PPARα Agonists Inhibit Tissue Factor Expression in Human Monocytes and Macrophages

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Background—Monocytic tissue factor (TF) expression may contribute to thrombogenicity associated with plaque rupture and may propagate thrombus formation at the site of vascular lesions. Induction of monocytic TF expression by endotoxin is mediated by the activation of transcription factors such as AP-1 and NF-κB. Both these signaling pathways are modulated by peroxisome proliferator–activated receptor-α (PPARα). Therefore, we have studied the effects of fibrates and other PPARα agonists on the expression of TF.

Methods and Results—We show that PPARα protein, like primary human monocytes, is also expressed in the human monocytic THP-1 cell line. Fenofibric acid, WY14643, and GW2331 inhibited TF mRNA upregulation after stimulation of THP-1 cells with lipopolysaccharide or interleukin-1β. In primary human monocytes and macrophages, the lipopolysaccharide- or interleukin-1β-mediated induction of TF activity was also inhibited by fenofibric acid, WY14643, or GW2331.

Conclusions—These data indicate that activation of PPARα results in the downregulation of the TF gene. Our results suggest a novel role for PPARα in the control of atherosclerotic plaque thrombogenicity through its effects on TF expression in monocytes and macrophages. (Circulation. 2001;103:207-212.)

Key Words: tissue factor ■ lipopolysaccharide ■ receptors ■ monocytes ■ fibrates

Tissue factor (TF), a membrane-anchored glycoprotein, plays an important role in promoting coagulation and thrombosis.1 TF initiates blood coagulation by forming a complex with circulating factors VII and VIIa.2 Although physiologically absent from all intravascular cells, TF can be induced in monocytes by external signals such as growth factors, inflammatory cytokines (interleukin [IL]-1β and tumor necrosis factor [TNF]-α), oxidized LDLs, and endotoxin.2 Monocytic TF activity has been implicated in several diseases associated with inflammation.3 In particular, TF antigen and mRNA have been localized in atheromatous plaques.4,5 Both monocytes and macrophages are involved in the progression of atherosclerosis and in the pathogenesis of thrombosis.6 Monocytic TF expression may contribute to thrombogenicity associated with plaque rupture.7 Moreover, TF from monocytes present in peripheral blood may propagate thrombus formation at the site of a vascular lesion.8

Peroxisome proliferator–activated receptor-α (PPARα) is a ligand-dependent transcription factor that, on heterodimerization with the retinoid X receptor, binds to specific peroxisome proliferator response elements (PPREs) in the promoter of target genes, thus regulating the transcription of these genes. Transcription of affected genes may also be modulated by PPARα via interference with other transcription factor pathways. Activation of PPARα negatively interferes with nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT), and activator protein-1 (AP-1) pathways.9–11

PPARα, which plays an important role in the metabolism of fatty acids, lipids, and lipoproteins, has also been implicated in interference with atherogenic and inflammatory processes. PPARα-deficient mice show a prolonged response to inflammatory stimuli.12 PPARα has been shown to inhibit transcription of several inflammatory response genes, which also occur in atherosclerotic plaques. In human aortic smooth muscle cells, fibrates inhibit the IL-1β–induced expression of cyclooxygenase (COX)-2 and IL-6 by inhibiting the NF-κB and AP-1 signaling pathway.9,10 In human vascular endothelial cells, PPARα inhibits the thrombin-mediated activation of endothelin-1 via negative interference with the AP-1 signaling pathway.13 Moreover, PPARα activators prevent TNF-α–induced VCAM-1 expression in human saphenous vein endothelial cells, partly via inhibition of the NF-κB pathway.14 This PPARα action may lead to a decreased recruitment of monocytes to early atherosclerotic lesions. In
addition, PPARα is present in primary human monocytes, and its expression increases on differentiation into macrophages. Furthermore, PPARα activators induce apoptosis of TNF-α-activated macrophages, most likely by inhibiting the antiapoptotic NF-kB pathway.

Although the TF promoter does not contain a PPRE, it contains Sp1-, Egr-1-, AP-1-, and NF-kB–responsive elements. Induction of monocytic TF expression by endotoxin is mediated by the interaction of transcription factors such as AP-1 and NF-kB with its promoter. Because both these signaling pathways are modulated by PPARα, we hypothesized that PPARα may modulate TF expression in human monocytes and macrophages. In the present report, the effect of several PPARα agonists on the endotoxin- and IL-1β–mediated induction of TF expression was studied. We demonstrate that PPARα is also expressed in human monocytic THP-1 cells and that LPS- and IL-1β–induced TF expression in THP-1, primary human monocytes, and macrophages is downregulated by activation of PPARα.

