that LDL oxidation enhances secretion of PAI-1 from cultured SMCs and that a similar mechanism may be involved in vascular expression of PAI-1. (Arterioscler Thromb Vasc Biol. 1999;19:3025-3032.)

Key Words: plasminogen activator inhibitor-1 ■ smooth muscle cells ■ lipid oxidation ■ lysophosphatidylcholine ■ activator protein-1

In healthy vessels, plasminogen activator inhibitor-1 (PAI-1) is produced predominantly by the endothelium, whereas high levels of PAI-1 mRNA and PAI-1 immunoreactivity are also observed in smooth muscle cells (SMCs) and macrophages in atherosclerotic arteries.1-4 The main function of PAI-1 is to regulate both tissue-type and urokinase-type plasminogen activator-induced activation of plasmin to plasmin.5 This activation plays a key role in the fibrinolytic system. Increased secretion of PAI-1 from the endothelium leads to impaired fibrinolytic function and may predispose to formation of occlusive thrombosis.6,7 However, PAI-1 appears to be important not only on the surface but also inside the vascular wall as a regulator of cell growth and matrix degradation. PAI-1 inhibits plasmin-dependent activation of matrix-degrading metalloproteinases.8 Accordingly, inactivation of the PAI-1 gene in mice results in a more rapid migration of SMCs into the intima of injured vessels and in increased neointima formation.9

Interactions between lipoproteins and vascular cells are believed to be of key importance in atherosclerosis. In particular, interest has focused on the role of oxidative modification of LDL, which is believed to occur in the matrix of the arterial intima.10,11 This modification is thought to be responsible for the uptake of LDL by macrophages and subsequent formation of macrophage foam cells.12 Oxidative modification of LDL is associated with formation of a number of reactive substances, such as peroxides, aldehydes, lysophospholipids, and oxysterols.13 Exposure of vascular cells to these substances has major effects on cell function. High levels are generally cytotoxic, whereas low levels may activate cells, presumably through interaction with redox-regulated transcription factors and phospholipid-dependent messenger pathways.14 The biological effects of mildly modified LDL or low levels of fully oxidized LDL include stimulation of endothelial cell adhesion molecule expres-
sion, release of leukocyte chemoattractants, monocyte-macrophage differentiation, and activation of monocyte tumor necrosis factor-α secretion. Oxidized LDL has also been shown to activate DNA synthesis in cultured SMCs. This effect is primarily explained by potentiation of the mitogenic effects of other growth factors and appears to involve activation of the transcription factor activator protein-1 (AP-1) by lysophosphatidylcholine and peroxides.

Recent studies have demonstrated that injection of human LDL in rats results in accumulation of apoB immunoreactivity in arterial tissues within 4 to 6 hours, followed by expression of oxidized LDL-specific immunoreactivity a few hours later. The expression of oxidized LDL immunoreactivity, but not apoB immunoreactivity, was inhibited by preincubating LDL with the antioxidant probucol. In the study described here, we used this in vivo model for LDL oxidation as well as cultured human SMCs to demonstrate that oxidized LDL and one of its active components, lysophosphatidylcholine, influence the production of PAI-1 in SMCs.

**Methods**

**Materials**

Human PAI-1 cDNA was a gift from Dr Tor Ny. A 1.2-kb Sfi/I, BgII fragment of this cDNA clone was used as a probe for Northern blotting. PAI-1 antigen was determined by means of a chromogenic assay kit (Spectrolyse/fibrin, Biopool). [α-32P]cTTP, [γ-32P]ATP, and the Rediprime random prime labeling system were from Amersham Pharma- cia Biotech. Sera and culture media were purchased from Gibco. The following antibodies were used: monoclonal mouse anti-human apoB, from Boehringer Mannheim; rabbit anti-rat PAI-1, derived from Amer- ican Diagnostica; and mouse monoclonal antibody NA-59, specific for c-Fos and c-Jun subunits were obtained from Oncogene Research Products/Calbiochem. Sephadex G-25M PD-10 columns were obtained from Pharmacia. A 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, 1-α-lysophosphatidylcholine (from egg yolk; contains primarily palmitic and stearic acid), and limulus amebocyte lysate (LAL) assay (E-Toxate) were purchased from Sigma Chemical Co. Male Sprague-Dawley rats were obtained from B&K Universal AB (Sollentuna, Sweden). Probucol was kindly provided by Dr Ann-Margret Lindquist.

