Oxidized Cholesteryl Linoleates Stimulate Endothelial Cells to Bind Monocytes via the Extracellular Signal–Regulated Kinase 1/2 Pathway

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Abstract**—**Oxidation products of cholesteryl esters have been shown to be present in oxidized low density lipoprotein and in atherosclerotic lesions. Monocyte adhesion to the endothelium is an initiating crucial event in atherogenesis. Here, we show that in vitro oxidized cholesteryl linoleate (oxCL) stimulated human umbilical vein endothelial cells (HUVECs) to bind human peripheral blood mononuclear cells as well as monocyte-like U937 cells but not peripheral blood neutrophils or neutrophil-like HL-60 cells. Among the oxidation products contained in oxCLs, 9-oxononanoyl cholesterol (9-ONC) and cholesteryl linoleate hydroperoxides stimulated U937 cell adhesion. OxCL-induced U937 cell adhesion was inhibited by an antibody against the connecting segment-1 region of fibronectin. Neither oxCL nor 9-ONC induced activation of the classical nuclear factor-κB pathway. In contrast, stimulation of HUVECs with oxCL resulted in phosphorylation of the extracellular signal–regulated kinase 1/2. Moreover, U937 cell adhesion induced by 9-ONC and oxCL was blocked by a mitogen-activated protein kinase/extracellular signal–regulated kinase inhibitor and a protein kinase C inhibitor. Taken together, oxCLs stimulate HUVECs to specifically bind monocytes, involving endothelial connecting segment-1 and the activation of a protein kinase C– and mitogen-activated protein kinase–dependent pathway. Thus, oxidized cholesteryl esters may play an important role as novel mediators in the initiation and progression of atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2002;22:581-586.)**

Key Words: oxidized lipids ■ cholesteryl linoleate ■ endothelial cells ■ monocyte adhesion ■ atherosclerosis

Oxidative modification of lipids may play a role in the pathogenesis of various diseases, including atherosclerosis, diabetes, cancer, and rheumatoid arthritis, as well as aging.[1] Lipid peroxidation leads to the generation of lipid hydroperoxides,[2] which undergo carbon-carbon bond cleavage, resulting in the formation of short-chain unesterified aldehydes[3,4] and of aldehydes still esterified to the parent lipid, termed core aldehydes.[5–7]

Cholesteryl linoleate (CL) has been shown to be the major cholesteryl ester contained in LDL[8] and atherosclerotic lesions.[9] In oxidized lipoproteins and advanced human atherosclerotic plaques, 9-oxononanoyl cholesterol (9-ONC) was found to be one of the most abundant oxidation products derived from CL.[5,7,9–11] Moreover, 9-ONC was detected after decomposition of CL-hydroperoxides,[6,9,10,12] which were found in plasma from healthy humans[13] and in human atherosclerotic plaques.[14,15] However, little is known about the (patho)physiological role of oxidation products derived from CL.

Other forms of oxidized lipids, such as oxidized phospholipids or isoprostanes, were shown to specifically induce monocyte-endothelial interactions,[16–18] an initiating event in the development of the atherosclerotic plaque.[19] Activation of mitogen-activated protein (MAP) kinases rather than the classical nuclear factor (NF)-κB pathway by oxidized phospholipids or isoprostanes seems to mediate specific monocyte adhesion to endothelial cells (ECs).[16,20]

In the present study, we show that oxidation products of CL stimulate human umbilical vein ECs (HUVECs) to specifically bind human peripheral blood mononuclear cells and monocyte-like U937 cells. The activation of HUVECs leading to U937 cell binding was dependent on protein kinase C, extracellular signal–regulated kinase (ERK) 1/2, and MAP kinase/ERK kinase (MEK)-1/2 but not on the NF-κB pathway. Thus, we suggest that oxidized cholesteryl esters represent novel mediators that may promote chronic inflammatory processes, such as atherosclerosis.

