FEBS Letters 471 (2000) 34–38 FEBS 23502

Oxidized phospholipids activate PPARα in a phospholipase A2-dependent manner

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Received 1 March 2000

Edited by Marco Baggiolini

Abstract The peroxisome proliferator-activated receptor α (PPARα) is a transcription factor belonging to the PPAR subfamily of nuclear receptors. Fatty acids and eicosanoids are natural PPARα ligands. Here, we show using transient transfection assays that oxidized (oxLDL) but not native low-density lipoproteins (LDL) dose-dependently activate PPARα in endothelial cells without affecting PPARα protein expression. Fractioning of oxLDL lipids followed by transactivation experiments demonstrated that the oxidized phospholipid component in oxLDL is responsible for PPARα activation. Using specific inhibitors, it is shown that oxLDL-mediated PPARα activation requires phospholipase A2 activity and that the oxidized fatty acids 9- and 13-HODE activate PPARα directly. Finally, we found that, similar to the synthetic PPARα ligand Wy-14643, oxLDL induced expression of the fatty acid transport protein-1 in human primary endothelial cells. Our findings define a novel group of PPARα activators and provide a molecular basis for certain effects of these biologically active phospholipids on gene transcription.

Key words: Peroxisome proliferator-activated receptor; Phospholipid; Phospholipase A2; Oxidized low-density lipoprotein

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors which belong to the nuclear receptor superfamily [1]. Three different PPAR subtypes have been identified to date: PPARα, PPARδ and PPARγ. PPARα and PPARγ are the best characterized PPARs. PPARα stimulates the β-oxidative degradation of fatty acids whereas PPARγ promotes lipid storage via its effects on adipocyte differentiation and function (see [2] for review). Recently, PPARα and PPARγ have also been shown to play a role in inflammatory control (see [2,3] for review).

PPARs regulate gene expression by binding with the 9-*cis*-retinoid X receptor (RXR) as heterodimeric partner to specific DNA sequence elements termed PPAR response elements (PPRE) [4]. In addition, PPARs regulate gene transcription in a DNA binding-independent manner by negatively interfering with the AP-1 and NF-κB transcriptional activities [5]. The lipid-lowering fibrates and the anti-diabetic glitazones are synthetic ligands for, respectively, PPARα and PPARγ [6,7]. Long chain fatty acids such as linoleic, linolenic and arachidonic acid [6,8–10] have been identified as natural PPAR activators which bind and activate both PPARα and PPARγ. PPARs are furthermore activated by fatty acid derivatives. A cyclooxygenase product of arachidonic acid, 15d-Δ12,14-PGJ2, has been identified as a selective PPARγ ligand [11,12] whereas its lipoxygenase product, 8(*S*)-hydroxy-(5*Z*,9*E*,11*Z*,14*Z*)-eicosatetraenoic acid (HETE), is a stereoselective PPARα agonist [6,8,9]. Moreover, Devchand et al. [13] showed that the pro-inflammatory arachidonic-acid derivative, LTB4, is a PPARα ligand. Recently, it has been shown that oxidized low-density lipoproteins (oxLDL) regulate macrophage gene expression through ligand activation of PPARγ [14]. By screening oxidized sterols and fatty acids contained in the oxLDL particle, Nagy et al. identified two oxidized metabolites of linoleic acid, 9- and 13-hydroxyoctadecadienoic acid (9-HODE and 13-HODE), as potent PPARγ activators [14]. Finally, Huang et al. found that interleukin-4 (IL-4)-induced PPARγ activity in macrophages requires the 12/15 lipoxygenase pathway which catalyzes the conversion of arachidonic acid and linoleic acid to generate the PPARγ ligands 15-HETE and 13-HODE, respectively [15]. However, to date it is unknown whether any of these oxLDL-derived fatty acids also affect PPARα activity.