**Cell Culture**

Human aortic SMCs were purchased from Cytotech (Copenhagen, Denmark). Cells were maintained in DMEM medium containing 10% FCS and 50 μg/mL penicillin-streptomycin with incubation in 5% CO2 in air at 37°C. Cells were passaged by trypsinization and used in the third to ninth passages.

**Lipoprotein Preparation**

LDL (density, 1.025 to 1.050 kg/L) was isolated from human plasma by density gradient ultracentrifugation in a SW 40 swinging bucket rotor in a Beckman L8-55 ultracentrifuge at 1°C overnight. The LDL was subsequently desalted by running it through a Sephadex G-25M PD-10 column. The protein concentration of LDL was determined according to the method of Lowry et al. LDL was oxidatively modified by incubation in 5 μmol/L CuSO4 in PBS for 18 hours at 37°C. Oxidative modification was verified as enhanced mobility by use of agarose gel electrophoresis and by increased absorbance at 234 nm due to the formation of conjugated dienes. For all experiments, freshly prepared lipoproteins were used within 24 hours after ultracentrifugation. Endotoxin content in the lipoprotein preparations was tested by use of the E-Toxate assay. Briefly, lipoprotein samples were diluted 1:10 in endotoxin-free water and heated at 65°C for 5 minutes to inactivate the LAL inhibitor found in plasma. E-Toxate working solution containing LAL was added, and samples were incubated at 37°C for 1 hour. Positive controls containing 0.06 endotoxin units/mL (derived from Escherichia coli 0.55:B5 lipopolysaccharide) yielded formation of a hard gel, which permitted complete inversion of the tube without disruption of the gel. All LDL samples, both native and oxidized, were found to be endotoxin-free by this assay.

**Isolation and Analysis of PAI-1 mRNA Expression**

Confluent 100-mm plates of SMCs were serum-starved in DMEM with 0.1% FCS for 24 hours. After 4 hours of exposure to the different experimental substances, total RNA was isolated from the cells by using RNasea (Qiagen) according to the manufacturer’s instructions. Northern blotting and hybridization on Dupont GeneScreen Plus nylon membranes were performed using ExpressHyb hybridization solution (Clontech) according to the manufacturer’s protocols. Twenty-five micrograms of cDNA samples for PAI-1 or β-actin was labeled with [α-32P]dCTP using the Rediprime random prime labeling system. Blots were hybridized with a 1000 dpm probe/mL hybridization solution.

**Determination of PAI-1 Secretion**

Confluent cultures of aortic SMCs were incubated for 24 hours at 37°C in DMEM containing 0.1% FCS with or without additions. At the end of the incubation period, the conditioned medium was removed, immediately frozen in precooled Eppendorf caps, and stored at −80°C until analysis. The amount of active PAI-1 in the medium was determined by means of a chromogenic assay kit. Total protein levels of the cells were determined according to the method of Lowry et al and used as a reference to determine the amount of secreted PAI-1. Mean values (n=6) are expressed in picograms per microgram of total protein along with the SD. Groups were compared by using ANOVA.

**Probucol Enrichment of LDL**

Plasma was incubated for 2 hours at 37°C with probucol (0.05 mmol/L). LDL was then isolated and concentrated, and the protein concentration was determined using the methods described above. Roughly 45% of the added probucol is incorporated into the LDL fraction with this protocol, which uses plasma with a total cholesterol level of 6.5 to 7.0 mmol/L.