Methods

Reagents

CL, dimethyl sulfoxide (DMSO), butylated hydroxytoluene, medium 199, E-Toxate (*Limulus* amebocyte lysate), and *O*-phenylene diamine

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were purchased from Sigma Chemical Co. CL-hydroperoxides, 13*R*-hydroxy-9*Z*,11*E*-octadecadienoic acid (13R-HODE) cholesteryl ester and 9*R*-hydroxy-10*E*,12*Z*-octadecadienoic acid (9R-HODE) cholesteryl ester were from Cayman Chemical; oleic acid and cholesterol were from Aldrich Chemical Co; *N*,*N*′-dicyclohexyl-carbodiimide was from Fluka AG; and PD098059 and bisindolylmaleimide I were obtained from Calbiochem. Tumor necrosis factor (TNF)-α was from Boehringer-Mannheim; mouse anti-human E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 antibodies (all IgG) were from R&D Systems; U937 cells and HL-60 cells were from American Type Culture Collection; supplemented calf serum was from HyClone; polyclonal rabbit antibodies against nonphosphorylated and phosphorylated ERK 1/2 and LumiGLO were from New England BioLabs; and polyclonal rabbit antibodies against nonphosphorylated and phosphorylated IκBα were from Santa Cruz Biotechnology. Peroxidase-conjugated secondary anti-rabbit and anti-mouse antibodies were from Amersham Pharmacia Biotech. Anti-human connecting segment-1 (CS-1) and anti–*Treponema pallidum* antibodies (both IgM) were gifts from Dr Judith Berliner (Department of Cardiology, University of California at Los Angeles, Calif) and Dr Otto Majdic (Department of Immunology, University of Vienna, Austria), respectively.

Tissue Culture

HUVECs were prepared and cultured as described previously.[21] HUVECs were used for experiments at passages 2 to 5.

Oxidation of CL

OxCL was prepared by exposing dried CL to air at 100°C for 45 minutes[10] or to air at room temperature for 7 days. Each preparation showed a reproducible stimulation of HUVECs to bind peripheral blood mononuclear cells and monocyte-like U937 cells and was characterized by high-performance liquid chromatography as described.[9]

Synthesis of 9-ONC

9-ONC was prepared by ozonolysis of oleic acid and subsequent condensation of the resulting 9,9-dimethoxy nonanoic acid with cholesterol in the presence of *N*,*N*′-dicyclohexyl-carbodiimide as described previously.[22]

Reduction of OxCL and 9-ONC by Using $NaBH_4$

OxCL and 9-ONC were reduced as described before.[23] Briefly, 60 μg of oxCL and CL-hydroperoxides or 27 μg of 9-ONC dissolved in DMSO was suspended in 0.5 mL of a 0.1-mol/L borate buffer and treated with $NaBH_4$ at room temperature for 30 minutes. Lipids were extracted by the addition of chloroform/methanol (2:1 [vol/vol]) supplemented with 0.01% butylated hydroxytoluene,[23,24] then dried under a stream of nitrogen, and dissolved in DMSO before suspension in media and the subsequent addition to HUVECs for experiments.

All lipid preparations used for the tissue culture experiments were examined for endotoxin content by *Limulus* amebocyte lysate assay (Sigma). Only preparations containing <50 pg/mL endotoxin were used.

