Chemical Probes That Differentially Modulate Peroxisome Proliferator-activated Receptor α and BLTR, Nuclear and Cell Surface Receptors for Leukotriene B₄*

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Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor for various fatty acids, eicosanoids, and hypolipidemic drugs. In the presence of ligand, this transcription factor increases expression of target genes that are primarily associated with lipid homeostasis. We have previously reported PPARα as a nuclear receptor of the inflammatory mediator leukotriene B₄ (LTB₄) and demonstrated an anti-inflammatory function for PPARα in vitro (Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1997) Nature 384, 39–43). LTB₄ also has a cell surface receptor (BLTR) that mediates proinflammatory events, such as chemotaxis and chemokinesis (Yokomizo, T., Izumi, T., Chang, K., Takuwa, Y., and Shimizu, T. (1997) Nature 387, 620–624). In this study, we report on chemical probes that differentially modulate activity of these two LTB₄ receptors. The compounds selected were originally characterized as synthetic BLTR effectors, both agonists and antagonists. Here, we evaluate the compounds as effectors of the three PPAR isotypes (α, β, and γ) by transient transfection assays and also determine whether the compounds are ligands for these nuclear receptors by coactivator-dependent receptor ligand interaction assay, a semi-functional in vitro assay. Because the compounds are PPARα selective, we further analyze their potency in a biological assay for the PPARα-mediated activity of lipid accumulation. These chemical probes will prove invaluable in dissecting processes that involve nuclear and cell surface LTB₄ receptors and also aid in drug discovery programs.

Hormones and nutrient-derived molecules, such as retinoids and fatty acid derivatives, are important signals in many biological processes. Dysregulation or disruption of their signaling pathways can manifest in various ways, with defects that range in severity, rate of onset, and organ systems affected. For instance, a prolonged disturbance of lipid homeostasis is often associated with many late-onset inflammatory conditions, obesity, diabetes, and cardiovascular disease. In order to efficaciously treat and prevent these prominent metabolic problems, a better understanding of the mechanisms involved in lipid regulation is required.

Intracellular targets for lipid mediators have been postulated for many years (1). However, it is only recently that we have seen the emergence of reports describing nuclear receptors for fatty acids and their derivatives (see Refs. 2 and 3 and references therein). Particular attention has focused on a group of ligand-activated transcription factors called peroxisome proliferator-activated receptors (PPARs). The three PPAR isoforms (α, β/δ, and γ) form a distinct subclass of the nuclear hormone receptor superfAMILY (4). The functional complex is a heterodimer of PPAR and the retinoid X acid receptor (RXR) that binds to a consensus sequence in the promoter of target genes and can up-regulate transcription in the presence of a PPAR ligand. Although the PPAR target genes identified so far, are generally associated with lipid homeostasis, the extent of their involvement in biological processes related to disease are yet to be elucidated.

In transient transfection experiments, PPARα activity can be induced by a range of structurally diverse compounds (see Ref. 5 and references therein). Many of these natural and synthetic compounds are bona fide PPARα ligands (6–11). Although it is now clear that PPARα is a nuclear receptor for various fatty acids (e.g. linoleic and arachidonic acid), eicosanoids (e.g. 8(S)-HETE and LTB₄) and hypolipidemic drugs (e.g. fibrates and Wy14,643), we are only beginning to understand the functional relevance of these receptor-ligand interactions.

Our knowledge of the biology of PPARα is still superficial but is increasing rapidly. Extensive analyses of PPARα knockout mice indicate that PPARα can be associated with many homeostatic functions of peroxisomes and also with response to various compounds termed peroxisome proliferators (for review, see Ref. 12). For example, wild-type mice respond to hypolipidemic drugs such as fibrates and Wy14,643; but the PPARα(–/–) mice display neither lowering of blood lipid levels nor the proliferation of peroxisomes in the liver (13, 14). Consistent with this, PPARα(–/–) mice exhibit normal basal levels of hepatic fatty acid ω- and β-oxidation. However, they lack the ability to increase expression of these genes in response to various peroxisome proliferators (13). In the liver, these induc...
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ible pathways serve not only to regulate normal dietary fatty acids but also as a detoxification or degradation process for xenobiotics and potent eicosanoid mediators, such as the chemotactrant leukotriene B₄ (15, 16).

PPARα has also been evaluated for its ability to induce adipogenesis (17). In vitro, NIH 3T3 fibroblasts can be retrovirally infected to express high levels of PPARα and then challenged with potent activators to promote lipid droplet accumulation. High levels of lipid accumulation can in turn potentiate a pathway mediated by the adipogenic regulator, PPARγ.