**Methods**

**Materials**

Fenofibric acid was a kind gift from A. Edgar (Fournier, Daix, France). WY14643 came from Chemsyn, and GW2331 was a kind gift from T. Willson (GlaxoWellcome Inc, Durham, NC). *Escherichia coli* lipopolysaccharide (LPS) and all *trans*-retinoic acid (ATRA) were purchased from Sigma Chemical Co and IL-1β from Peprotech.

**THP-1 Culture**

Suspensions of human monocytic leukemia THP-1 cells were maintained in RPMI 1640 medium containing 25 mmol/L HEPES buffer and 10% FCS. The cell suspensions were grown at 37°C in a humidified 5% CO2 atmosphere. Suspensions were diluted 1:1 when they reached a concentration of ~1.5 x 10^6 cells/mL. Cells were incubated 1 hour before LPS stimulation with either 100 μmol/L fenofibric acid, 10 μmol/L WY14643, 0.5 μmol/L GW2331, or 0.5 μmol/L ATRA. After addition of 10 μg/mL LPS, cells were further incubated for 2 hours. Then 5 mL of cell suspension was centrifuged (15 seconds, 10 000g), washed with PBS, and used for further RNA extraction.

**Isolation and Culture of Human Monocytes**

Venous blood obtained from healthy donors was anticoagulated with EDTA, and mononuclear cells were isolated by gradient centrifugation (separation medium MSL, d=1.077±0.001, Eurobio), washed 2 times with PBS, and resuspended in RPMI 1640 (1 x 10^6 cells/mL). Monocytes were isolated from lymphocytes by adherence (1 hour at 37°C in a humidified 5% CO2 atmosphere) to 96-well microplates. In separate experiments, monocytes were differentiated by culturing for 12 days in the presence of 10% human serum at 37°C in a humidified 5% CO2 atmosphere. Cells were either preincubated with 100 μmol/L fenofibric acid, 10 or 100 μmol/L WY14643, 1 μmol/L GW2331, or 1 μmol/L ATRA for 4 hours before a 16-hour stimulation with LPS (0.4 ng/mL) or IL-1β (10 ng/mL) at 37°C in a humidified 5% CO2 atmosphere. At the end of the incubation period, the medium was removed, and plates were washed with cold PBS and assayed for TF activity. All reagents and culture supplies used were free of endotoxin (chromogenic limulus amoebocyte lysate assay; sensitivity, 0.025 endotoxin units/mL).

**TF mRNA Analysis**

Total RNA was prepared from THP-1 cells by acid guanidinium thiocyanate–phenol-chloroform extraction. Fifty micrometers of RNA was separated by electrophoresis and transferred to nylon membranes. Northern blots were hybridized at 68°C with radiolabeled TF or 36B4 cDNA probes in ExpressHyb according to the manufacturer’s instructions (Clontech Laboratories). For the TF probe, a 641-bp cDNA product identical to the probe reported in the literature was isolated after reverse transcription–polymerase chain reaction amplification of RNA from human monocytes (primers, 5′-CTAGAATTCTCACAATACTGTTGCAGCATA-3′ and 5′-ACGGATTCCCTTTCCTCTGCC-3′). The fragment was cloned into a pBSKS vector and its identity verified by sequence analysis.

**TF Activity Assay**

TF activity was determined by a modified amidolytic assay. Briefly, cells were mixed with 0.25 mol/L CaCl2 (50 μL) and prothrombin concentrate complex (Laboratoire de Fractionnement et des Biotechnologies) as a source of factor VII (50 μL, 3 IU/mL). After addition of 50 μL of the chromogenic substrate S2765 (Biogenic), the change in optical density at 405 nm was quantified with a microplate reader and converted to units of TF activity by being plotted log to log with readings from standard dilutions of tissue thromboplastin. Arbitrarily, 1 mL of thromboplastin was assigned a value of 1000 U/mL of TF activity.

**Statistics**

Statistically significant differences between groups were reported when P<0.05 through an ANOVA test followed by a Bonferroni correction.

**Results**

To determine the conditions for studying the effects of PPARα activators, the LPS-mediated induction of TF expression was monitored in monocytic THP-1 cells. As in other studies, LPS increased TF mRNA expression in these cells several-fold. After 2 hours of incubation with LPS, both the 2.2- and 3.4-kb mRNA species of TF were observed (Figure 1A). The 3.4-kb transcript may contain intron-1 and is probably not translated to protein. The 2.2-kb TF mRNA transcript was transiently induced, with a maximum after 2 hours of LPS stimulation. After 3 hours of incubation with LPS, the 2.2-kb transcript level was already decreased.

