Review Article

PPARα/γ-Independent Effects of PPARα/γ Ligands on Cysteinyl Leukotriene Production in Mast Cells

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Received 3 March 2008; Revised 5 June 2008; Accepted 15 September 2008

Recommended by Francine M. Gregoire

Peroxisome proliferator-activated receptor (PPAR) α ligands (Wy-14,643, and fenofibrate) and PPARγ ligands (troglitazone and ciglitazone) inhibit antigen-induced cysteinyl leukotriene production in immunoglobulin E-treated mast cells. The inhibitory effect of these ligands on cysteinyl leukotriene production is quite strong and is almost equivalent to that of the anti-asthma compound zileuton. To develop new aspects for anti-asthma drugs the pharmacological target of these compounds should be clarified. Experiments with bone-marrow-derived mast cells from PPARα knockout mice and pharmacological inhibitors of PPARγ suggest that the inhibitory effects of these ligands are independent of PPARs α and γ. The mechanisms of the PPAR-independent inhibition by these agents on cysteinyl leukotriene production are discussed in this review.

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1. INTRODUCTION

Asthma is defined as “a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation” [1]. Many types of inflammatory cells, neutrophils, eosinophils, lymphocytes, and mast cells contribute to the development of asthma.

Mast cells are differentiated from bone marrow stem cells and release various mediators of inflammation, such as histamine, through degranulation and arachidonic acid metabolites through de novo synthesis in response to pathological stimuli in asthma, atopic dermatitis, and other conditions. Immunoglobulin (Ig) E, a protein from B lymphocytes, increases in the serum of patients with type I allergic diseases [2].

Arachidonic acid is metabolized into many biologically active lipids, such as prostaglandins via cyclooxygenase, and leukotrienes (LTs) via 5-lipoxygenase (5-LOX). Arachidonic acid liberated from membrane phospholipids by phospholipase A2 is then metabolized into LTA4 by the 5-LOX/FLAP activating protein (FLAP) complex (Figure 1). LTA4 is metabolized into LTC4 by conjugating cysteine, glycine, and glutamic acid via LTC synthase [3]. LTC4 is subsequently metabolized into LTD4 and LTE4 via the contribution of dipeptidases [4] or cytochrome P450 [5] by glutamic acid and glycine degradation (Figure 2). The LTs C4, D4, and E4 are called cysteinyl LTs (cysLTs) because they contain cysteine in their molecules. The cysLTs are regarded as main mediators of asthma because of their potent constricting effects on bronchiolar smooth muscle [6]. Specific receptors of cysLT are known [7, 8], and the inhibitors of the receptor [9] and the inhibitors of 5-LOX/FLAP activity [10–12] have been used to treat asthma.

Peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that are part of the nuclear receptor superfamily. The PPARs have 3 subtypes from the independent genes α, β (also called δ), and γ. A group of hypolipidemic agents, such as clofibrate and fenofibrate, are known to be ligands for PPARα, and some agents used to treat type 2 diabetes mellitus, such as rosiglitazone, pioglitazone, and ciglitazone, are known to be ligands for PPARγ. Some physiological fatty acids, such as leukotriene B4 and 15-deoxy-D12,14-prostaglandin J2, are reported to be ligands for PPARα and PPARγ, respectively [15, 16].
2. LIGANDS FOR PPARγ INHIBIT cysLT PRODUCTION IN MAST CELLS

Troglitazone (1 μM), a PPARγ ligand formerly used to treat type 2 diabetes mellitus, inhibits LTD₄, LTC₄, and LTE₄ production induced by the type 1 allergy mechanism in a mast cell line, RBL-2H3 [17]. The inhibitory effects of troglitazone on these LTs are strong and similar to those of the clinically-used 5-LOX inhibitor zileuton (1 μM) [17]. Another PPARγ ligand, ciglitazone (30 μM), also inhibits LTC₄ production [18]. Neither troglitazone nor ciglitazone affects hexosaminidase release, the index for mast cell degranulation, or prostaglandin D₂ production via cyclooxygenase [17, 18]. The observations that 0.1 μM of the PPARγ antagonist GW9662, which inhibits the PPARγ activation of (AOx)₃-TK-Luc promoter induced by the PPARγ antagonist GW9662, also inhibits LTC₄ production [18]. Neither troglitazone nor ciglitazone affects hexosaminidase release, the index for mast cell degranulation, or prostaglandin D₂ production via cyclooxygenase [17, 18]. The observations that 0.1 μM of the PPARγ antagonist GW9662, which inhibits the PPARγ activation of (AOx)₃-TK-Luc promoter induced by the PPARγ antagonist GW9662, also inhibits LTC₄ production [18].

