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SHORT COMMUNICATION

The Expression of the Lectin-like Oxidized Low-Density Lipoprotein Receptor (LOX-1) on Human Vascular Smooth Muscle Cells and Monocytes and its Down-regulation by Lovastatin

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ABSTRACT. Accumulation of oxidatively modified low-density lipoprotein (oxLDL) in the vascular wall is a characteristic feature of atherosclerosis. oxLDL can be taken up into monocytes, smooth muscle cells, and endothelial cells by several known scavenger receptors such as scavenger receptor class A I and II, CD36, and CD68. A new lectin-like oxLDL receptor (LOX-1) was recently found in bovine and human endothelial cells. We studied whether LOX-1 is also expressed in other cells present in the atherosclerotic lesion and whether its expression can be modified. We found LOX-1 expression in human blood monocytes, umbilical smooth muscle and endothelial cells, and 3T3 fibroblasts. LOX-1 mRNA expression in monocytes could be significantly suppressed by lovastatin. Thus, LOX-1 expression is not restricted to endothelial cells and its down-regulation by HMG-CoA reductase inhibitors could contribute to the clinical benefits of these drugs. BIOCHEM PHARMACOL **57**;4:383–386, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. arteriosclerosis; scavenger receptor; oxLDL; LOX-1; lovastatin; monocytes

The generation and accumulation of oxLDL† in the vascular wall is regarded as an important step in atherogenesis. OxLDL, which is no longer a substrate for the feedback-regulated apoB-LDL-R [1], can enter cells by alternative scavenger receptor pathways [2]. The first identified scavenger receptors, class A type I and II (SRC-A I+II) [3–5], are expressed HMDM [6], smooth muscle cells, and fibroblasts [7]. Several other receptors, initially characterized by other functions, have been shown to internalize oxLDL [8–10]. Recently, a new oxLDL receptor with homology to lectins was detected in bovine and human endothelial cells [11]. The expression of this new oxLDL receptor has so far not been clarified in other cell types present in the atherosclerotic lesion. In order to assess whether LOX-1 could contribute to the accumulation of oxLDL in the subendothelial vessel wall, we studied LOX-1 expression in HMDM and smooth muscle cells. We also studied whether LOX-1 expression could be influenced by HMG-CoA reductase inhibitors, which can alter LDL-R [12], SRC-A I+II [13], and CD36 [14] expression.

MATERIALS AND METHODS

Cell culture media, ingredients, and all chemicals were purchased from Sigma. Lovastatin was a kind gift from Merck, Sharp & Dohme. Phenol was from Amresco. Murine leukemia virus reverse transcriptase was from Life Technologies and random hexamer primers were from Boehringer Mannheim. Taq polymerase and the diethylaminoethyl column were from Applied Biosystems. Ficoll was from Biochrom. Falkon Primaria cell culture dishes were from Becton Dickinson.

Cell Culture

Blood from healthy, nonsmoking, and normolipemic male volunteers was collected on sodium citrate (0.16 % w/v final concentration). Peripheral blood mononuclear cells were isolated by Ficoll density-gradient separation [15]. HMDM were separated from other mononuclear cells by adhesion to culture dishes in serum-free medium (RPMI 1640) containing penicillin/streptomycin and 4 mM glutamine for 2 hr at 37°, 95% humidity and 5% CO_2. After the first wash, cells were grown in RPMI medium containing sterile-filtered autologous serum (20%) and either lovastatin (10 μM) or its carrier (DMSO, < 0.1%). The medium was changed after three days. HUASMC prepared from umbilical cords, were cul-

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† *Abbreviations:* HUASMC, human umbilical arterial smooth muscle cells; HUVEC, human umbilical endothelial cells; HMDM, human monocyte-derived macrophages; LDL-R, LDL receptor; LDL, low-density lipoprotein; oxLDL, oxidized LDL; LOX-1, lectin-like oxLDL receptor; RT-PCR, reverse transcriptase polymerase chain reaction; and SRC-A I+II, scavenger receptor class A I and II.

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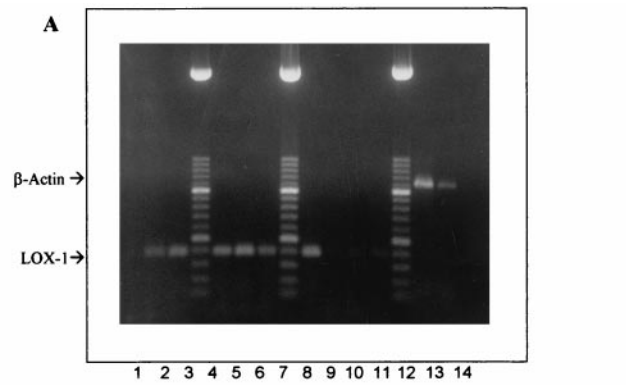
tivated in Waymouth medium MD752/1, containing 15% fetal bovine serum and antibiotics. HUVEC were isolated and cultured as described [16]. The monocytic cell line THP-1, fibroblast cell line 3T3, and epithelial cell line A431 were cultured as in [13, 17, 18], respectively.