The aim of this study was therefore to determine the effects of oxLDL on PPARα activity in human endothelial cells which express high levels of PPARα compared to PPARγ [16]. Our results of transient transfections demonstrate that oxLDL induce PPARα activity in a dose-dependent manner without affecting its protein expression. Furthermore, by LDL lipid fractioning, oxidized phospholipids were identified as PPARα activators. Using specific inhibitors it was demonstrated that phospholipase A2 (PLA2) but not lipoxygenases nor cyclooxygenases are required for this activation. Moreover, 9- and 13-HODE were found to activate PPARα in transient transfection experiments. Finally, we demonstrate that oxLDL, as well as the synthetic PPARα ligand Wy-14643, regulate the expression of the PPAR target gene, fatty acid transport protein-1 (FATP-1), in human primary endothelial cells.

2. Materials and methods

2.1. Cell culture and chemical reagents

Human coronary artery endothelial cells (CAEC) (Clonetics), cultured in endothelial cell basal medium supplemented with 5% fetal calf serum (FCS) and various growth factors as described by the

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PII: S0014-5793(00)01364-8

manufacturer, were used for RNA and Western blot analysis. Murine aortic endothelial cells (MAE) (ATCC, Rockville, MD, USA), cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS, 600 ng/ml glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, were used for the transient transfection experiments.

Nordihydroguaiaretic acid (NDGA) and 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC) were provided by Sigma, Saint-Quentin, France. Ketoprofen was from Rhône-Poulenc Rorer, Vitry, France. Arachidonyltrifluoromethyl ketone (AACOCF3) was from Euromedex, Strasbourg, France and 9- and 13-HODE were from Cayman Research. The goat polyclonal PPARα antibody was from Research Diagnostics, Flanders, NJ, USA.

2.2. Protein extraction and Western blot analysis

CAEC cells were washed twice in ice cold phosphate buffered saline (PBS) and harvested in ice cold lysis buffer containing PBS, 1% Triton X-100 and a freshly prepared protease inhibitor cocktail (ICN, Orsay, France) (10 mg/ml 4-(2-aminoethyl)-benzenesulphonyl fluoride (AEBSF), 1 mg/ml leupeptin, 1 mg/ml pepstatin, 5 mg/ml EDTA-Na_2) to which 1 mM phenylmethylsulfonyl fluoride was added. Cell homogenates were collected by centrifugation at 13 000 rpm at 4°C. Protein concentrations were determined using the bicinchoninic acid assay (Pierce Interchim, Montlucon, France). Electrophoresis of the indicated amount of protein lysate was performed through a 10% polyacrylamide gel under reducing conditions (sample buffer containing 10 mM dithiothreitol). Proteins were transferred onto nitrocellulose membranes and membranes were checked for equal loading by Ponceau red staining. Non-specific binding sites were blocked overnight at 4°C with 10% skim milk powder in TBST (20 mM Tris–HCl, 55 mM NaCl, 0.1% Tween 20). Membranes were subsequently incubated for 4 h at room temperature in 5% skim milk–TBST containing a goat polyclonal PPARα antibody. After incubation with a secondary peroxidase conjugated antibody, signals were visualized by chemiluminescence (Amersham, Buckinghamshire, UK).

2.3. RNA analysis

RNA preparation and Northern blot hybridizations were performed as previously described [17]. FATP-1 [18] and 36B4 cDNA fragments were used as probes.

2.4. Lipoprotein isolation and oxidation

LDLs ($d = 1.03$–1.053) were isolated from freshly drawn blood from healthy normolipidemic volunteers, dialyzed, sterilized and stored at 4°C as previously described [19]. The protein concentration was determined by using Peterson's method with bovine serum albumin as standard. LDLs (1 mg protein/ml) were oxidized with 1.66 mM $CuSO_4$ at 37°C for 24 h. Native and oxLDL were screened for lipopolysaccharide (LPS) contamination by using a limulus lysate assay. All the different LDL preparations used in this study contained less than 0.75 IU of LPS/ml. LDL contained 20 ± 4 nmol peroxides/mg protein and 0.163 nmol TBARS (thiobarbituric acid-reactive substances)/mg protein whereas oxLDL contained 215.2 ± 32 nmol peroxides/mg protein and 46 ± 4 nmol TBARS/mg protein.

