PPARα Activators Inhibit Cytokine-Induced Vascular Cell Adhesion Molecule-1 Expression in Human Endothelial Cells

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Background—Adhesion molecule expression on the endothelial cell (EC) surface is critical for leukocyte recruitment to atherosclerotic lesions. Better understanding of transcriptional regulation of adhesion molecules in ECs may provide important insight into plaque formation. Peroxisome proliferator–activated receptor-α (PPARα), a member of the nuclear receptor family, regulates gene expression in response to certain fatty acids and fibric acid derivatives. The present study investigated PPARα expression in human ECs and their regulation of vascular cell adhesion molecule-1 (VCAM-1).

Methods and Results—Immunohistochemistry revealed that human carotid artery ECs express PPARα. Pretreatment of cultured human ECs with the PPARα activators fenofibrate or WY14643 inhibited TNF-α–induced VCAM-1 in a time- and concentration-dependent manner, an effect not seen with PPARγ activators. Both PPARα activators decreased cytokine-induced VCAM-1 mRNA expression without altering its mRNA half-life. Transient transfection of deletional VCAM-1 promoter constructs and electrophoretic mobility shift assays suggest that fenofibrate inhibits VCAM-1 transcription in part by inhibiting NF-κB. Finally, PPARα activators significantly reduced adhesion of U937 cells to cultured human ECs.

Conclusions—Human ECs express PPARα, a potentially important regulator of atherogenesis through its transcriptional control of VCAM-1 gene expression. Such findings also have implications regarding the clinical use of lipid-lowering agents, like fibric acids, which can activate PPARα. **(*Circulation*. 1999;99:3125-3131.)**

Key Words: atherosclerosis ■ endothelium ■ leukocytes

Adhesion of circulating leukocytes to the endothelium is a critical early step in atherogenesis.[1–5] This process depends on the interaction between adhesion molecules on the endothelial cell (EC) surface and their cognate ligands on leukocytes. These EC adhesion molecules include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), E-selectin, and P-selectin.[6,7] Increased adhesion molecule expression by ECs in human atherosclerotic lesions may contribute to further leukocyte recruitment to sites of atherosclerosis.[6,8,9] Although inducers of EC adhesion molecule expression, such as the inflammatory mediators tumor necrosis factor (TNF)-α and interleukin (IL)-1,[10] have received much attention, less is known about the negative regulation of adhesion molecule transcription. Such understanding may provide important insight into plaque formation.

Certain polyunsaturated fatty acids, for example, docosahexaenoic acid (DHA), can inhibit cytokine-induced VCAM-1 expression in ECs, although the underlying mechanism remains unclear.[11] Interestingly, some polyunsaturated fatty acids can activate the peroxisome proliferator–activated receptor-α (PPARα), a nuclear receptor involved with transcriptional responses to fatty acids. Fibric acid derivatives, such as fenofibrate, are also thought to act as specific activators for PPARα.[12–14] In addition to PPARα, the PPAR family also includes PPARγ and PPARδ. PPARs, activated by binding of specific agonists, form heterodimers with the retinoid X receptor and associate with PPAR response elements in the promoter region of target genes whose expression they regulate.[15] We have demonstrated expression of PPARγ in human ECs and identified plasminogen activator inhibitor-1 as a potential PPARγ target gene in these cells.[16] Although PPARα mRNA expression in human ECs has been reported,[17] its role in EC biology, including candidate target genes, remains essentially unexplored.

We hypothesized that PPARα might regulate VCAM-1 expression in human ECs, thus potentially modulating leukocyte adhesion. To this end, we investigated the presence of PPARα in human ECs, studying the effect of well-established

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PPARα and PPARγ activators on adhesion molecule expression in these cells.

Methods

Immunohistochemistry of Human Carotid Artery Specimens

Staining for PPARα was performed on acetone-fixed serial cryostat sections of human carotid arteries (protocols approved by Brigham and Women's Institutional Review Board) with a polyclonal goat anti-human PPARα antibody (Santa Cruz). ECs were identified by staining with anti-CD31 antibodies (Dako). Sections were blocked with PBS/5% serum, and incubated with appropriate biotinylated secondary antibody (Vector Laboratories), then avidin-biotin-peroxidase complex (Vectastain ABC kit). Antibody binding was visualized with True Blue peroxidase substrate (Kirkegaard & Perry Laboratories) and counterstained with Gill's hematoxylin or contrast red (Kirkegaard & Perry Laboratories).