Quantitative RT-PCR

Total RNA was isolated from cells grown on 10-cm dishes or in culture flasks as described [17], and quantified on a spectrophotometer (Kontron) at 260 nm. Complementary DNA (cDNA) was reverse transcribed from 0.3 μg of total RNA using murine leukemia virus reverse transcriptase primed with random hexamers. PCR was performed using specific primers for LOX-1 (195 bp) and LDL-R (258 bp), as described in [11] and [14], respectively. Beta-actin (540 bp) served as internal standard [19]. cDNA was amplified using Taq (Thermus aquaticus) polymerase in a Perkin-Elmer Cetus thermocycler 480 (Perkin-Elmer) set to the following profile: 95° melting (5 min), 58° annealing (60 sec), and 72° extension (60 sec) followed by 95° (30 sec), 58° (60 sec) and 72° (60 sec) for 25 cycles and finished by an extension step at 72° for 10 min. Specific mRNA levels were quantified by HPLC separation of the reaction products on a nonporous diethylaminoethyl column with a 0.3 to 0.6 M NaCl gradient, buffered at pH 9.0. The amplificates were quantified by UV detection at 260 nm and integration of corresponding peak areas. LOX-1 and LDL-R mRNA amounts were normalized to levels of β-actin mRNA. Appropriate controls were always coprocessed to exclude unspecific amplification or false-negative results. Samples were also amplified without prior reverse transcription to exclude amplification of potential contaminating genomic DNA. DNA electrophoresis was done in a 1.25% agarose gel stained with ethidium bromide.



B

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                                                TTCAGTTTCT TTACTCTCCA TGGTGGT
1   GCCTGGCTGC TGCGACTCTA GGGGTCCTTT GCCTGGGATT AGTAGTGACC
121 GCCTGGCTGC TGCGACTCTA GGGGTCCTTT GCCTGGGATT AGTAGTGACC

51  ATTATGGTGC TGGGCATGCA ATTATCCCAG GTGTCTGACC TCCTAACACA
181 ATTATGGTGC TGGGCATGCA ATTATCCCAG GTG TCTGACC TCCTAACACA

101 AGAGCAAGCA AACCTAACTC ACCAGAAAAA GAAACTGGaG GGACAGATCT
241 AGAGCAAGCA AACCTAACTC ACCAGAAAAA GAAACTGGAG GGACAGATCT

151 CAGCCCGGCA ACAAGCAGAA GGTCATAGCT GTTTC
301 CAGCCCGGCA ACAAGCAGAA GAAGCTTCAC AGG     AGTCAGAAAA   360
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FIG. 1. (a) Representative specific LOX-1 (195 bp, lanes 1–11) and β-actin (540 bp, lanes 13 and 14) mRNA amplificates separated on an ethidium bromide-stained agarose gel. Lanes 3, 7 and 12: 50 bp ladder, more highly concentrated at 500 and 250 bp; lane 1: HMDM, lovastatin-treated; lane 2: control HMDM; lane 4: HUVEC; lane 5: 3T3 fibroblasts; lane 6: HUASMC; lane 8: control HMDM; lane 9: epithelial A431 cells; lane 10: the same sample as in lane 8 amplified without the RT step; lane 11: the same sample as in lane 4 amplified without the RT step; lane 13: β-actin amplified from HMDM; lane 14: β-actin amplified from A431 cells. (b) Sequence identity between the amplificate obtained from mRNA of HMDM by RT-PCR with LOX-1 primers (upper row) and the cDNA sequence recorded from the EMBL gene bank with the accession No. D89050 (lower row). Annealing sites for primers used are underlined.

RESULTS

As previously described for aortic and carotid endothelial cells [11], we found LOX-1 to be expressed in HUVEC. In addition, LOX-1-specific mRNA was also detected in HUASMC and the human fibroblast cell line 3T3. No expression was detected in the human epithelial cell line A431 and in phorbol 12-myristate 13-acetate-differentiated human THP-1 macrophages, known to express both SRC-A I+II and CD36 (Fig. 1A).

However, HMDM clearly showed expression of LOX-1 mRNA. The identity of the amplificate obtained from HMDM was verified by completely sequencing it (MWG Laboratory). The sequence obtained was 100% identical to that which could be predicted from the published sequence of LOX-1 (EMBL, GenBank) and the primers used (Fig. 1B). No amplificates were obtained in samples amplified without prior reverse transcription, excluding the amplification of potential contaminating genomic DNA.

To study the pharmacological effects of HMG-CoA reductase inhibitors on LOX-1 expression, HMDM were incubated with 10 μM lovastatin for two and five days. Compared to controls, LOX-1 mRNA expression was significantly suppressed to 48% and 69% of control in lovastatin-treated cells on days two and five, respectively (Fig. 2). There was no evidence of a toxic effect of either lovastatin or carrier as assessed by ethidium bromide and acridine orange staining. A consistent but statistically nonsignificant increase in LDL-R mRNA expression in lovastatin-treated cells was also observed. Furthermore, lovastatin also significantly increased the uptake of ^{125}I-labeled native LDL in these cells, rendering a toxic effect of lovastatin very unlikely [20]. Interestingly, LOX-1 expression in control cells decreased between two and five days in culture. Thus, similarly to many other surface proteins and receptors, LOX-1 expression is influenced by the state of differentiation of monocytes in culture.