2.5. Lipid extraction

Total lipid extracts of 1 ml of native or oxLDL (1 mg protein/ml) were extracted with 2 ml of $CHCl_3$–MeOH (2:1, v/v) containing 0.01% butylated hydroxytoluene, the pooled organic fractions were evaporated to dryness under nitrogen and the residue was dissolved in 100 μl of MeOH (for use on cells) or in 100 μl of hexane for further separation. Fractionation of the total lipid extract in hexane was accomplished with Bakerbond spe Diol (Baker) extraction columns (3 ml) preconditioned by washing with hexane (5 ml) [20,21]. The column was eluted with 2 ml of increasingly polar solvent mixtures to give eight fractions: fraction 1, 1% ethyl acetate in hexane; fraction 2, 5% ethyl acetate in hexane; fraction 3, 15% ethyl acetate in hexane; fraction 4, 30% ethyl acetate in hexane; fraction 5, 60% ethyl acetate in hexane; fraction 6, 100% ethyl acetate; fraction 7, 30% MeOH in CH_2Cl_2 and fraction 8, 100% MeOH. The eluates were evaporated to dryness and reconstituted in 100 μl of MeOH. Fraction 1 contained esterified cholesterol, fraction 4 triglycerides and free cholesterol and fraction 7 phospholipids. In some studies, LDL solutions were reduced with sodium borohydride (10 mg) [22], the samples were kept on ice for 20 min and then at room temperature for a further 100 min. At this time, 20 μl glacial acetic acid was added followed by 1 ml water. The non-reduced samples were subjected to the same conditions except that sodium borohydride was omitted. Oxidized PAPC (oxPAPC) was obtained by transferring 1 mg PAPC in 100 μl of chloroform and evaporating the solvent under a stream of nitrogen. The lipid residue was allowed to autoxidize on exposure to air for 48 h.

2.6. Plasmids and transient transfection assays

The plasmids pGal5-TK-pGL3, J6-TK-pGL3, Gal4-hPPARα and pSG5-hPPARα have been previously described [5]. MAE cells, grown in 24-well plates to 50–60% confluence in DMEM supplemented with 10% FCS, were transiently transfected using a lipid cationic technique (RPR120535B, Rhône-Poulenc Rorer, Vitry, France) with reporter and expression plasmids, as stated in the figure legends. Phosphoglycerate kinase (PGK) β-galactosidase expression plasmid (50 ng) was cotransfected as a control for transfection efficiency. Two hours post-transfection, the medium was refreshed and supplemented with the various PPAR activators as stated in the figure legends. After 48 h, cells were collected and the luciferase and β-galactosidase assays were performed as previously described [5]. Reporter gene activity is presented as fold induction over vehicle control. All the experiments were performed at least three times.

2.7. Cell viability assay

Cell viability was determined by using the Celltiter 96 Aqueous one solution cell proliferation assay (Promega, Charbonnières, France) based on the bioreduction of a tetrazolium compound (MTS) into a colored formazan product. The quantity of formazan product measured at 490 nm is directly proportional to the number of living cells.

3. Results

3.1. oxLDL activate PPARα in a dose-dependent manner

To determine whether oxLDL modulate PPARα-dependent transcription, MAE cells were transfected with a multiple PPRE-driven promoter construct in the presence or absence of PPARα and RXRα (Fig. 1A). In the absence of cotransfected PPARα, LDL and oxLDL (100 μg/ml) did not influence PPRE-driven reporter activity which is consistent with the absence of endogenous PPARα in MAE cells (data not shown). Cotransfection of PPARα led to a two-fold increase of basal promoter activity which was not affected by LDL treatment. oxLDL incubation resulted in a significant (seven-fold) increase of the promoter activity. Cotransfection of both PPARα and RXRα led to a drastic induction (almost 30-fold) of reporter activity. LDL failed to activate the