Leukocyte Adhesion Assays

Adhesion assays were performed as described previously.[25] Human peripheral blood mononuclear cells were isolated by using Ficoll-Paque Plus (Amersham Pharmacia Biotech) and Leucosep tubes (Greiner) as described by the manufacturer. Human peripheral blood neutrophils were isolated as described previously.[26] Confluent HUVECs in 48-well plates were incubated with lipids for 4 hours at 37°C. Lipids were dissolved in DMSO before suspension in medium 199 containing 10% supplemented calf serum. In some experiments, HUVECs were pretreated in the presence/absence of inhibitors for 30 minutes and then stimulated with lipids in the presence/absence of inhibitors for 4 hours. After incubation, HUVECs were washed, and a suspension of unstimulated monocyte-like U937 cells, neutrophil-like HL-60 cells, isolated peripheral human blood mononuclear cells, or neutrophils (10^5 cells per well) was added to HUVECs for 15 minutes. U937 cells and HL-60 cells used in the adhesion assays were shown to behave like human monocytes and neutrophils, respectively.[16,25,27] For blocking experiments, HUVECs were treated for 30 minutes with 8 μg/mL of either a monoclonal IgM against the CS-1 region of fibronectin or an irrelevant IgM against *Treponema pallidum* before the addition of U937 cells.[28] Nonadherent leukocytes were removed by washing, and adherent cells were counted microscopically.

Cell ELISA

Confluent HUVECs in 96-well plates were treated with lipids as described above for 4 hours at 37°C. The assay was then performed as described previously.[29]

In all leukocyte adhesion assays and ELISA experiments, TNF-α (20 U/mL) was used as a positive control and yielded a reproducible 3- to 5-fold increase compared with cells treated with medium containing DMSO (control).

Western Blotting

After stimulation, HUVECs were lysed in Laemmli buffer, and proteins were separated by electrophoresis in 10% SDS-polyacrylamide gels. Proteins were transferred onto polyvinylidene difluoride membranes (Millipore), blocked with 5% dry milk/0.1% Tween 20, and incubated with primary antibodies in the same solution. Bound antibodies were incubated with secondary anti-IgG conjugated with peroxidase and subsequently detected by chemiluminescence (LumiGLO).

Statistical Analysis

Results are expressed as mean±SEM. Statistical analysis was performed by using 1-way ANOVA. A value of $P<0.05$ was considered statistically significant.

Results

OxCL Stimulates ECs to Bind Peripheral Blood Mononuclear Cells and Monocyte-Like U937 Cells

In vitro oxidation of CL generates a mixture of oxidation products (oxCLs).[10] To examine whether these products could influence leukocyte-endothelial interactions, we used a leukocyte adhesion assay in which HUVECs were stimulated for 4 hours at 37°C. OxCL stimulated the HUVECs to bind increased levels of peripheral blood mononuclear cells (Figure 1A). In addition, oxCL, but not native CL, dose-dependently stimulated HUVECs to bind monocyte-like U937 cells (Figure 1B). At concentrations that were effective in stimulating peripheral blood mononuclear and U937 cell adhesion, oxCL and native CL did not stimulate HUVECs to bind human peripheral blood neutrophils or neutrophil-like HL-60 cells (Figure 1C). Because of similar behavior in adhesion experiments, monocyte-like U937 cells and neutrophil-like HL-60 cells were used as models for peripheral blood monocytes and neutrophils, respectively.[16,27]

Characterization of Biologically Active Component(s) Contained in OxCL

Oxidation of CL leads to the formation of a substantial amount of 9-ONC, among other oxidation products.[9,10] To identify the active components contained in oxCL, several possible oxidation products were tested individually. Although 9-ONC and CL-hydroperoxides stimulated U937 cell adhesion, 9R-HODE cholesteryl ester and 13R-HODE cholesteryl ester (Figure 2A) and 9S-HODE- and 13S-HODE cholesteryl esters (data not shown) were not active. Resem-