So far, two studies have reported a role for PPARα in inflammation. Recently, Staels et al. (18) have proposed that in vascular walls, PPARα has an anti-inflammatory action that is mediated via repression of NF-κB signaling. We have previously shown, in vivo, that PPARα has a role in inflammation control (6, 19). Compared with wild-type mice, the PPARα(--/--) mouse exhibits a prolonged inflammatory response when challenged by the eicosanoid LTB₄ and its precursor arachidonic acid, but not the phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Based on the available data, a potential mechanism proposed for this anti-inflammatory role of PPARα is a negative feedback loop. LTB₄ would effectively control its own degradation by initiating the up-regulation of the fatty acid oxidation pathways. This catabolic inactivation of the eicosanoid is facilitated by direct interaction and activation of PPARα, a nuclear receptor for LTB₄ (K₉₀ of 60 and 90 nM, determined by two independent groups using fluorometric (20) and radioligand (6) binding assays).

Leukotriene B₄ also binds to a cell surface receptor, BLTR (21). A full-length cDNA for BLTR has recently been isolated from human HL-60 leukemia cells and characterized (22). The predicted protein contains the classical seven membrane-spanning domains but shows little amino acid homology to other known proteins. The proinflammatory effects of LTB₄ are thought to be triggered by high affinity binding to the BLTR on immune cells (K₉₀ = 0.15 nM). Analyses of downstream signaling pathways in stably transfected Chinese hamster ovary cells indicate that BLTR potentially couples to different G-proteins.

At the mechanistic level, little is known about the LTB₄ signaling pathways. Elucidation of these pathways would be greatly facilitated by the use of chemical probes that could differentially target cell surface and nuclear LTB₄ receptors. PPARα and BLTR are promising targets in therapeutic intervention for lipid-related disorders. Indeed, the pharmaceutical industry has deployed considerable resources to the development of antagonists of BLTR as anti-inflammatory drugs and agonists of PPARα as lipid-lowering compounds. Here, we investigate some of these compounds to find useful probes for BLTR and PPARα function, the reasoning being that if PPARα and BLTR share a ligand (LTB₄), then one would expect to find some overlap in recognition of other ligands. We report the effect of some BLTR effectors on transcriptional activation by the three PPAR subtypes. We then established whether the effects are mediated by direct interaction with the PPARs. Finally, we used NIH 3T3-mPPARα cells as a biological assay to evaluate the potential of these activators in the PPARα-associated activity of lipid accumulation.

**EXPERIMENTAL PROCEDURES**

**Transient Transfections**—Transfections were performed using the calcium phosphate precipitation technique in HeLa cells, as described previously (6). Transfected cells were incubated with indicated concentrations of test compound (ZK151657, ZK158252, ZK183838 (Schering), LTB₄ (Cascade Biochemicals Ltd.), or Wy14,643 (Campro Scientific)) or solvent (Me₂SO). Chloramphenicol acetyltransferase (CAT) assays were normalized using β-galactosidase activities (internal standard).

**Fusion Protein Constructs and Protein Expression**—The LB4 fragment (shown in Fig. 2a) for mPPARα and mPPARβ were amplified by PCR from clones containing the full-length sequences, whereas mPPARγ fragment was isolated as a BamHI-XbaI fragment from the vector pβG5-mPPAR-γ-stop. The fragments were cloned in-frame into BamHI site of either pGEX-1 (for mPPARα) or pGEX-SX-3 (for mPPARβ and mPPARγ).

The GST-PPAR (LBD) fusion proteins were expressed in Escherichia coli BL2 DE3 (pLysS) bacteria. Briefly, a freshly transformed colony was used to start a 2-ml inoculum (in log phase) for a 500-ml culture. After 12 h, cultures were diluted with 500 ml of medium and induced with either 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 4–6 h (for mPPARβ and mPPARγ) or 0.1 mM isopropyl-1-thio-β-D-galactopyranoside for 9 h (for mPPARα). All cultures were grown at 30 °C in LB medium containing 17 μg/ml chloramphenicol and 50 μg/ml ampicillin. Bacteria pellets were stored at −70 °C as aliquots equivalent to 50 ml of culture.

**CARLA**—The CARLA was performed as described by Krey et al. (11) with the following modifications. The above bacterial pellets were resuspended in 10 ml of lysis buffer (phosphate-buffered saline A containing 1% Triton X-100 and 0.5 mM phenylmethylsulfonyl fluoride) and lysed by repeated freeze-thaw. The DNA and insoluble matter were removed by centrifugation. Fusion proteins were purified onto glutathione-Sepharose beads at 4 °C (Amersham Pharmacia Biotech), washed three times in lysis buffer, and equilibrated in NETN buffer (20 mM Mes, 0.1% SDS, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, and 1 mM dithiothreitol) supplemented with 1% (w/v) of dry 2% milk powder prior to use in CARLA. The amount of protein used per reaction was 1 μg for mPPARα and 2–3 μg for mPPARβ and mPPARγ. Reactions were performed in NETN buffer supplemented with 1% (w/v) of dry 2% milk powder, and the first wash contained 0.5% milk powder. The exposure times for autoradiography were 12–24 h for mPPARα and 1–4 h for mPPARβ and mPPARγ.

**Adipogenesis Assay**—Generation of virally infected stable NIH mPPARα cell lines and adipogenesis assays were performed as described by Brun et al. (17). All test compounds were dissolved in Me₂SO. Cells were used for either Oil Red O staining of neutral lipids or RNA isolation followed by Northern blot analysis of the p2 message.