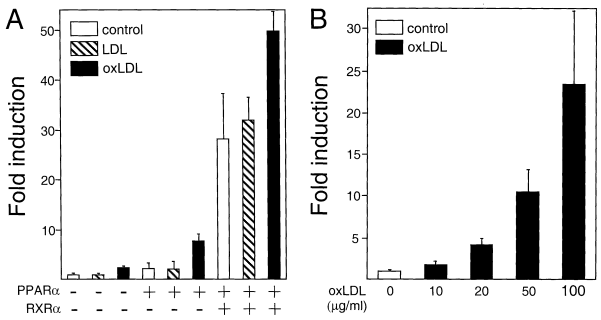

Fig. 1. oxLDL activate PPARα in a dose-dependent manner. A: MAE cells were transfected with J6-TK-pGL3 (100 ng) in the presence of hPPARα (30 ng) and RXRα (30 ng) or empty (pSG5) expression plasmids as described in Section 2. After transfection, cells were refed with DMEM supplemented with 0.2% FCS in the presence of LDL or oxLDL (100 μg/ml) or vehicle (PBS) for 36 h. B: MAE cells were transfected with the pGal5-TK-pGL3 reporter (100 ng) and Gal4-hPPARα expression plasmids (20 ng). After transfection, cells were refed with DMEM supplemented with 0.2% FCS in the presence of increasing concentrations oxLDL (0–100 μg/ml) for 36 h.

PPARα–RXRα heterodimer whereas incubation with oxLDL resulted in a further induction of reporter activity (Fig. 1A). In a second set of experiments, the influence of oxLDL was tested on a chimeric construct comprising the DNA binding domain of the Gal4 yeast transcription factor fused to the ligand binding domain of PPARα. This system is completely independent of endogenous RXR. Incubation of MAE cells with increasing concentrations of oxLDL resulted in a dose-dependent increase of Gal4-driven promoter activity (25-fold with 100 μg/ml) (Fig. 1B). As a control, native LDL at similar concentrations failed to activate this promoter construct (not shown). These results indicate that oxLDL activates PPARα-dependent transcription in a dose-dependent manner in MAE cells.

3.2. *Oxidized phospholipids activate PPARα-dependent transcription*

In order to identify which chemical components of the oxLDL particle are involved in PPARα activation, total lipids from LDL and oxLDL were extracted and tested in the transactivation assays. First, it was demonstrated that the protein moiety is unable to activate PPARα (data not shown). Therefore, bonded phase columns were used to separate the different classes of lipids in oxLDL. When the Gal4-chimeric/reporter system was used to test the effect of these different lipid components, unoxidized triglycerides, cholesteryl esters and phospholipids were not found to transactivate the PPARα chimera (Fig. 2A). By contrast, oxidized phospholipids significantly activated PPARα-driven transcription (almost eight-fold) albeit to a lesser extent compared to entire oxLDL (11-fold) (Fig. 2A). Oxidized cholesteryl esters and triglycerides did not activate PPARα. Since it has been reported that oxidation products of PAPC mediate the action of oxidized LDL on endothelial cells [23], the influence of PAPC and oxPAPC on PPARα-dependent transcription was further tested. oxPAPC treatment in MAE cells resulted in a dose-dependent induction of the PPRE-driven promoter activity whereas PAPC, even when tested at high concentrations, had almost no effect (Fig. 2B). These results suggest that oxidized phospholipids mediate oxLDL-induced PPARα activation in MAE cells.

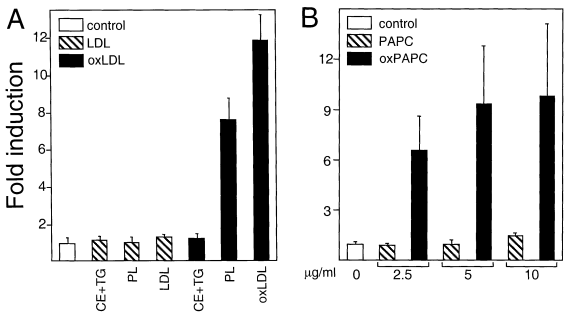

Fig. 2. Oxidized phospholipids activate PPARα. A: MAE cells were transfected with the pGal5-TK-pGL3 reporter (100 ng) and Gal4-hPPARα expression (20 ng) plasmids. After transfection, cells were refed with DMEM supplemented with 0.2% FCS and the indicated lipid fractions (equivalent to 50 μg/ml LDL) (CE: cholesteryl esters; TG: triglycerides; PL: phospholipids), LDL and oxLDL (50 μg/ml) for 36 h. B: MAE cells were transfected with J6-TK-pGL3 (100 ng) in the presence of hPPARα (30 ng). After transfection, cells were refed with DMEM supplemented with 0.2% FCS in the presence of increasing concentrations of PAPC or oxPAPC (2.5–10 μg/ml) for 36 h.