Cell Culture

Human saphenous vein ECs were isolated from explants from unused portions of saphenous veins harvested at coronary artery bypass surgery. Cells, cultured as described before,[11] were >99% von Willebrand factor–positive by flow cytometry, exhibited typical EC cobblestone growth pattern, and were of low passage number (p2–5). Bovine aortic ECs (BAECs) and human fibroblasts were cultured in DMEM (Biowhittaker) containing 1% glutamine, 1% penicillin-streptomycin, and 10% FCS. The hematopoietic cell line U937 was cultured in RPMI medium (Biowhittaker) containing 1% glutamine, 1% penicillin-streptomycin, and 10% FCS.

Preparation of Nuclear and Cytosolic Extracts and Western Blot Analysis

For Western blotting, nuclear and cytosolic extracts of 10^7 cells were prepared as previously described.[18] Processed samples were applied to 10% SDS-PAGE and transferred to nitrocellulose membranes (Millipore) by use of semidry blotting.[18] Membranes were treated overnight with TBS-Tween/5% dry milk and incubated with goat anti-human PPARα antibodies (Santa Cruz) for 1 hour. After washing, membranes were incubated with horseradish peroxidase–conjugated rabbit anti-goat monoclonal antibodies. Antigen detection was performed via chemiluminescence (NEN); Nuclear extracts from human fibroblasts transfected with a PPARα expression construct (provided by Dr Bruce Spiegelman, Dana Farber Cancer Institute, Boston, Mass) served as a positive control.

Cell-Surface Enzyme Immunoassays

For determination of cell-surface expression of adhesion molecules, ECs were pretreated with PPAR activators [PPARα activators: fenofibrate (Sigma) and WY14643 (Biomol); PPARγ activators: 15-deoxy-$^{\Delta 12,14}$-prostaglandin J_2 (15d-PGJ_2) (Calbiochem), troglitazone (Parke-Davis), and BRL 49653 (SmithKline Beecham)] at the times and concentrations indicated and then stimulated with the specified cytokines (8 hours). Enzyme immunoassay (EIA) was performed by incubating EC monolayers first with specific monoclonal antibodies against VCAM-1 (E1/6), ICAM-1 (HU5/3), or E-selectin (H18/7), then with biotinylated goat anti-mouse IgG (Vector Laboratories), and finally with streptavidin–alkaline phosphatase (Zymed Laboratories). (All monoclonal antibodies were a generous gift from Dr Michael Gimbrone, Brigham and Women's Hospital, Boston, Mass). Cells were washed in PBS/1% BSA after each incubation step, and the integrity of the cellular monolayer was ensured by phase-contrast microscopy. Surface expression of each adhesion molecule was measured spectrophotometrically at 410 nm 15 to 30 minutes after addition of the chromogenic substrate (para-nitrophenylphosphate, Sigma). Experiments were performed in triplicate for each condition.

Adhesion Assay

ECs were grown to confluence in 96-well plates, pretreated with PPARα activators for 24 hours, and stimulated with TNF-α for 8 hours, then adhesion assays were performed.[19] Briefly, U937 cells were labeled with 2′,7′-bis(2-carboxy)-fluorescein acetoxymethyl ester (Molecular Probes) and then added, under rolling conditions (63 rpm, 23°C, 15 minutes), to a rinsed EC monolayer (2×10^6 cells/mL) in RPMI medium/10% FCS/1 mmol/L $CaCl_2$. Nonadherent cells were removed by inverting the plate under rotation (20 minutes). After solubilization of well contents, fluorescence intensity was measured in a microtiter plate fluorimeter (Pandex, FCA). A standard curve using dilutions of labeled U937 cells was determined, and results were expressed as cells/cm^2.

RNA Extraction and Northern Blot Analysis

For Northern blot experiments, human ECs were pretreated with PPARα activators for 24 hours and then stimulated with the specified cytokines for 3 hours. Total RNA (10^7 cells) was isolated by the guanidinium thiocyanate–phenol-chloroform method (RNAzol, Tel-Test) and 5 μg of RNA used in standard Northern blot analysis with a VCAM-1 probe.

VCAM-1 mRNA half-life was determined by stimulating ECs with TNF-α for 3 hours before blocking transcription by treatment with actinomycin D 5 μg/L. Cells then received fenofibrate for the times indicated; mRNA levels were compared with those of untreated cells.