3. LIGANDS FOR PPARα ALSO INHIBIT cysLT PRODUCTION IN MAST CELLS

Whether PPARα ligands affect LT production in mast cells has been examined, and the PPARα ligands fenofibrate (100 μM) and Wy-14,643 (30 μM) have been reported to inhibit calcium ionophore A23187-induced cysLT production by the RBL-2H3 mast cell line [13]. However, Wy-14,643 does not significantly inhibit cysLT production by the IgE-sensitized and Ag-treateBL-2H3 mast cell line. Neither fenofibrate (100 μM) nor Wy-14,643 (30 μM) affects radioactivity released from the IgE sensitized [³H]-arachidonic acid prelabeled RBL-2H3 mast cell line following treatment with Ag, which is an index of arachidonic acid release from mast cells. Neither fenofibrate (100 μM) nor Wy-14,643 (30 μM) affects lipid peroxidation, which is an index of 5-LOX activation, whereas troglitazone (1 μM) and zileuton (1 μM) strongly inhibit lipid peroxidation [13].

4. ARE THE INHIBITORY EFFECTS OF THESE PPARs LIGANDS VIA PPARs?

Subsequently, the mRNA levels of PPARα and γ were examined in mast cells. There were no significant PPARα [13] and PPARγ (our unpublished data) bands on Northern blot analysis of the RBL-2H3 mast cell line or of mouse bone marrow-derived mast cells (BMMCs). Then, PPARα [13] and γ [14] mRNA levels in RBL-2H3 mast cell line were measured with the real-time semiquantitative polymerase chain reaction (PCR) and compared with levels in other organs. The PPARα mRNA level is less than the level in 1000-times diluted liver, and the PPARγ mRNA level is almost the same as the level in 100-times diluted white adipose tissue (Figure 3).

These observations that mast cells have very low levels of PPARα/γ mRNA lead to another question: are these PPARs in mast cells effective?

Studies have examined whether fenofibrate (100 μM) raises acyl-CoA oxidase mRNA levels, which are known to be induced by PPARα activation [20, 21], and have shown that fenofibrate does not increase acyl-CoA oxidase mRNA levels in the RBL-2H3 mast cell line [13]. The effects of these PPARα ligands on BMMCs from PPARα-null mice were thoroughly examined, and both fenofibrate (100 μM) and Wy-14,643 (30 μM) were found to inhibit cysLT production [13]. It has been concluded that these compounds inhibit cysLT production independently of PPARα.

We have observed that the immunoreactivity of anti-PPARγ IgG in the RBL-2H3 mast cell line though ciglitazone (30 μM) does not induce the mRNA level of acyl-CoA binding protein [18], which is a target gene of PPARγ [22]. Diaz et al. [23] have examined PPARγ protein in mouse BMMCs by SDS-PAGE immunoblot analysis and reported that the amount of PPARγ in BMMCs is equivalent to that in the Jurkat T-cell line, which is known to have effective PPARγ [24]. Maeyama et al. [25] have demonstrated that rosiglitazone (1–30 μM) increases the proliferation of BMMCs, but that the proliferation is not observed in BMMCs from PPARγ heterozygous deficient mice. Ward and Tan [26] have reviewed the contents of PPARs in various types of cells and have concluded that the PPARγ in mast cells might play a role, and Paruchuri et al. [27] have recently reported that LTE₄-induced COX-2 induction, prostaglandin D₃ production, and ERK phosphorylation are sensitive for the interference of PPARγ in the human mast cell sarcoma line LAD2 and may indicate a role of PPARγ in mast cells. Further studies of the role of PPARγ in mast cells are necessary.

5. WHAT IS THE TARGET?

The experimental findings that PPARα and γ in mast cells seem not to be effective at very low mRNA levels lead to another question: what is the target of these compounds?