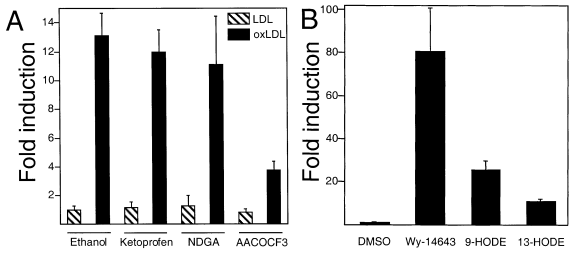

Fig. 3. PLA2 is required for oxLDL-mediated PPARα transactivation. A: MAE cells were transfected with pGal5-TK-pGL3 (100 ng) and Gal4-hPPARα (20 ng). 12 h after transfection, cells were pretreated with ketoprofen (100 μM), NDGA (10 μM), AACOCF3 (10 μM) or solvent (0.1% ethanol) for 3 h and subsequently refed with DMEM supplemented with 0.2% FCS and LDL or oxLDL (50 μg/ml) for 24 h. B: MAE cells were transfected as described in (A). After transfection, cells were refed with DMEM supplemented with 0.2% FCS in the presence of 9- or 13-HODE (30 μM) or Wy-14643 (50 μM) or solvent (0.1% ethanol).

3.3. *PLA2 is required for oxLDL-mediated PPARα transactivation*

To determine whether oxLDL-derived lipids require intracellular modifications to activate PPARα, their effects on PPARα transactivation were further studied in the presence of specific inhibitors of cyclooxygenase (ketoprofen), lipoxygenase (NDGA) and PLA2 (AACOCF3). Incubation of oxLDL (50 μg/ml) led to a strong activation of the chimeric Gal4-PPARα/reporter system (13-fold) which was not significantly affected by pretreatment (3 h) with cyclooxygenase nor lipoxygenase inhibitors (Fig. 3A). Interestingly, the specific PLA2 inhibitor (AACOCF3) reduced significantly oxLDL-induced promoter activation (Fig. 3A). During these experiments, cell viability was determined using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-derived assay. Incubation for 3 h with the various inhibitors did not lead to a reduced cell viability suggesting that the effect of the

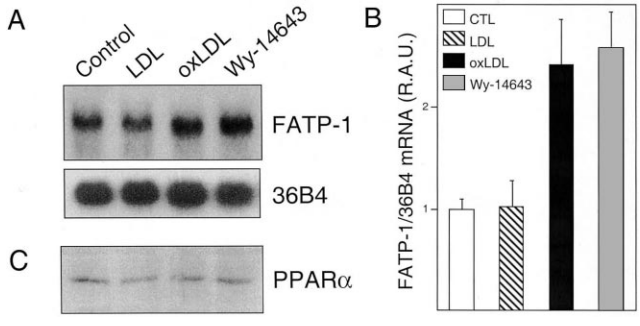

Fig. 4. oxLDL induce the PPARα target gene FATP-1 in CAEC cells without affecting PPARα protein expression. A: CAEC cells were incubated for 24 h in standard medium supplemented with LDL or oxLDL (100 μg/ml) or Wy-14643 (50 μM) or vehicle (DMSO 0.1%). FATP-1 and 36B4 mRNA were measured by Northern blot analysis. FATP-1 mRNA levels were quantified and normalized to 36B4 mRNA levels by densitometric analysis. The results of three different experiments are expressed in relative arbitrary units (R.A.U.) (B). C: CAEC were cultured for 24 h similarly as described in A. Total protein extracts were prepared and proteins (50 μg) were separated by SDS–PAGE, transferred to a Hybond membrane and probed with a polyclonal PPARα antibody.