Transient Transfections

To investigate the effect of PPARα activators on VCAM-1 promoter activity, we transiently transfected BAECs with a series of deletional VCAM-1 promoter constructs, all containing the chloramphenicol acetyltransferase (CAT) reporter.[20] [−755]F0.CAT is the putative full-length human VCAM-1 promoter containing AP-1, GATA, and NF-κB binding sites. [−98]F3.CAT lacks the AP-1 and GATA sites but retains NF-κB binding sites. [−44]F4.CAT lacks NF-κB binding sites (Figure 5A). BAECs, which are more easily transfectable than human ECs, were cotransfected via calcium phosphate precipitation[21] with each reporter construct (5 μg) and a pCMV.β-GAL (4 μg) as an internal control. Cells were stimulated (48 hours after transfection) with TNF-α 10 μg/L with or without fenofibrate 100 μmol/L. BAECs were then harvested after 36 hours, and lysates were subjected to CAT and β-galactosidase assay (Tropix) as described.[22] Normalized CAT activity was calculated as the ratio of CAT activity to β-galactosidase activity. Results for each reporter construct were expressed as multiples of induction compared with transfected, unstimulated cells.

Electrophoretic Mobility Shift Assay

For electrophoretic mobility shift assays (EMSAs), human ECs were preincubated for 24 hours with fenofibrate 100 μmol/L and then stimulated for 2 hours with TNF-α 10 μg/L before nuclear extracts were prepared. The NF-κB oligonucleotide (CCTGGGTTTCCCCTTGAAGGGATTTCCCTCC) (Genosys Biotechnologies) spanning the 2 tandem NF-κB sites (as underlined above) in the human VCAM-1 promoter was end-labeled with [γ-^{32}P]ATP (3000 Ci/mmol) by T4 polynucleotide kinase (New England Biolabs) and purified (Sephadex G-25 columns, Pharmacia LKB Biotechnology). Nuclear extracts (5 μg) were incubated with the labeled NF-κB oligonucleotide under standard conditions.[11] In the indicated experiments, nuclear extracts were incubated with anti-p50 [polyclonal rabbit anti-p50 (NLS)X, Santa Cruz] or anti-p65 (polyclonal rabbit anti-p65 AX, Santa Cruz) or nonspecific IgG before the addition of radiolabeled NF-κB probes. DNA-protein complexes were electrophoretically separated (5% nondenaturing polyacrylamide gel). Specificity was determined by addition of an excess of unlabeled (cold) NF-κB oligonucleotide to the nuclear extracts before formation of DNA-protein complexes.

Assessment of Total Protein Synthesis

Total protein synthesis was assessed as ^{35}S-methionine incorporation as described previously.[11]

Statistical Analysis

Results of the experimental studies are reported as mean±SEM. Differences were analyzed by 1-way ANOVA followed by Fisher's protected least significant difference test. A value of $P<0.05$ was regarded as significant.

Results

Human ECs Express PPARα In Vivo and In Vitro

Immunohistochemistry of human carotid artery specimens (n=6) revealed PPARα staining in the EC nuclei (Figure 1A; blue staining, arrowheads). Parallel sections stained with goat IgG showed no immunostaining (Figure 1B). ECs were identified by immunoreactive CD31 (platelet and endothelial cell adhesion molecule-1) in parallel sections (Figure 1C; red staining).

To demonstrate PPARα expression in vitro in a homogeneous population of human ECs, Western blot analysis of cultured human saphenous vein ECs was performed. Consistent with the in situ findings, PPARα protein was detected in nuclear but not cytosolic fractions. The identity of the detected band was confirmed by comigration with a band from fibroblasts transfected with a PPARα expression construct (Figure 1D); untransfected fibroblasts reveal no such band (data not shown).

