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 PRE, 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. 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. Fifteen micrograms of RNA was separated by electrophoresis and transferred to nylon membranes. Northern blots were hybridized at 68°C with radiolabeled TF or 36B4cDNA 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’-CTAGAATTCACAAATACTGTTGGCAGCAT-3’ and 5’-AAGGGATCCCTTTTCTCCTGGCC-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 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
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 monocyctic 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

**Figure 3.** Effect of different PPARα agonists and ATRA on TF activity in unstimulated and LPS- or IL-1β-stimulated human monocytes. Cells were incubated for 4 hours with either fenofibric acid (Fenofibr., 100 μmol/L), WY14643 (100 μmol/L), GW2331 (1 μmol/L), or ATRA (1 μmol/L) and then incubated without (A) or with LPS (0.4 ng/mL) (B, D) or IL-1β (10 ng/mL) (C) for 16 hours. Bars are mean ± SEM from 3 to 4 independent experiments. *Significant difference vs control (P < 0.05). D, TF activity expressed in monocytes incubated with or without 0.4 ng/mL LPS for indicated periods of time with 1 μmol/L ATRA.

**Figure 4.** Effect of PPARα activators and ATRA on TF activity in human macrophages stimulated with LPS. Primary human monocytes were differentiated for 12 days in presence of 10% human serum. Thereafter, cells were incubated in serum-free conditions with either fenofibric acid (Fenofibr., 100 μmol/L), WY14643 (10 μmol/L), GW2331 (1 μmol/L), or ATRA (1 μmol/L). After a 4-hour preincubation, 0.4 ng/mL LPS was added, and cells were incubated for another 16 hours before TF activity was measured. In parallel, cells were preincubated for 30 minutes with ATRA before 16-hour LPS stimulation.
present in primary human monocytes. Here, we extend these observations by showing that PPARα protein is also expressed in THP-1 cells. Furthermore, our study demonstrates that PPARα activation can inhibit TF upregulation in THP-1 cells and in primary human monocytes and macrophages. Thus, these results extend previous observations identifying a role for PPARα in human monocytes and, moreover, for the first time point out a possible role for PPARα in the control of atherosclerotic plaque thrombogenicity.

The activity of PPARα agonists on the downregulation of TF expression was compared with that of ATRA. Retinoic acid has been shown to inhibit the LPS-induced TF expression in human THP-1 cells, monocytes, and macrophages. We confirmed these effects for LPS- and IL-1β-induced TF mRNA expression in THP-1 cells. As previously demonstrated for macrophages, the inhibitory effect of ATRA on LPS-stimulated TF activity in monocytes appeared to depend on the time of preincubation with retinoids. Whereas preincubation of monocytes with ATRA for 4 hours inhibited IL-1β-induced TF activity, no inhibition of LPS-induced TF activity was observed. However, a limited preincubation of 30 minutes with ATRA completely prevented the LPS induction of TF activity in monocytes and macrophages. The bases for these apparent kinetic differences, as well as the mechanism by which ATRA inhibits the induction of TF mRNA expression, are poorly known.

PPARα may interfere with proatherogenic processes at different levels. First, PPARα exerts beneficial effects on atherosclerosis by changing plasma lipid and lipoprotein profiles toward less atherogenic levels. Second, PPARα interferes with the development of atherosclerosis by inhibiting inflammatory responses at the level of the vascular wall. PPARα may interfere with the early stages of atherosclerotic lesion development by affecting monocyte recruitment by inhibiting TNF-α-induced VCAM-1 expression in endothelial cells. Furthermore, PPARα may also influence later stages of atherosclerosis by inducing apoptosis of activated human macrophages. Our results demonstrate that PPARα activation also inhibits its TF expression, which is a major initiator of thrombosis. In addition, TF may mediate adhesion and migration of monocytes. Thus, inhibition of monocyte TF expression is another way by which PPARα may modulate atherogenic processes.

We have shown that PPARα activators inhibit the expression of TF after induction by the inflammatory stimuli LPS and IL-1β. Induction of TF mRNA by LPS has been shown to occur via Jun phosphorylation and NF-κB translocation in a rapid but transient way. PPARα inhibits the proinflammatory AP-1 and NF-κB signaling pathways by repression of both c-Jun and p65 transcription activity. Because these factors also control TF promoter transcription, it is likely that PPARα modulates TF expression also by interfering negatively with the AP-1 and/or NF-κB activation pathway. Further molecular studies are necessary to determine whether the repression of TF gene expression by PPARα agonists indeed occurs via 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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