PLA2 inhibitor is not due to cytotoxicity (data not shown). This experiment indicates that PLA2 is required for oxLDL-stimulated PPARα activation in endothelial cells. Since it has been demonstrated that hydrolysis of oxidized phospholipids by PLA2 may produce biologically active oxidized fatty acids [24] and since 9- and 13-HODE have been identified as potential PPARγ ligands [14], we tested the influence of these compounds on PPARα-mediated transactivation. 9- and 13-HODE activated 25- and 10-fold the Gal4-driven promoter activity in the presence of cotransfected PPARα chimera, respectively (Fig. 3B). As a control, the synthetic PPARα agonist Wy-14643 drastically activated the reporter construct. Taken together, these data demonstrate that 9- and 13-HODE are PPARα activators as similarly reported for PPARγ [14].

3.4. oxLDL regulate FATP gene expression in CAEC cells

To determine whether oxLDL could also activate PPARα in endothelial cells in vivo, the influence of oxLDL on known PPARα target gene expression was tested in CAEC cells and compared to those of the PPARα ligand Wy-14643. The fatty acid transporter/scavenger receptor CD36/FAT has been reported to be a PPAR-responsive gene [25,26] whose expression is regulated by oxLDL in macrophages [26]. Unfortunately, Northern blot analysis failed to detect CD36/FAT mRNA in CAEC cells. Therefore, the effect of oxLDL and Wy-14643 was studied on FATP-1 which is ubiquitously expressed and is positively regulated by PPAR activators [18,25]. Untreated CAEC cells exhibit a basal FATP-1 mRNA level which was significantly increased by Wy-14643 treatment (Fig. 4A). Incubation of CAEC cells with oxLDL (100 μg/ml) but not with LDL resulted also in a significant induction of FATP-1 mRNA (Fig. 4A). These data suggest that oxLDL regulate FATP-1 gene expression in endothelial cells via PPARα activation. Since it has been reported that oxLDL induce PPARγ expression in macrophages [26], we tested the influence of LDL and oxLDL (100 μg/ml) for 24 h on PPARα protein expression in primary human CAEC. Neither LDL nor oxLDL modified PPARα protein (Fig. 4C) nor mRNA (data not shown) levels in CAEC indicating that oxLDL regulate FATP-1 gene expression without altering PPARα protein expression.

4. Discussion

In this study, it is shown, for the first time, that oxLDL but not native LDL activate PPARα in a PLA2-dependent manner. Furthermore, oxLDL incubation resulted in the induction of the well-characterized PPARα target gene, FATP-1. This induction by oxLDL was similar as the one obtained using the synthetic PPARα ligand Wy-14643 in primary CAEC cells. In contrast to the previously reported induction of PPARγ expression in macrophages [26], oxLDL treatment of endothelial cells did not influence PPARα protein expression levels.

Using transient transfection assays, it was found that oxLDL but not native LDL provide PPARα activators to endothelial cells. Thus, as similarly reported for PPARγ in macrophages, the ability of LDL and oxLDL to activate PPARα is directly related to the oxidation state of the particle [14]. The mechanism by which endothelial cells take up the oxLDL or their components to generate endogenous PPARα activators, remains largely unknown. Nagy et al. [14] reported that PPARγ-dependent gene regulation by oxLDL in macrophages is mediated via the CD36/FAT scavenger receptor. However, CD36/FAT expression was undetectable in CAEC cells thereby excluding a role for this receptor in oxLDL uptake in endothelial cells. However, it is possible that endothelial cells take up oxLDL via the lectin-like oxidized LDL receptor-1 (LOX-1) which is highly expressed in human CAEC cells [27] and displays a high affinity for oxLDL [28]. Further studies will be required to delineate more precisely whether oxLDL-induced PPARα activation occurs via this mechanism.