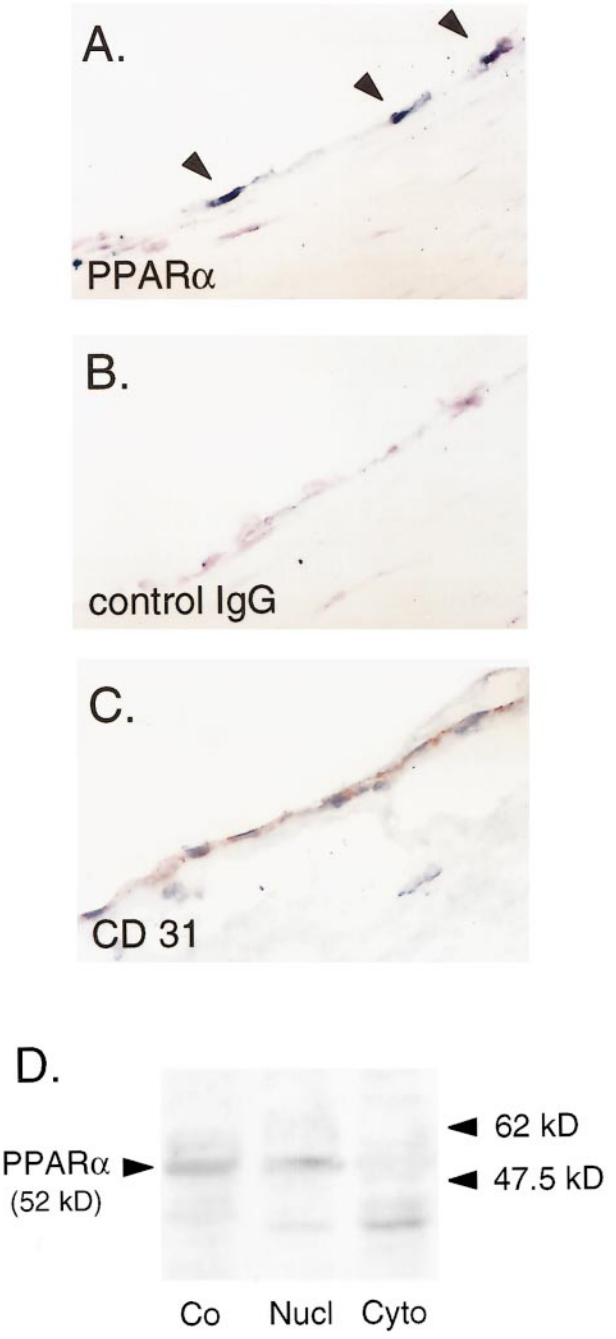

Figure 1. ECs express PPARα in vivo and in vitro. A, PPARα expression in nuclei of ECs of human carotid arteries (positive nuclei stained blue, arrowheads). B, Parallel sections stained with goat IgG show no signal, which suggests that staining for PPARα is specific. C, Immunostaining of parallel sections with CD31 identifies endothelial cell layer at luminal surface of artery (stained red). Magnification ×400 in all. Analysis of 6 separate carotid sections revealed similar results. D, Western blot analysis of cultured human saphenous vein ECs reveals PPARα protein expression in nuclear extracts (Nucl). Identity of detected band was confirmed by comigration with a band from fibroblasts transfected with a PPARα expression construct (Co). PPARα is not seen in cytosolic fraction of ECs (Cyto). Three independent experiments showed similar results.

PPARα but Not PPARγ Activators Reduce EC Surface Expression of VCAM-1

As expected, cell surface EIAs of human ECs revealed a marked increase of VCAM-1 expression in response to stimulation with TNF-α 10 μg/L. Pretreatment of ECs with the PPARα activator fenofibrate 100 μmol/L or WY14643 250 μmol/L reduced VCAM-1 expression levels significantly, to 33±9% ($P<0.01$) or 52±2% ($P<0.01$) of TNF-α–stimulated cells, respectively (Figure 2A). Similar results were obtained by flow cytometry (data not shown). None of 3 different PPARγ activators (troglitazone, 10 μmol/L; 15d-PGJ_2, 10 μmol/L; or BRL49653, 10 μmol/L); significantly affected TNF-α–induced VCAM-1 expression (Figure 2A). Treatment of unstimulated human ECs with PPARα or PPARγ activators did not alter VCAM-1 expression (data not shown). Fenofibrate did not affect EC viability (>95% excluded trypan blue) or total protein synthesis ($263\pm5\times10^3$ cpm/cm^2 well in TNF-α–treated cells versus $283\pm22\times10^3$ cpm/cm^2 well in TNF-α– and fenofibrate-treated cells; P=NS).

Neither PPARα nor PPARγ activators significantly reduced the TNF-α–induced cell surface expression of ICAM-1 (Figure 2B, solid bars) or E-selectin (Figure 2B, open bars) in ECs.

Fenofibrate Reduces Cytokine-Induced VCAM-1 Expression in a Time- and Concentration-Dependent Manner

To investigate the time- and concentration-dependence of PPARα activator treatment on VCAM-1 expression, human ECs were pretreated with fenofibrate for different times or concentrations before stimulation with TNF-α and subsequent EIA determination of VCAM-1 expression. Inhibition of TNF-α–induced VCAM-1 expression depended on the time of fenofibrate exposure, with a maximal reduction after 24 hours of fenofibrate pretreatment (Figure 2C). In addition,