**Animal Protocol**

Male Sprague-Dawley rats (300 to 400 g) were injected intravenously with PBS or human LDL while under anesthesia with ether. The rats were subsequently euthanized at 0, 12, and 24 hours while under anesthesia (ketamine, 30 mg/kg; Rompun, 3 mg/kg) by intracardiac injection of KCL. Rats for immunohistochemical studies and in situ hybridization were perfused with 0.9% saline containing 0.02 mmol/L of butylated hydroxytoluene (BHT) for 5 minutes and fixed by perfusion for 10 minutes with 4% formaldehyde/0.02 mmol/L BHT. The aorta was removed, placed in 4% formaldehyde/0.02 mmol/L BHT for 3 hours, transferred to a 15% sucrose/0.02 mmol/L BHT solution, kept at 4°C over night, and embedded in paraffin. For electrophoretic mobility shift assays, aortic tissue sections were removed rapidly without previous perfusion, washed in cold PBS containing 0.02 mmol/L BHT, and frozen in liquid nitrogen. The study was approved by the Institutional Animal Care and Use Committee of the Karolinska Institute.

**Immunohistochemical Studies**

Tissue sections were deparaffinized with xylene and dehydrated with graded ethanol. The membranes were permeabilized in 0.2% Triton X-100. Endogenous peroxidase activity was quenched by incubating the sections in 0.3% H2O2, and 80% methanol for 30 minutes at room temperature. PBS was used as washing buffer for NA-59 and PAI-1 antibodies, whereas Tris buffer, pH 7.4, was used for apoB.
were rinsed 3 times in washing buffer and then incubated with 10% horse serum (for apoB and NA-59) or 10% goat serum (for PAI-1) for 30 minutes. Primary antibodies were diluted in PBS or Tris buffer (final concentrations: anti-apoB, 10 μg/mL; anti-PAI-1, 2 μg/mL; and NA-59, 1:500) and incubated overnight at 4°C. On the next day, sections were washed and incubated with biotinylated secondary antibodies (diluted 1:200) for 30 minutes and then washed. Sections were incubated for 30 minutes with a peroxidase-labeled avidin-biotin complex and washed again. The sections were developed using 3'-diaminobenzidine tetrahydrochloride (Vector) and counterstained in hematoxylin. Negative controls included substitution of the primary antibody with either PBS or irrelevant antibody.

**In Situ Hybridization**

Deparaffinized sections were hybridized at 42°C for 20 hours with a 32P-end-labeled antisense oligonucleotide probe (5×10⁶ cpm/mL) and the corresponding nonbinding sense oligonucleotide probe together with 0.5 mg/mL salmon sperm DNA and 0.2 μL/mL diethiothreitol in a solution containing 50% formamide, 1× Denhardt’s solution, 4× SSC, 1% sarcosyl, 0.02 mol/L sodium phosphate buffer (pH 7.0), and 10% dextran sulfate. They were then rinsed 4 times in 1× SSC and once in distilled water, dehydrated in ethanol, and dried. The slides were then dipped in photographic emulsion (NTB-2, Eastman Kodak) and developed after 4 weeks of exposure. Four antisense oligonucleotides were used: (1) 5'-GGC AGG AGC TGT GCC CCT CTC ACT GAT ATT GAA TCA TTC CAT AGC ATC-3', spanning the border between exons 2 and 3 of the rat PAI-1 gene; (2) 5'-CCT TGG CCA GTA AGT CAC TGA TCA TAC CCT TGG TGT GCC TCT CCA-3', spanning the border between exons 3 and 4; (3) 5'-GGT CCC ATC CCG AGT GGT GAA CTA AGT GAT GAA TCT GGT GTC-3', spanning the border between exons 4 and 5; and (4) 5'-CC TCA CCT CCA GAG GAA ACT TAG GCA GGA TGA GCA GGC GGG GCA-3', spanning the border between exons 5 and 6. The sense sequence 5'-dGCA TTY AGT CAG CGG GAA-3' was used as a negative control.