The experiments using isolated lipid fractions of oxLDL led us to identify oxidized phospholipids as natural PPARα activators in endothelial cells. The fact that a specific PLA2 inhibitor can blunt oxLDL-induced PPARα activation, suggests that oxidized phospholipids are not PPARα ligands per se but are precursors for the true endogenously generated activators. PLA2 belongs to a family of signal transduction enzymes which catalyze fatty acid hydrolysis from the *sn*-2 position of phospholipids with concomitant production of lysophospholipids [29]. Cleavage of oxidized phospholipids by PLA2 leads to the release of oxidized fatty acids, such as oxidized-arachidonic or oxidized-linoleic acid. Chatidis et al. demonstrated that PLA2 is able to liberate 15-HETE from 15-lipoxygenated PAPC [24]. It is therefore likely that the PPAR ligands 9- and 13-HODE or 15-HETE may be produced by such hydrolysis of oxLDL-derived phospholipids. Our results demonstrate that 9- and 13-HODE are not only PPARγ but also PPARα activators suggesting that oxidized fatty acids might be responsible for PPARα activation by oxLDL. Using PLA2-specific inhibitors, Couturier et al. demonstrated that PLA2 is involved in IL-1-mediated sPLA2 gene induction in rat aortic smooth muscle cells [30]. They found that IL-1-activated PLA2 liberates fatty acids which are oxidized by lipoxygenases leading to the formation of PPARγ ligands such as 15-HETE, 9- and 13-HODE [30]. Their data and our results emphasize the role of PLA2 as one of the first steps involved in the generation of PPAR ligands in endothelial cells and smooth muscle cells. In addition, in a recent paper, Huang et al. found that IL-4-induced CD36/FAT expression in macrophages requires the 12/15 lipoxygenase which catalyzes the conversion of arachidonic acid and linoleic acid to 15-HETE and 13-HODE, respectively [15], thus resulting in the generation of PPARγ ligands. Altogether these reports indicate that natural PPARα and PPARγ activators may be generated by various complex pathways in different cells.

Oxidative stress is one of the main regulators of gene expression in the vasculature (see [31] for review). The role of oxLDL as an important oxidative signal in the pathogenesis of atherosclerosis has been well established [32]. oxLDL act by activating the redox-sensitive transcription factors AP-1 [33] and NF-κB [34] which drive the transcription of numerous genes involved in atherogenesis such as VCAM-1, MCP-1, etc. However, several reports demonstrated that oxLDL as well as oxidized phospholipids may also exert anti-inflammatory properties at the level of the vascular wall. Leitinger et al. demonstrated that oxPAPC dose-dependently inhibit LPS-induced neutrophil binding and E-selectin expression at the transcriptional level in endothelial cells [35]. Eligini et al. found that oxLDL-mediated inhibition of LPS-induced COX-2 expression in macrophages was mimicked by oxPAPC

[36]. We and others demonstrated that PPARα activators negatively regulate the vascular inflammatory response by reducing COX-2 [37], IL-6 [5] and VCAM-1 [38] in vascular cells. Based on these different observations, it is tempting to speculate that the anti-inflammatory activities of oxidized phospholipids and oxLDL are PPARα-mediated. Further studies are required to elucidate this question.

In addition, PPARα was reported to play a major role in the control of the cellular redox status [39,40]. Klucis et al. reported that administration of PPARα activators results in a drastic increase of the hepatic activity of catalase, an antioxidant enzyme [41]. It will be of interest to determine whether catalase may be induced by synthetic (fibrates) or natural ligands such as oxidized phospholipids in endothelial cells. This might contribute to a decrease of the cellular oxidative stress and therefore to a decrease of the NF-κB and AP-1 activities. Since PPARα has been proposed as a modulator of the inflammatory response and of the cellular redox status, the pro-inflammatory activities of oxLDL should thus be balanced by PPARα activation. The equilibrium of both oxLDL actions will therefore determine the inflammatory phenotype of the cell.

In conclusion, our data clearly show that oxLDL and oxidized phospholipids activate PPARα in endothelial cells in a PLA2-dependent manner. Moreover, oxLDL treatment resulted in the induction of FATP-1 gene expression in endothelial cells probably via PPARα activation. These results provide a possible molecular mechanism of anti-inflammatory action of certain oxLDL components in endothelial cells.

Acknowledgements: The authors would like to acknowledge the technical contribution of O. Vidal and M. Coevoet. The authors thank Dr. G. Martin for providing us with the FATP-1 cDNA. The authors are grateful to Dr. G. Byk (Rhône-Poulenc Rorer, Vitry, France) for providing us with the cationic lipid RPR 120535B. This work was supported by grants of the Institut Pasteur de Lille, INSERM, Rhône-Poulenc Rorer, ARCOL and the Région Nord-Pas-de-Calais. P. Delerive is supported by a grant of the Région Nord-Pas-de-Calais.

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