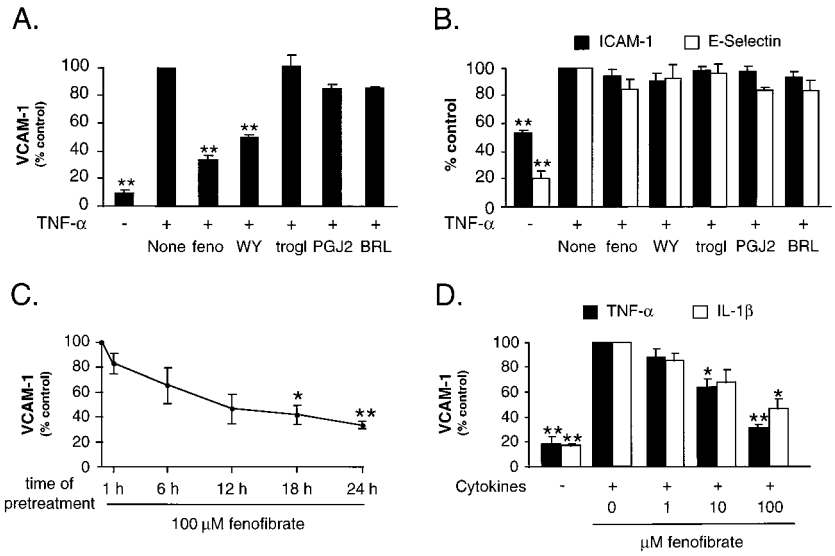

Figure 2. PPARα but not PPARγ activators inhibit cytokine-induced cell surface expression of VCAM-1 in human ECs. A and B, Cells were pretreated with PPARα [100 μmol/L fenofibrate (feno), 250 μmol/L WY14643 (WY)] or PPARγ [10 μmol/L troglitazone (trogl), 10 μmol/L 15d-PGJ2 (PGJ2), 10 μmol/L BRL49653 (BRL)] activators for 24 hours and then stimulated with TNF-α 10 μg/L for 8 hours before cell surface EIAs were performed for VCAM-1 (A), ICAM-1, and E-selectin (B). Results are expressed as percent of TNF-α–stimulated cells (% control). Bars represent mean±SEM (VCAM-1, n=8; ICAM-1/E-selectin, n=4); *$P<0.05$, **$P<0.01$ vs control. C and D, PPARα activators inhibit surface expression of VCAM-1 in human ECs in a time- and concentration-dependent manner. Cells were pretreated with fenofibrate 100 μmol/L for duration shown before stimulation with TNF-α 10 μg/L for 8 hours (C). ECs were pretreated with fenofibrate at concentrations shown for 24 hours, before stimulation with TNF-α 10 μg/L (D, solid bars), or IL-1α 10 μg/L (D, open bars). Results are expressed as percent of cytokine-stimulated cells (% control) as determined by cell surface EIA. Circles/bars represent mean±SEM (n=3); *$P<0.05$, **$P<0.01$ vs control.

fenofibrate inhibited VCAM-1 expression in human ECs induced by TNF-α (Figure 2D, solid bars) or IL-1α (Figure 2D, open bars) in a concentration-dependent manner with a maximal reduction at 100 μmol/L fenofibrate.

PPARα Activators Inhibit the Adhesion of Monocyte-Like Cells on Human ECs

To investigate the potential functional relevance of PPARα activator–reduced VCAM-1 expression in human ECs, we performed an in vitro adhesion assay using fluorescently labeled U937 cells and monolayers of human ECs. Stimulation of the EC monolayer with TNF-α increased the number of adherent cells from $9.1\pm1.5\times10^3$ cells/cm^2 to $73.2\pm2.4\times10^3$ cells/cm^2 ($P<0.01$) (Figure 3B). Pretreatment of ECs with fenofibrate or WY14643 before TNF-α stimulation reduced U937 cell adhesion significantly, to $36.7\pm2.2\times10^3$ cells/cm^2 ($P<0.01$) or $37.3\pm4.3\times10^3$ cells/cm^2 ($P<0.01$), respectively (Figure 3A and 3B). Preincubation of TNF-α–stimulated ECs with blocking anti-VCAM monoclonal antibody inhibited U937 cell adhesion almost completely (data not shown).

PPARα Activators Reduce Cytokine-Induced VCAM-1 mRNA Levels in Human ECs

Northern blot analysis revealed increased VCAM-1 mRNA levels after 3 hours of stimulation of human ECs with TNF-α 10 μg/L, which could be inhibited in a concentration-dependent manner by pretreatment with the PPARα activators fenofibrate or WY14643 (Figure 4A). Similar results were seen when ECs were stimulated with IL-1α instead of TNF-α (data not shown). In the presence of actinomycin D, fenofibrate did not significantly reduce VCAM-1 mRNA half-life compared with control cells (6.4±0.6 hours in control cells versus 6.4±1.1 hours in fenofibrate-stimulated cells, P=NS), indicating that the inhibitory effect of PPARα activators on VCAM-1 does not result from altered mRNA stability (Figure 4B).