Positive staining was obtained using all 4 antisense oligonucleotides, whereas no staining was obtained using the sense oligonucleotide. The strongest staining was achieved by using an equal mixture of the 4 antisense oligonucleotides, whereas no staining was obtained using the sense oligonucleotide. The strongest staining was achieved by using an equal mixture of the 4 antisense oligonucleotides. Image analysis was performed with use of Microimage image analysis software (Olympus Optical Co). In brief, different freeform areas of interest within the intima and media were chosen, and signals were detected by both a defined color range and roundness. The number of signals was then divided by the area. Groups were compared by using ANOVA.

**Preparation of Nuclear Extracts**

Nuclear extracts were prepared essentially as described by Cercek et al.26 2-Mercaptoethanol (5 mmol/L) and the protease inhibitors leupeptin (0.7 μg/mL), aprotinin (16.7 μg/mL), and PMSF (0.5 mmol/L) were added to all buffers just before use. Thawed samples of rat aorta were minced in cold PBS and homogenized with a Dounce glass homogenizer in ice-cold hypotonic lysis buffer (10 mmol/L Tris, pH 7.3, 10 mmol/L KCl, 1.5 mmol/L MgCl₂, and 0.4% Nonidet P-40) for 2 to 3 minutes. After centrifugation at 9000g for 1 minute, the pellet was washed in 20 mmol/L KCl buffer (20 mmol/L Tris, pH 7.3, 20 mmol/L KCl, 21.75% glycerol, 1.5 mmol/L MgCl₂, and 0.2 mmol/L EDTA). Isolated nuclei were resuspended in 15 μL of 20 mmol/L KCl buffer, and 60 μL of 600 mmol/L KCl buffer (20 mmol/L Tris, pH 7.3, 600 mmol/L KCl, 21.75% glycerol, 1.5 mmol/L MgCl₂, and 0.2 mmol/L EDTA) was added. Nuclear proteins were extracted by incubation on ice for 30 minutes. After centrifugation at 9000g (4°C) for 15 minutes, the supernatant containing nuclear proteins was transferred to a precooled microcentrifuge tube. An aliquot of the extract was diluted 40 times with 484 mmol/L KCl buffer (mixture of 20 mmol/L KCl buffer and 600 mmol/L KCl buffer to give the same glycerol and salt concentrations as in the undiluted nuclear extracts) for the protein assay. Protein concentration was determined spectrophotometrically according to the following equation: Concentration (μg/mL) = (183 × A₃₂₀ nm) / (75.8 × A₆₅₀ nm), where A is absorbance.

**Electrophoretic Mobility Shift Assay**

Equal amounts of protein from nuclear extracts of rat aortic tissue (20 μg) were incubated on ice with 2 μg of poly(deoxyinosin-deoxycytosin) and 1 μg of acetylated BSA in binding buffer (giving the final concentrations stated below) for 35 minutes. For supershift analysis, 2 μL of the relevant antibodies was added after the binding buffer solution. The oligonucleotide probe (50 000 cpm in 3 μL of 10 mm Tris, 1 mm EDTA (pH = 8.0) buffer) was added, and the reaction mixture (25 μL) was incubated for 25 minutes at room temperature. Final concentrations in binding reactions were as follows: 10% glycerol, 10 mmol/L HEPES (pH 7.9), 60 mmol/L KCl, 5 mmol/L MgCl₂, 0.5 mmol/L EDTA, 1 mmol/L dithiothreitol, and 1 mmol/L PMSF. DNA-protein complexes were separated from unbound DNA probe on a native 7% polyacrylamide gel (acrylamide:bisacrylamide [wt/wt], 80:1) in low ionic strength buffer (22.5 mmol/L Tris, 22.5 mmol/L borate, and 0.5 mmol/L EDTA, pH 8) by electrophoresis at 300 V for 2 hours. The sequence of the double-stranded AP-1 consensus oligonucleotide probe (labeled with T4 kinase and [γ-32P]ATP and purified using Pharmacia Nick columns) was as follows: 5'-CGC TTT ATG AGT CAC TGG CAG GAA-3'.