Fenofibrate (25 mg/kg p.o. for 10 days) induces proliferation of peroxisomes even in PPARα-null mice [28]. Wy-14,643 (75 μM) induces plasminogen activator inhibitor I with the induction of p38 and p42 mitogen-activated protein kinase (Figure 4).
kinase (MAPK) phosphorylation 5 minutes after treatment, which would be too early for the induction to occur via transcription [29]. The ligand Wy-14,643 (1 μM) leads to the phosphorylation of extracellular signal-regulated kinase (ERK) after 5 minutes of treatment but does not increase acyl-CoA oxidase mRNA levels [30].

The PPARγ ligands ciglitazone (20 μM) and 15-deoxy-D12-14 prostaglandin J2 (15 μM) induce ERK, c-Jun N-terminal kinase, and p38 MAPK after 15 minutes of treatment, which might be earlier than transcription occurs [31]. The inducible effects of PPARγ ligands on MAPK have been reported elsewhere [32, 33], and most authors have concluded that these effects are independent of PPARγ.

MAPK is reported to induce 5-LOX activity in human polymorphonuclear cells and the Mono Mac 6 human monocytic leukemia cell line [34], and these findings may support the presence of PPAR-independent effects of PPARα and γ ligands. However, MAPK phosphorylation has not been observed in mast cells treated with these PPAR ligands. The stimulating effect of these compounds on MAPK seems not to be the main mechanism of the PPAR-independent inhibition of cysLT production because it might increase the production of cysLTSs.

The cysLT concentration is determined by subtracting degradation from production, and the PPAR-independent activation of MAPK increases cysLT production in mast cells. The degradation of cysLTSs could be another mechanism of these drugs. The responsible enzymes of cysLT metabolism remain unclear. Recent findings that LTC4 is metabolized into LTD4 by γ-glutamyltransferase and γ-glutamylleukotrienease and that of double knockout mice of these enzymes do not metabolize LTC4 into LTD4 may indicate that these enzymes are the enzymes responsible for LTC4 degradation [35]. The degradation of LTD4 into LTE4 is reported to occur partly because of dipeptidase [36], but the responsible enzyme is still unclear. Induction of cytochrome P450 (CYP) 2B1/2 by phenobarbital in rats and the decrease in LTC4 concentrations in liver extract suggest the involvement of CYP2B1/2 in LTC4 degradation [37]. The CYP family comprises a large number of enzymes, and we do not yet have sufficient information on the contribution of CYP to cysLT metabolism.

Fujimura et al. [38] have reported that incubation with prostaglandin A1 (as PPARβ/δ ligand) and 15-deoxy-D12-14 prostaglandin J2 (as PPARγ ligand) for more than 6 hours decreases the surface IgE receptor FcεRI in the KU812 human basophilic cell line, whereas LTB4 (as PPARα ligand) does not. The PPARα and γ ligands were preincubated for 2 hours before antigen treatment in mast cells [13, 17, 18], and the decrease of FcεRI on the surface of mast cells is not the main mechanism of the PPAR-independent inhibition of cysLT production. Regulation of the sensitivity to antigens is of pathological interest in allergic diseases, including asthma, and the interaction of mast cells with other inflammatory cells in pathological conditions should be examined.

6. CONCLUSION

These findings show that some effects of ligands of PPARα and γ occur through a mechanism independent of PPARα and γ. The involvement of PPARα and γ should be examined in pharmacological experiments of PPAR ligands and of ligands of other nuclear receptors.

The involvement of PPARα in the effects of PPAR ligands can be investigated in PPARα-null mice [39] and at lower cost in mast cells, as described above.
PAR-null mice die at 10.5 to 11.5 days post coitum because of placental dysfunction [40], and the contribution of PPARγ cannot be examined in PPARγ-homozygous knockout mice. One of the mutants of the PPARγ2 sub-

**ACKNOWLEDGMENTS**

Part of this work, appearing in Figure 3, was performed in the NCI Intramural Research Program in Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA. The publication was supported by the special subsidies of subsidies for ordinary expenses of private school from the promotion and mutual aid corporation for private schools of Japan, and the Nihon University College of Bioresource Sciences Research Fund for 2008.

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