Fenofibrate Inhibits TNF-α–Induced VCAM-1 Promoter Activity

To determine potential sites of interaction of PPARα activators with the VCAM-1 promoter, we performed transient transfections of various deletional VCAM-1 promoter reporter CAT constructs in bovine ECs (Figure 5A). After stimulation for 36 hours, CAT activity, as well as the activity of a cotransfected β-galactosidase construct, was measured (Figure 5B). TNF-α stimulation of cells transfected with the full-length promoter construct (F0) led to a 5.9±1.6-fold increase in normalized promoter activity (CAT/β-galactosidase activity). Treatment with fenofibrate significantly reduced this response to 2.4±0.4-fold ($P<0.05$ compared with TNF-α–stimulated cells). Transfection studies with a VCAM-1 promoter deletion construct (F3) containing the 2 tandem NF-κB sites, but lacking the AP-1 and GATA sites, revealed similar PPARα agonist responsiveness. Stimulation of transfected cells with TNF-α enhanced relative CAT activity 3.4±0.6-fold; treatment with fenofibrate significantly inhibited this increase to 1.4±0.2 ($P<0.05$ compared with TNF-α–stimulated cells). Transfection studies with the VCAM-1 deletion construct (F4), lacking the 2 NF-κB sites, revealed no change in relative CAT activity after treatment with TNF-α or fenofibrate. In the case of all constructs, treatment with fenofibrate alone had no effect on relative CAT activity compared with control, consistent with the absence of consensus PPAR response elements in the VCAM promoter.

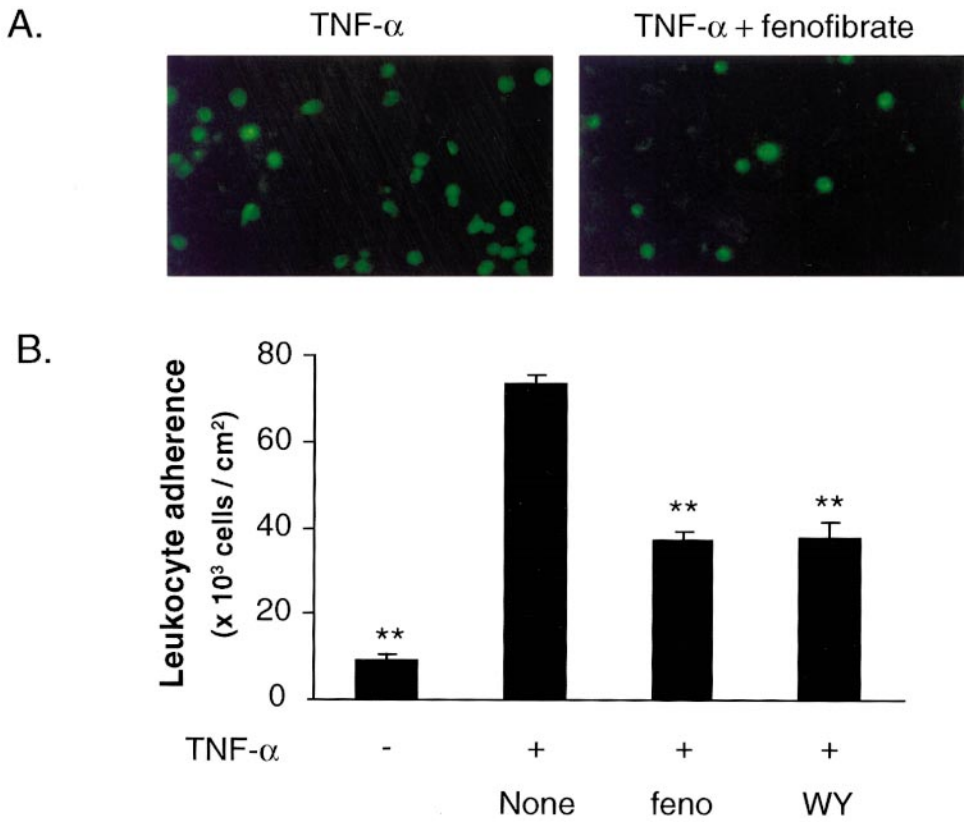

Figure 3. PPARα activators inhibit adhesion of U937 monocytoid cells to human ECs. A, Fluorescein-labeled U937 cells were added to TNF-α 10 μg/L–stimulated human EC monolayers with or without fenofibrate pretreatment. Fluorescence microscopy shows adherent U937 cells (green) on ECs. B, Quantification of U937 adherence on EC monolayers after EC pretreatment with fenofibrate (feno) 100 μmol/L or WY14643 (WY) 250 μmol/L as determined by fluorescence assay. Results are expressed as cells/cm^2. Bars represent mean±SEM (n=3); $^{**}P<0.01$ vs TNF-α stimulated cells.