**Results**

**Exposure of Human Aortic SMCs to Oxidized LDL and Lysophosphatidylcholine Increases Expression of PAI-1 mRNA**

The effects of native LDL, oxidized LDL, and lysophosphatidylcholine on expression of the PAI-1 gene in human aortic SMCs were analyzed by Northern blotting. Confluent plates were serum-starved in DMEM containing 0.1% FCS for 24 hours. Because refreshment of culture medium is known to affect PAI-1 mRNA levels in many cells, native LDL (10 μg/mL), oxidized LDL (10 μg/mL), or lysophosphatidylcholine (2 μg/mL) was directly added to the medium. Total RNA was extracted after 4 hours of exposure. As shown in Figures 1A and 1B, significant increases in PAI-1 transcripts were observed in human aortic SMCs exposed to oxidized LDL and the lipid peroxidation product lysophosphatidylcholine. Expression of the housekeeping gene β-actin as well as methylene blue stains of the corresponding blotting filters showing the 28S and 18S ribosomal RNAs demonstrate that approximately equal amounts of RNA were loaded. Densitometric analysis was performed by using Kodak Digital Science 1D image analysis software (Figure 1B). Mean values (±SD) of PAI-1 mRNA (3.2-kb transcript) expression relative to β-actin mRNA expression from 4 independent experiments are given as the percentage over control: control, 100%; native LDL, 119.9±47.7%; oxidized LDL, 166.4±41.9%; and lysophosphatidylcholine, 242.7±82.6%. Differences in continuous variables between 2 groups were tested by using an unpaired Student’s t test.

**Oxidized LDL and Lysophosphatidylcholine Enhance Secretion of PAI-1 From Human Aortic SMCs**

The effects of native LDL, oxidized LDL, and lysophosphatidylcholine on secretion of active PAI-1 protein from cultured human aortic SMCs were investigated using a chromogenic assay kit (Figure 2). Basal extracellular release of PAI-1 in cultures grown in medium for 24 hours was 34.2±7.6 pg/μg total cellular protein. Addition of 10 μg/mL native LDL increased mean PAI-1 secretion (56.8±44.7 pg/μg total cellular protein); however, because of large variances, this phenomenon did not reach statistical significance. In contrast, addition of 10 μg/mL oxidized LDL as well as 2 μg/mL...
Oxidative Modification of LDL in Rat Aorta Is Associated With Increased Vascular Expression of PAI-1

To study the effect of oxidized LDL on vascular expression of PAI-1 in vivo, we used a rat model previously developed in our laboratory. No immunoreactivity for apoB or oxidized LDL was present in arteries of control rats. Twelve hours after injection of 6 mg/kg human LDL, immunoreactivity for human apoB was evident throughout the arterial media. This staining was even more pronounced after 24 hours. Minor amounts of immunoreactivity to oxidized LDL could be observed in the subendothelial layer of the media 12 hours after injection of LDL, whereas strong staining for oxidized LDL was present throughout the media at 24 hours (Figure 3). PAI-1 immunoreactivity was detectable in the endothelium of control rats, whereas there was no PAI-1 staining in the media. The pattern of PAI-1 expression remained unchanged at 12 hours, but 24 hours after injection of LDL, PAI-1 immunoreactivity was abundant throughout the media. Much of the PAI-1 appeared to have accumulated in the extracellular matrix, whereas part of the immunoreactivity was clearly cell-associated (Figure 3). Preincubation of LDL with the antioxidant probucol before injection did not influence arterial accumulation of apoB but markedly reduced expression of oxidized LDL and PAI-1 immunoreactivity (Figure 4).