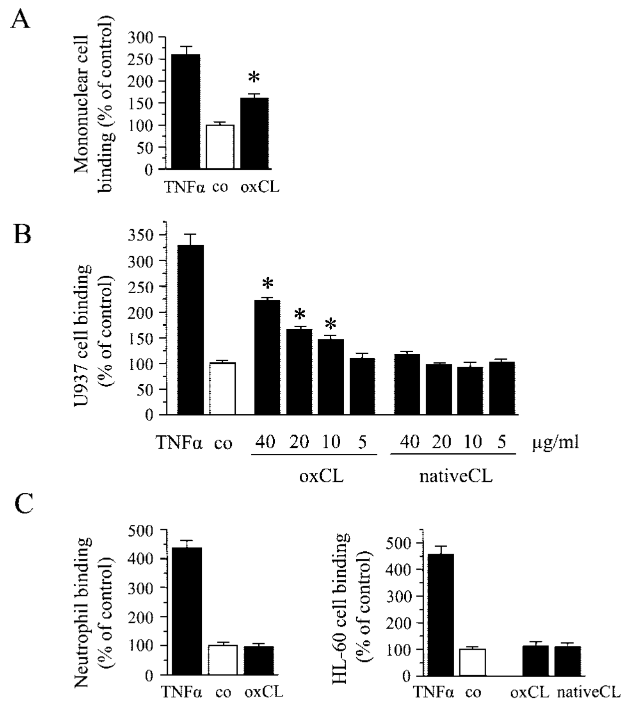

Figure 1. OxCL but not native CL activates HUVECs to bind peripheral blood human mononuclear cells and monocyte-like U937 cells. A, HUVECs were treated with oxCL (40 μg/mL), and the binding of unstimulated human peripheral blood mononuclear cells was determined. B, HUVECs were treated with oxCL or native CL at the indicated concentrations, and the binding of unstimulated monocyte-like U937 cells was determined. C, HUVECs were treated with oxCL or native CL (both 40 μg/mL), and the binding of unstimulated human peripheral blood neutrophils or of neutrophil-like HL-60 was determined. HUVECs were incubated with agonists for 4 hours at 37°C in 48-well plates in triplicate. Adhesion assays were performed as described in Methods. TNF-α was used as a positive control, and cells treated with DMSO were used as a negative control (co). Data are expressed as mean$\pm$SEM and are representative of 3 independent experiments (A through C). *$P<0.05$ compared with co.

bling the effects of oxCL, 9-ONC stimulated HUVECs to bind monocyte-like U937 cells (Figure 2B) but not neutrophil-like HL-60 cells (data not shown).

Reducible functional groups were shown to play an important role in the biological activity of oxidized lipids.[23] Therefore, we tested whether chemical reduction of oxCL, CL-hydroperoxides, or 9-ONC would affect their ability to stimulate U937 cell–EC interactions. Lipids were treated with sodium borohydride, which is well documented to reduce hydroperoxides, epoxides, aldehydes, and ketones to hydroxides. U937 cell adhesion to HUVECs induced by oxCL, CL-hydroperoxides, and 9-ONC was significantly inhibited after reduction with sodium borohydride (Figure 2C).

Mechanism That Leads to Specific Monocyte Binding

The adhesion of leukocytes to the endothelium requires increased expression of specific adhesion molecules on the surface of ECs.[30] Thus, we examined the expression of E-selectin, ICAM-1, and VCAM-1 by whole-cell ELISA after stimulation of HUVECs with oxCL, native CL, and 9-ONC. Neither oxCL, 9-ONC, nor native CL induced the expression of E-selectin, ICAM-1, or VCAM-1 on the surface of HUVECs at concentrations that were effective to induce monocyte-endothelial interactions (data not shown). The biological activity of oxCL and 9-ONC was reproducibly confirmed by U937 cell adhesion experiments that were performed in parallel (data not shown).