Fenofibrate Inhibits TNF-α–Induced NF-κB Activation

EMSAs that used radiolabeled oligonucleotides corresponding to the 2 tandem NF-κB sites in the VCAM-1 promoter were performed to investigate whether PPARα activators inhibit NF-κB activation. Fenofibrate decreased the amount of shifted complexes induced by TNF-α, which suggests that PPARα activators directly inhibit NF-κB activation (Figure 5C).

To further investigate these findings, supershift analysis was performed to define fenofibrate effects on the NF-κB transcriptional complex (Figure 5C). As described by others, TNF-α–induced NF-κB activation involves the p50 and p65 subunits. Fenofibrate treatment of similarly stimulated ECs resulted in a parallel decrease in the amount of supershifted p50 and p65 complexes.

Discussion

The present study reports expression of PPARα in ECs of human arteries and reduction of cytokine-induced VCAM-1 expression by PPARα agonists through inhibition of NF-κB. This inhibition of VCAM-1 expression by PPARα activators decreased adhesion of monocyte-like cells to stimulated ECs. PPARγ activators exhibited no such effects.

Initially, PPARα was thought to be limited to tissues such as liver and fat, in which it participates in the regulation of lipid, and in particular fatty acid, metabolism.[23,24] A recent study demonstrated PPARα expression in human vascular smooth muscle cells with inhibition of IL-6, cyclooxygenase-2, and prostaglandin gene expression by the same PPARα activators used here (WY14643, fenofibrate).[25] Human ECs, like vascular smooth muscle cells, express both PPARα and PPARγ,[16] with each PPAR probably having unique effects relevant to vascular biology in these cellular settings. We have previously shown PPARγ expression in ECs and suggested a role of PPARγ in the regulation of plasminogen activator inhibitor-1 gene expression.[16] We report here that PPARγ activation does not appear to be involved in the regulation of adhesion molecule expression (Figure 2).

In contrast, 2 different established PPARα activators, fenofibrate and WY14643, inhibit cytokine-induced VCAM-1 expression in ECs. These agents probably act in ECs by activating PPARα. Both of these agonists have high binding affinities to PPARα while selectively interacting with PPARα, with little to no activity on other PPAR isoforms.[12,26] The fibrates used here produced inhibitory effects at concentrations similar to those that induced established PPARα response genes, eg, apolipoprotein A-II.[27] In contrast, various PPARγ activators, among them the highly specific PPARγ agonist BRL49653,[12,26] added either before or after (data not shown) cytokine treatment, had no effect on VCAM-1 levels. Therefore, PPARγ activation by PPARα agonists seems an unlikely explanation for our results.

The reduction of VCAM-1 expression by PPARα activators appears at a transcriptional level because fenofibrate did

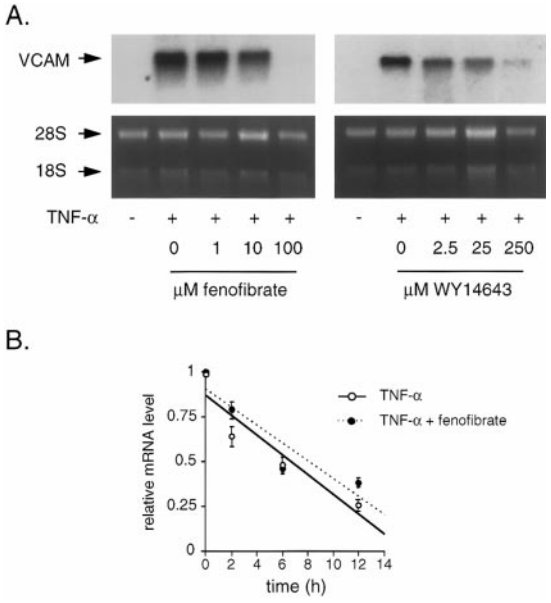

Figure 4. PPARα activators inhibit VCAM-1 mRNA expression but not mRNA half-life in human ECs. A, Northern blot analysis of ECs pretreated with fenofibrate or WY14643 at concentrations shown for 24 hours before stimulation with TNF-α 10 μg/L for 3 hours (top). Ethidium bromide staining (bottom) demonstrates equal loading of intact RNA. Three independent experiments yielded similar results. B, Densitometry analysis of TNF-α–induced VCAM-1 mRNA levels in absence or presence of fenofibrate 100 μmol/L as measured by Northern blot analysis of actinomycin D-treated ECs. Actinomycin D and fenofibrate were added to ECs 3 hours after TNF-α stimulation (0 hours); cells were harvested at times indicated. Amount of mRNA at each time point was compared with mRNA levels after 3 hours of TNF-α stimulation at time 0 hours (ordinate labeled as relative mRNA level). Results are shown as mean±SEM of 3 independent experiments.