Expression of PAI-1 mRNA in rat aorta after injection of LDL was studied by using in situ hybridization with 32P end-labeled antisense oligonucleotides. Basal expression of PAI-1 mRNA was observed in the endothelium of untreated animals as well as 24 hours after injection of PBS alone, whereas hardly no expression was found in the media (Figures 5 and 6). Twenty-four hours after injection of LDL, increased expression of PAI-1 mRNA was observed in some endothelial areas (Figure 5B) and to a higher extent in the media (Figure 5C).

Oxidative Modification of LDL in Rat Aorta Is Associated With Activation of Transcription Factor AP-1

Previous studies using the same animal model have shown activation of the transcription factor nuclear factor κB within the vascular wall in parallel with the expression of oxidized LDL–specific epitopes. The rat PAI-1 gene contains 7 sequences that are 85% identical to the consensus sequence for AP-1 sites (TGA \([C/G]\) TCA).27 In control rats, no AP-1–specific band in lane 2 due to addition of the different families. The decrease in sum intensity per area of the specific band in lane 2 due to addition of the different antibodies was quantitated by using Kodak Digital Science 1D image analysis software: anti–c-Fos, −22.6%; anti–

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**Figure 1.** A, Effects of native LDL (nLDL; 10 μg/mL), oxidized LDL (oxLDL; 10 μg/mL), and lysophosphatidylcholine (LysoPC; 2 μg/mL) on PAI-1 mRNA expression in human aortic SMCs. After 4 hours of incubation, total RNA was extracted and Northern blotting was performed with use of a human PAI-1 cDNA probe. B, Densitometric analysis was performed with use of Kodak Digital Science 1D image analysis software. Mean PAI-1 mRNA (3.2-kb transcript) expression values relative to β-actin mRNA expression together with the standard deviation from 4 independent experiments are given as the percentage over control.

**Figure 2.** Effects of native LDL (nLDL; 10 μg/mL), oxidized LDL (oxLDL; 10 μg/mL), and lysophosphatidylcholine (LysoPC; 2 μg/mL) on secretion of PAI-1 from aortic SMCs. Confluent cultures of human SMCs were incubated for 24 hours at 37°C in DMEM containing 0.1% FCS. The amount of active PAI-1 in the medium was determined by use of a chromogenic assay kit. Values are mean±SD (n=6).
c-Jun, −69%; anti-JunB, −32%; and anti-JunD, −75.9%. These findings suggest that the described complex consisted of Jun homodimers rather than Fos/Jun heterodimers.

**Discussion**

The observations made in this study indicate that SMC synthesis of PAI-1 is stimulated by oxidized LDL through one of its major components, lysophosphatidylcholine. Exposure of cultured human aortic SMCs to 10 μg/mL oxidized LDL or 2 μg/mL lysophosphatidylcholine significantly increased both PAI-1 mRNA level and secretion, whereas native LDL at a concentration of 10 μg/mL had only weak enhancing effects that were found to be more variable and did not reach statistical significance. Because incubation of native LDL with cultured SMCs...
has been shown to result in oxidative modification, it is likely that stimulated PAI-1 SMC production and release after exposure to native LDL is also mediated by oxidation products. Different amounts of naturally occurring antioxidants like vitamin E in LDL preparations derived from different subjects might explain the inconsistent induction of PAI-1 production by native LDL in cultured SMCs.