The adhesion of monocytes to ECs is also mediated by the CS-1 domain of fibronectin, which was recently described to be expressed on the surface of ECs after stimulation with oxidized lipids.[28,31] To determine the role of CS-1–containing fibronectin in oxCL-induced monocyte adhesion, HUVECs stimulated with oxCL (40 μg/mL) were treated either with a monoclonal antibody against the CS-1 region of fibronectin or an irrelevant antibody before the addition of U937 cells. OxCL-stimulated HUVECs that had been treated with an antibody against the CS-1 region of fibronectin showed a significant ($P<0.05$) reduction in monocyte-like U937 cell binding (control 100$\pm$8%, oxCL 202$\pm$25%, oxCL+CS-1 antibody 130$\pm$19%, and oxCL+irrelevant antibody 189$\pm$18%; data are representative of 3 independent experiments and are expressed as percentage of control$\pm$SEM). The antibodies did not influence U937 cell

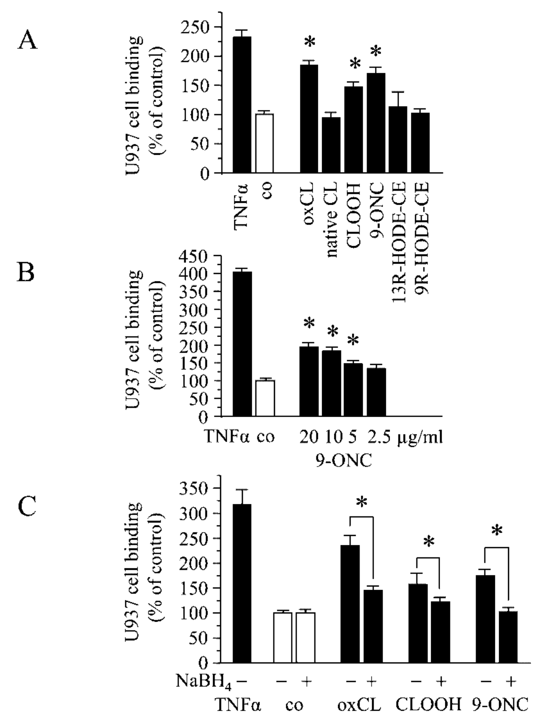

Figure 2. Characterization of biologically active components in oxCL. A, HUVECs were stimulated with oxCL, native CL, and CL-hydroperoxides (CLOOH, all 20 μg/mL) and 9-ONC, 13R-HODE cholesteryl ester (13R-HODE-CE), and 9R-HODE-CE (all 10 μg/mL) for 4 hours at 37°C. Monocyte-like U937 cells were then added. B, HUVECs were stimulated with 9-ONC at the indicated concentrations for 4 hours at 37°C before addition of monocyte-like U937 cells. C, OxCL, CLOOH, and 9-ONC were either reduced with $NaBH_4$ or treated with buffer only. HUVECs were then treated with either reduced or nonreduced lipids for 4 hours at 37°C before addition of monocyte-like U937 cells. For panels A through C, experiments were performed in 48-well plates in triplicate. TNF-α was used as a positive control, and cells treated with DMSO were used as a negative control (co). Data are expressed as mean$\pm$SEM. *$P<0.05$ compared with co (A and B) or as indicated (C). Data are representative of 3 independent experiments (A and B) or 2 independent experiments (C).

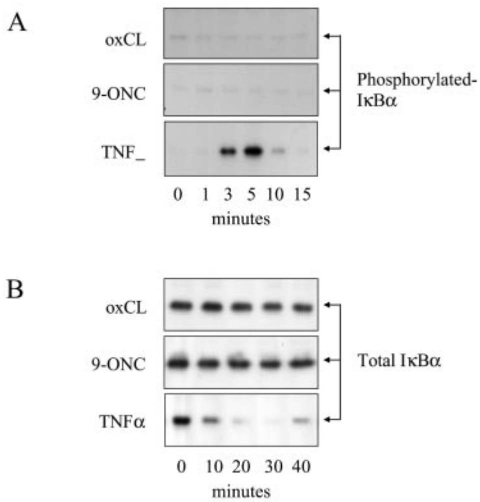

Figure 3. Neither IκBα degradation nor IκBα phosphorylation was induced by oxCL or 9-ONC. HUVECs were treated with TNF-α (200 U/mL), oxCL (40 μg/mL), or 9-ONC (10 μg/mL) for the indicated time periods. After the blotting procedure, membranes were stained for phosphorylated IκBα (A) or total IκBα (B). Data are representative of 2 independent experiments.

binding to untreated cells or to cells treated with TNF-α (data not shown).