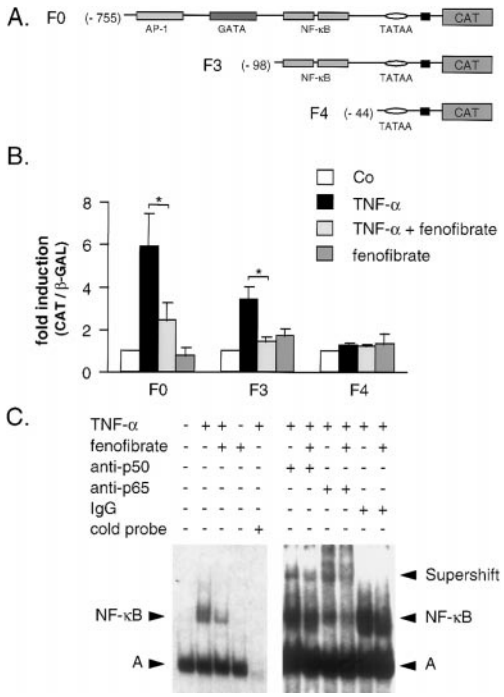

Figure 5. Fenofibrate inhibits TNF-α–induced activation of human VCAM-1 promoter and activation of NF-κB. A, Deletional series of VCAM-1 promoter constructs used in transfection experiments. B, Bovine ECs were cotransfected with VCAM-1 promoter deletion constructs indicated and a β-galactosidase expression construct (pCMV.β-GAL). Transfected cells were stimulated with agents indicated for 36 hours, and assays were performed for CAT- and β-galactosidase activity. Results for each construct were normalized to β-galactosidase activity and expressed as multiples of induction compared with unstimulated cells. Bars represent mean±SEM (n=3); *$P<0.05$ vs TNF-α–stimulated cells. C, EMSA of human ECs pretreated with fenofibrate 100 μmol/L for 24 hours before TNF-α stimulation for 2 hours. Specificity was determined by addition of 40 ng unlabeled NF-κB oligonucleotide (cold probe). Supershift analysis was performed with anti-p50 and anti-p65 antibodies. As a control, nuclear extracts were incubated with nonspecific IgG. Similar results were seen in 3 independent experiments.

not alter VCAM-1 mRNA half-life but did inhibit TNF-α–induced VCAM-1 promoter activity. This effect appears to stem from inhibition of NF-κB activation, as suggested by the reduction of CAT activity of the promoter construct lacking NF-κB binding sites ([−44]F4) and gel shift assays. The inhibition of NF-κB activation by PPARα could result from direct interference with NF-κB binding to the VCAM-1 promoter, as postulated for the interaction of NF-κB with the estrogen receptor.[28] Alternatively, the inhibitory effects might occur through competitive binding of transcriptional coactivators by PPARα or by PPARα-induced transcription factors. Such "negative crosstalk" has been suggested between other nuclear receptors and the transcription factor AP-1.[29] In fact, one such coactivator, p300, involved in VCAM-1 expression[30] reportedly interacts with PPARα.[31] Our data also do not exclude a PPARα effect on IκB or an effect on the transcription of NF-κB subunits p50 and p65.

The genes encoding ICAM-1 and E-selectin have NF-κB sites in their promoter; nonetheless, PPARα activators did not alter ICAM-1 or E-selectin expression. This result may be explained in several ways. It could derive from the distinct nature of the VCAM-1 promoter, either its NF-κB sites or another undefined VCAM-1 transcriptional element,[32] ie, the interferon regulatory factor-1 site. We saw no effect of fenofibrate on the known TNF-α induction of interferon regulatory factor-1 expression in ECs (data not shown).[22] Other mechanisms might include competition for transcriptional coactivators as described above. Interestingly, retinoic acid, acting through the retinoic acid receptor, another nuclear receptor family member, also appears to inhibit activation of the NF-κB site of the VCAM-1 promoter, but not NF-κB activation of either the ICAM-1 or E-selectin promoters.[33]

Inhibition of VCAM-1 expression in human ECs by PPARα activators, with a consequent decrease in monocyte adherence to ECs, has important implications regarding atherogenic mechanisms as well as the treatment of atherosclerosis, especially given the similarity of fenofibrate concentrations used here and those achieved in patients.[34] Human angiographic studies have reported that fenofibrate treatment reduces coronary artery stenoses.[35] Epidemiological[36] as well as experimental work[37,38] suggests that the intake of polyunsaturated fatty acids, some of them also known PPARα agonists,[12] reduces the incidence of cardiovascular events. Given the likely involvement of VCAM-1 in monocyte recruitment to early atherosclerotic lesions,[6] our findings suggest PPARα as a potential mediator of critical inflammatory processes in the vessel wall.

Acknowledgments

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