To examine whether specific effects of PPARα activators could be expected in THP-1 cells, we analyzed the expression
of PPARα in these cells. Both PPARα mRNA (not shown) and protein (Figure 1B) were detected in control and LPS-stimulated THP-1 cells. LPS had no effect on the level of PPARα protein expression.

Because PPARα is expressed in THP-1 cells, we further examined the effects of several PPARα activators on TF mRNA expression. Incubation of THP-1 cells with PPARα activators 1 hour before LPS stimulation for 2 hours resulted in a decreased level of TF mRNA compared with cells incubated with only LPS (Figure 2A). The PPARα agonists fenofibric acid and WY14643 inhibited LPS-mediated induction of TF mRNA to 61% and 46%, respectively, of the mRNA levels of cells incubated with LPS alone (Figure 2A). Moreover, coinubcation of THP-1 cells with LPS and the potent PPARα agonist GW2331 (EC₅₀, 50 nmol/L) decreased TF mRNA expression to 39% of the level of LPS-stimulated cells.

To verify the extent of the TF mRNA inhibition, we compared the effects of the PPARα activators with those of ATRA, a previously identified negative regulator of TF expression.25 ATRA inhibited the LPS-mediated induction of TF mRNA expression in THP-1 cells to 21% of the control level.

To investigate whether PPARα activators could also inhibit TF mRNA induction by other inflammatory stimuli, we studied their effects on THP-1 cells stimulated with IL-1β. Like LPS-induced TF mRNA expression, PPARα agonists and ATRA inhibited IL-1β–induced TF expression (Figure 2B). Both WY14643 and GW2331 decreased TF mRNA levels to ~63% and 48%, respectively, of levels in cells incubated with IL-1β alone. ATRA diminished the mRNA level to 19% of the IL-1β–stimulated control.

Next, we studied the influence of PPARα agonists on TF activity in primary human monocytes. Incubation of unstimulated monocytes with fenofibric acid, WY14643, GW2331, or ATRA did not influence basal TF activity (Figure 3A). Incubation of monocytes with LPS or IL-1β resulted in a 10- and 5-fold increase of TF activity, respectively. Preincubation with different PPARα agonists significantly inhibited both the LPS- and IL-1β–induced TF activity (Figure 3B and 3C). The TF activity in LPS-stimulated monocytes decreased to 67%, 57%, and 56% on preincubation with fenofibric acid, WY14643, or GW2331, respectively. In IL-1β–stimulated monocytes, the TF expression after fenofibric acid, WY14643, or GW2331 preincubation was decreased to 72%, 60%, and 45%, respectively, compared with IL-1β only. The inhibitory effects of PPARα agonists on TF activity levels at

![Image](https://example.com/image.png)
the doses used correlated well with their relative potency for PPARα activation (GW2331 > WY14643 > fenofibric acid). ATRA inhibited IL-1β–induced TF activity to 33% of the activity in stimulated monocytes. ATRA had no detectable effect on LPS-induced TF activity when preincubated, however, analogous to the PPARα agonists, for 4 hours (Figure 3C). Preincubation with ATRA for 30 minutes followed by an incubation for 16 hours with LPS, however, resulted in a pronounced downregulation (to 11%) of TF activity compared with LPS incubation alone.

Finally, we studied the effects of PPARα agonists on TF activity in human monocyte–derived macrophages (Figure 4). Preincubation of differentiated macrophages with the PPARα agonists fenofibric acid, WY14643, and GW2331 resulted in an inhibition of LPS-stimulated TF activity to 78%, 35%, and 13%, respectively, of the activity in cells incubated with LPS alone (Figure 4). Like the effects in LPS-stimulated monocytes, only a 30-minute and not a 4-hour preincubation of the LPS-stimulated macrophages with ATRA decreased the TF activity level (12% of control).

**Discussion**

Induction of monocytic TF expression is mediated by signaling pathways, which can be modulated by PPARα activation.

In the present study, we determined whether incubation with PPARα agonists modulates TF expression in the human monocytic THP-1 cell line and in human monocytes and macrophages. It was previously demonstrated that PPARα is
cross talk of PPARα with other transcription factors, such as Jun-Fos and NF-κB.

In conclusion, the PPARα agonists fenofibric acid, WY14643, and GW2331 all inhibit the upregulation of TF expression, which occurs after stimulation of THP-1 cells or human monocytes with LPS or IL-1β. The effect of PPARα stimulation on monocyte and macrophage TF expression suggests a novel role for PPARα in atherosclerosis by influencing atherosclerotic plaque thrombogenicity. In vivo studies using atherosclerotic animal models may elucidate whether PPARα is able to reduce the thrombogenicity of atherosclerotic plaques by lowering TF expression.

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