The in vivo experiments demonstrated that oxidative modification of LDL in rat arteries was associated with marked induction of vascular expression of PAI-1 together with activation of the transcription factor AP-1. Increased PAI-1 mRNA expression in response to LDL accumulation and modification was evident in some endothelial areas and to a higher extent in the media. The observation that preincubation of LDL with the antioxidant probucol inhibited AP-1 activation and decreased both oxidized LDL and PAI-1 immunoreactivity but had no effect on arterial accumulation of apoB suggests a link between LDL oxidation, AP-1 activation, and PAI-1 expression. Some of the vascular PAI-1 immunoreactivity induced by LDL injection was clearly associated with the endothelium, but the major part was found in the underlying media and appeared to be associated with SMCs. Although it cannot be excluded that medial PAI-1 is produced by the endothelium and transported to the media by diffusion, these findings are in agreement with the results of cell culture studies showing that oxidized LDL activates SMC PAI-1 expression as well as with the in situ hybridization data.

Effects of lipoproteins on PAI-1 secretion have previously been studied in human umbilical vein endothelial cells and hepatocytes (HepG2 cells). VLDL, particularly VLDL isolated from hypertriglyceridemic subjects, increases the secretion of PAI-1 from human umbilical vein endothelial cells, an effect attributed to interaction between VLDL and the normal (B or E) LDL receptor and induction of previously unknown transcription factors. Furthermore, Latron et al showed that limited oxidation of LDL by exposure to UV radiation is associated with formation of factors that stimulate endothelial PAI-1 secretion and that this effect is independent of LDL binding to the B and E receptors. Similar observations were made by Tremoli et al, who used LDL modified by acetylation. These investigators also reported stimulatory activity of high concentrations (>50 μg/mL) of native LDL. Both effects were shown to be independent of the B and E receptors. Minor stimulation was observed in response to 25 μg/mL of LDL fully oxidized by exposure to copper, whereas higher concentrations of oxidized LDL were inhibitory. All of these data are in accordance with our findings and the concept that lipid oxidation is associated with formation of factors inducing PAI-1 secretion.

Oxidation of LDL is associated with formation of lipid peroxides and other reactive oxygen intermediates, oxy-
sclerosis produce increased amounts of PAI-1. Increased PAI-1 expression in SMCs in arteries affected by atherosclerosis, increased neointima formation, and migration of SMCs into the intima of injured vessels and to the site of balloon injury in rabbit and rat arteries. 

Endotoxin is a potent stimulus to secretion of PAI-1 from endothelial cells. 

Lipid peroxidation and oxidative stress are important in the pathogenesis of atherosclerosis. The role of oxidized LDL (oxLDL) in the production of PAI-1 remains to be fully understood but may represent a limiting factor in the recruitment of medial SMCs to the intima.

Immunohistochemical and in situ hybridization studies have demonstrated that SMCs in arteries affected by atherosclerosis produce increased amounts of PAI-1. Increased expression of PAI-1 has also been observed in SMCs in the media and neointima of balloon-injured rabbit and rat arteries. 

The pathophysiological role of PAI-1 produced by SMCs under these conditions remains speculative. One possibility is that PAI-1 modulates SMC function by interfering with the plasmin-dependent activation of latent matrix-degrading enzymes, such as stromelysin, collagenase, and gelatine. These enzymes are believed to be required for matrix remodeling in association with cell replication and migration in vivo. Synthetic inhibitors of these enzymes inhibit SMC proliferation in rabbit aortic explants. Inactivation of the PAI-1 gene in mice results in a more rapid migration of SMCs into the intima of injured vessels and to increased neointima formation. 

Moreover, recent investigations by Bochaton-Piallat et al. have shown that rat arterial SMCs with the epithelioid phenotype produce less PAI-1 and more plasminogen activators than their spindle-shaped counterparts, which might render them prone to migrate into the intima after endothelial injury. The same study also supports our finding that normal rat media produces very little or no PAI-1.

In summary, the findings of this study strongly suggest that oxidative modification of LDL is associated with formation of factors like lysophosphatidylcholine that increase secretion of PAI-1 from SMCs. The pathophysiological role of this induction remains to be fully understood but may represent a limiting factor in the recruitment of medial SMCs to the intima.

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