OxCL and 9-ONC Do Not Activate NF-κB but a MAP Kinase–Dependent Signaling Pathway

Stimulation of ECs by various proinflammatory agonists leads to phosphorylation and degradation of IκBα, which then allows translocation of NF-κB to the nucleus (see review[32]). To investigate whether oxCL or 9-ONC caused activation of NF-κB, cellular protein extracts of stimulated HUVECs were stained for phosphorylated and total IκBα after blotting. Neither oxCL nor 9-ONC led to IκBα phosphorylation (Figure 3A) or IκBα degradation (Figure 3B), in contrast to TNF-α, which was used as a positive control.

ERK 1/2 kinase–mediated signaling was shown to play an important role in ECs stimulated with oxidized lipids.[16,33] To determine whether treatment of HUVECs with oxCL leads to phosphorylation of ERK 1/2, HUVECs were stimulated with oxCL, and phosphorylation of ERK 1/2 was detected by Western blotting. Within 20 minutes, oxCL stimulated the phosphorylation of ERK 1/2, which was reversed after 40 minutes (Figure 4A). TNF-α, which was used as a positive control, also induced a reversible phosphorylation of ERK 1/2 that began after 10 minutes of incubation (Figure 4A). Staining for nonphosphorylated ERK 1/2 was used to confirm equal loading (data not shown).

The activation of ERK 1/2 depends on upstream MEK 1/2 and may require activation of protein kinase C.[34] To determine the functional role of MEK 1/2 and protein kinase C, HUVECs were treated in the presence or absence of a specific MEK 1/2 or protein kinase C inhibitor (PD098059 and bisindolylmaleimide I, respectively). OxCL-induced phosphorylation of ERK 1/2 was reduced in the presence of bisindolylmaleimide I and PD098059 (Figure 4B). In addition, monocyte-like U937 cell binding induced by oxCL and 9-ONC was blocked by PD098059 (Figure 5A) and bisindolylmaleimide I (Figure 5B). Taken

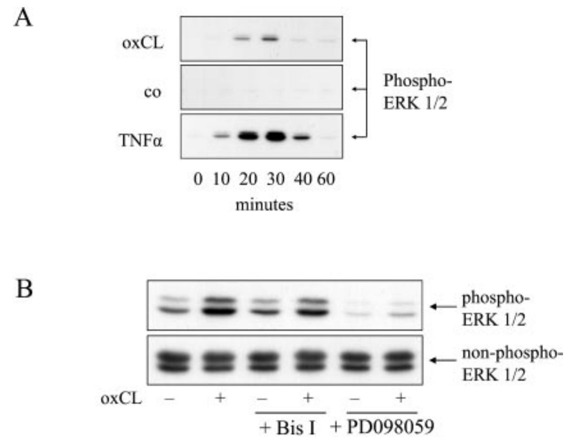

Figure 4. Intracellular signaling induced by oxCL and 9-ONC. A, HUVECs were treated with oxCL (40 μg/mL), TNF-α (200 U/mL), or medium containing DMSO (co) for the indicated time periods at 37°C. After the blotting procedure, membranes were stained for phosphorylated ERK 1/2 (phospho-ERK 1/2). Similar results were obtained in 3 different experiments. B, HUVECs were preincubated in the presence or absence of bisindolylmaleimide I (Bis I, 20 μmol/L) or PD098059 (10 μmol/L) and then stimulated with oxCL (60 μg/mL) in the presence or absence of respective inhibitors for 20 minutes at 37°C. After the blotting procedure, membranes were stained for phospho-ERK 1/2 and nonphosphorylated ERK 1/2 (non-phospho-ERK 1/2). Results were obtained in 3 different experiments.

together, these data indicate that stimulation of HUVECs leading to monocyte adhesion involved the activation of protein kinase C, MEK 1/2, and ERK 1/2.