DISCUSSION

LOX-1, the newly characterized scavenger receptor containing a lectin domain [11], had up to now only been

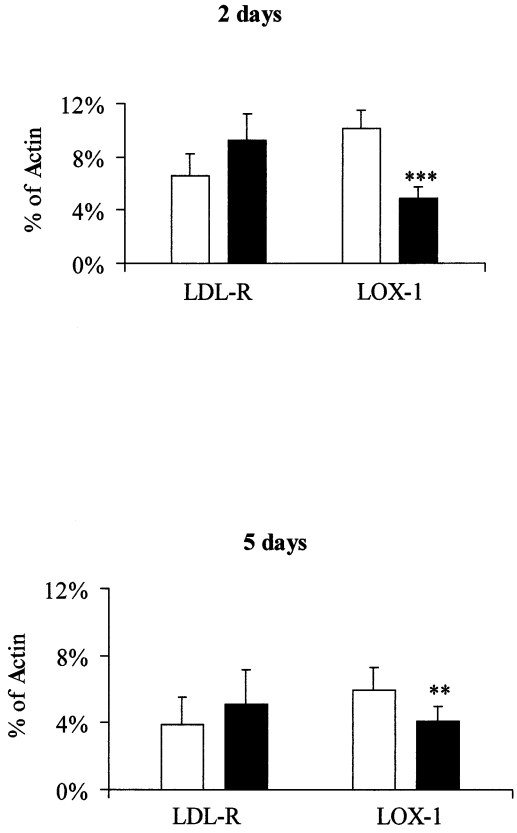

FIG. 2. Specific mRNA expression of LDL-R and LOX-1 in human monocytes, cultured under control conditions (white columns) and in the presence of lovastatin (10 μM) (black columns). Monocytes were prepared by Ficoll separation and incubated for 2 days (top panel, N = 11) or 5 days (bottom panel, N = 9), respectively. mRNA expression was quantified by RT-PCR and ion-exchange HPLC with UV detection of the amplificates (expressed in % of beta-actin). Mean + SEM *: 2P < 0.001; **: 2P < 0.01.**

described in cultured bovine and human endothelial cells and in mRNA extracts of endothelium-rich organs such as lung and liver. In the present study, we found LOX-1 mRNA expressed in two- and five-day-old HMDM, as well as in human umbilical endothelial and smooth muscle cells and in the human 3T3 fibroblast cell line. In contrast, LOX-1 was not expressed in the epithelial cell line A431 and in phorbol 12-myristate 13-acetate-differentiated THP-1 macrophages. Therefore, the distribution of LOX-1 appears to be more widespread, and is not restricted to the endothelial lining of vessels. Its presence in other subendothelial cell types suggests its involvement in lipid accumulation in the vessel wall.

Studies with SRC-A I+II-deficient macrophages indicate the role of several alternative receptors in the cellular uptake of oxLDL [21]. In HMDM, CD68 [22] and CD36 [23] are two major candidates and are both induced by oxidative stimuli such as oxLDL [24]. The binding and degradation of oxLDL by LOX-1 in endothelial cells is in the same order of magnitude as that mediated by SRC-A I+II or CD36 in other cell types and can also be induced by its ligand, oxLDL [11]. A similar up-regulation of LOX-1 expression in HMDM could contribute to continued lipid accumulation in atherosclerotic lesions.

Recent investigations into the clinical benefit of statins suggest further complex mechanisms in addition to a lowering of cholesterol [25]. We and others have shown that these may include a reduction in SRC-A I+II and CD36 expression [13, 14, 20]. Lovastatin also caused a significant decrease in LOX-1 mRNA in HMDM. Lovastatin, therefore, may provide a pharmacological option of limiting endothelial macrophage oxLDL uptake via three different scavenger receptor pathways.

To date, members of at least six protein families-SRC-A, SR-B1, CD36, LOX-1, CD68, and most recently SREC (scavenger receptor expressed by endothelial cells) [26], have been shown to act as multifunctional scavenger receptors. They differ in their cellular distribution and presumably their physiological function. As we found that LOX-1 mRNA levels decrease with macrophage differentiation, LOX-1 may be responsible for uptake of oxLDL in an early stage of this process. The molecular structure of LOX-1, with its lectin-like binding domain and several potential phosphorylatin sites, suggests that this protein may also be involved in cellular functions such as cell–cell interaction, adhesion and transmembrane signaling. The levels of LOX-1 expression in endothelial cells have recently been reported to be increased by hypertension and salt overloading in a rat model [27]. Together with the widespread expression of LOX-1, this might contribute to the epidemiologic link between hypertension and atherosclerosis.

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