Discussion

Many lines of evidence suggest that LDL is transported into the arterial wall and becomes atherogenic as a result of oxidation.[19,35,36] Oxidized lipids in oxidized LDL, including isoprostanes and oxidized phospholipids, have been detected in human atherosclerotic lesions[23,37] and have been shown to

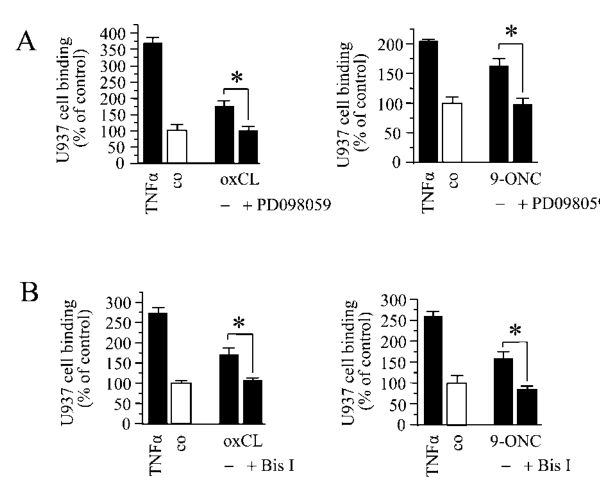

Figure 5. A and B, HUVECs were preincubated in the presence or absence of PD098059 (10 μmol/L, A) or Bis I (25 μmol/L, B) for 30 minutes and then stimulated with oxCL (40 μg/mL) or 9-ONC (10 μg/mL) in the presence or absence of inhibitors for 4 hours at 37°C. U937 cell-binding experiments were performed in 48-well plates in triplicate. Data show mean±SEM of 3 independent experiments. *$P<0.05$.

stimulate ECs to bind monocytes.[16–18,38] Adherent monocytes subsequently transmigrate into the subendothelial space and mature into macrophages, an important process in atherogenesis.[19,35] There is increasing evidence of the presence of oxidation products of cholesteryl esters, such as oxCL, in atherosclerotic lesions.[9,10] OxCL contains core aldehydes, ie, cholesteryl or oxysteryl esters of 9-oxononanoate, as the most abundant components.[10] In the present study, we show that oxCL, but not native CL, stimulates HUVECs to bind U937 cells, which are used as a model for peripheral blood monocytes, implicating a pathophysiological role for oxidized cholesteryl esters in atherogenesis. We further show that at least 2 different components contained in oxCL, ie, CL-hydroperoxides and 9-ONC, stimulate HUVECs to bind monocyte-like U937 cells. However, cholesteryl ester hydroperoxides decompose into cholesteryl ester–core aldehydes,[6,9] such as 9-ONC. Thus, the biological activity of CL-hydroperoxides may also be attributed to fragmentation products that are due to further oxidation. Whether the biological activity of CL-hydroperoxides was due to reactive oxygen species derived from hydroperoxides was not determined in the present study.

We hypothesized that functional groups present in oxCL were responsible for the biological activity. Reduction of oxCL and CL-hydroperoxides with sodium borohydride resulted in a partial decrease of biological activity, evidenced by decreased induction of monocyte-like U937 cell adhesion to ECs. These findings are in agreement with data from a previous study showing that reduction of oxidized phospholipids decreased biological activity by 60% to 70%.[23] Remaining stimulatory effects were probably due to biological activity of nonreducible oxidation products or due to further oxidation of lipids during incubation with HUVECs. Reduction of 9-ONC completely abolished its ability to induce U937 cell adhesion, indicating that reduction of the aldehyde resulted in complete loss of activity. Recently, it was demonstrated that enzymes such as paraoxonase 1 or aldose reductase lead to reduction of oxidized cholesteryl esters or phospholipid core aldehydes.[39,40] Thus, the reductive activity of these enzymes may be potentially protective by decreasing the activity of oxidized cholesteryl esters.

An important characteristic of atherosclerotic lesions is that they contain essentially no neutrophils.[41] We demonstrate that oxCL and 9-ONC specifically induce monocyte and monocyte-like U937 adhesion, whereas neutrophil and neutrophil-like HL-60 cell adhesion was not induced. Furthermore, the expression of adhesion molecules E-selectin, VCAM-1, and ICAM-1, which are important for the interaction of ECs with leukocytes,[30] was increased neither by oxCL nor by 9-ONC. However, a splice product of fibronectin, CS-1, which interacts with very late antigen-4 on monocytes, was recently described to be expressed on ECs after stimulation with oxidized lipids.[28,31,42] In the present study, we show that treatment of oxCL-stimulated HUVECs with an antibody against the CS-1 region of fibronectin strongly inhibits the binding of monocyte-like U937 cells, indicating a role for this adhesion molecule in this interaction.

NF-κB is highly activated at sites of inflammation in various diseases.[43,44] It has been proposed that oxidized lipoproteins can activate the NF-κB pathway in ECs.[45,46] Yet, we have clearly demonstrated that stimulation of HUVECs with oxCL or 9-ONC does not result in activation of the NF-κB pathway, as shown by a lack of IκBα phosphorylation and degradation. However, we have demonstrated that stimulation of HUVECs with oxCL leads to phosphorylation of ERK 1/2. Inhibition of protein kinase C and MEK 1/2, which have been shown to act upstream from ERK 1/2,[34] blocked oxCL-induced and 9-ONC–induced monocyte-like U937 cell adhesion to HUVECs. These results indicate that the activation of HUVECs by oxCL and 9-ONC is mediated via a protein kinase C/MEK 1/2/ERK 1/2–dependent signaling pathway. However, increased activity of additional kinases and/or other members of the MAP kinase family[47] cannot be excluded from playing a role in the oxCL–induced and 9-ONC–induced activation of HUVECs.

Homogenates of human atherosclerotic plaques contain very large amounts of oxidized lipids, with $\approx$30% of the fatty acid moiety of CL being present in oxidized forms.[14] The most abundant cholesteryl ester quantified in advanced lesions was CL, with mean concentrations of $\approx$0.50 mol/mol cholesterol.[9] In each of these plaque samples, 9-ONC was analyzed at mean concentrations of 29 μmol/mol cholesterol, corresponding to 60 μmol/mol CL or 11 nmol/g lipid extract, respectively.[9] Because these data represent normalized mean values, local concentrations of oxidized cholesteryl esters could be severalfold higher in areas of the lesion in which lipids are concentrated. In addition, endothelial activation by oxidized lipids is most likely due to an additive effect of a variety of oxidation products derived from several cholesteryl esters with different polyunsaturated fatty acids, as well as other biologically active lipid oxidation products.

Transportation of these rather hydrophobic lipids to the endothelium and subsequent stimulation thereof remains speculative. Oxidation products of CL have been shown to bind to serum proteins[5,10] or to interact with phospholipids.[9] In the core region of lesions, cholesterol-rich and cholesteryl ester–rich lipid deposits form.[48] Moreover, oxidized cholesteryl esters have been shown to be resistant to hydrolysis in macrophages.[10] Thus, intact oxidized cholesteryl esters may be released into the extracellular space as a result of apoptosis and necrosis of foam cells,[49] thereby directly stimulating the endothelium overlying the plaque.

Taken together, our findings indicate that oxidized cholesteryl esters, such as oxCL or one of its core aldehydes, ie, 9-ONC, represent a novel class of oxidized lipids that are capable of stimulating the endothelium to bind monocytes and, thus, contributing to the progression of the inflammatory process in atherosclerosis.

Acknowledgments

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