**LIC Assay**—The ligand-induced complex (LIC) assays were performed similar to Forman et al. (8). Briefly, reaction volumes were 20 μl. The two proteins, baculovirus-expressed mRXRβ (0.4 μl) and mPPARα translated mPPARα (0.7 μl), were incubated for 10 min on ice in 10 mM Tris-HCl, pH 8.5, 100 mM KCl, 2 mM MgCl₂, 1 mM dithiothreitol, 10% glycerol, 0.1% Nonidet P-40, and 3.5 μg of poly(dI-dC). After addition of test compounds (eicosanoids, drugs, or solvent control) and a 30-min incubation on ice, 1 ng of radiolabeled probe (the PPAR response element consensus site) was added, and the reaction was incubated for 20 min at room temperature. Complexes were resolved on 5% nondenaturing polyacrylamide gels in 0.5× TBE. Gels were dried, and results were visualized by autoradiography.

**RESULTS**

**BLTR Effectors Are Partial Agonists of PPARα—**LTB₄ acts as a potent chemotactrant for leukocytes (23). This process involves binding and activation of the LTB₄ to the cell surface receptor, BLTR (22). Based on this biological activity, one can evaluate effectors of BLTR by their ability to modulate LTB₄-induced neutrophil chemotaxis. Compounds that enhance chemotaxis are classified as BLTR agonists, and those that inhibit chemotaxis are classified as antagonists. In this study, we used four BLTR effectors (Ref. 24 and Table I): two agonists (LTB₄ itself and ZK151657) and two antagonists (ZK158252 and ZK183838). For convenience, the synthetic compounds ZK151657, ZK158252 and ZK183838 are referred to as BLTR agonist 1 (BAg1), BLTR antagonist 1 (BAntag1), and BAntag2, respectively.

The effects of the compounds on the three PPAR isotypes were evaluated by transient transfection experiments. In this system, HeLa cells are co-transfected with three constructs: a PPAR expression vector, a reporter construct containing the CAT gene driven by a PPAR-responsive promoter element (see Fig. 1A), and a vector that constitutively expresses β-galactosidase to standardize between different samples. After exposure to test compounds for 48 h, cells were harvested and lysed, and the CAT enzymatic activity per β-galactosidase unit was...
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Xenopus (24).

TABLE I
Effect of chemical probes on BLTR function
Chemotaxis of human PMNs in a modified Boyden chamber assay (24).

| BLTR   | Chemotactic coefficient† |
|--------|---------------------------|
| Agonists |                           |
| LTB4    | 3.4 × 10⁻⁹               |
| ZK 151 657 | 2.0 × 10⁻⁶               |
| (BAg1)   |                           |
| ZK 158 252 | 2.1 × 10⁻⁸               |
| (BAntag1) |                           |
| ZK 183 838 | 7.1 × 10⁻⁸               |
| (BAntag2) |                           |

† Induction by agonists shown as EC₅₀; inhibition by antagonists shown as Kᵢₐ.

measured. A test compound that increases standardized CAT activity in a PPAR-dependent manner was classified as a PPAR activator.

We first determined the effect of 10 µM concentrations of the synthetic compounds on the three isotypes of mouse PPARs (mPPARs, see Fig. 1B). Transfection results of empty pSG5 expression vector indicate that in the absence of exogenous PPAR, none of the compounds induced a positive response from the reporter construct. However, when the different mPPARs were cotransfected, isotype-specific responses to the synthetic compounds were observed. BAg1 and BAntag1 are activators that are selective for mPPARα. At a 10 µM concentration, BAg1 also induced a small response in the presence of mPPARβ. The third compound, BAntag2, did not induce mPPAR activity. Interestingly, mPPARγ activity was not induced by these compounds. Similar activation profiles were observed from PPARγ from other species (Xenopus and human) (data not shown).

The level of mPPARα activation observed with 10 µM of BAg1 and BAntag1 was 40–50% that of the standard activator and ligand Wy14,643. This could reflect either that higher doses of the compounds are required for maximal activation or that the compounds are partial agonists. An activation profile showing the mPPARα response in the presence of increasing amounts of compound, helps to differentiate between the two scenarios (Fig. 1C). In these transient transfection assays, BAg1 and BAntag1 are partial agonists with equal efficacy; at 10 µM, they gave quantitatively indistinguishable responses. Log dose curves show that BAg1 (EC₅₀ = 0.5 µM) is more potent than BAntag1 (EC₅₀ = 8 µM) and LTB₄ (EC₅₀ > 10 µM).

BAg1 and BAntag1 Are PPARα Ligands—The transient transfection assays report the activity of PPARs when cells are challenged with a given compound. This increased transcriptional activity could conceivably be a consequence of different mechanisms: a direct interaction of the compound with the nuclear receptor, an indirect mechanism of activation, such as production of a metabolite, or a combination of both. We used a semifunctional assay to evaluate whether the compounds can directly bind to the PPARs and render them functionally active.

Transcriptional activation by nuclear hormone receptors can be mediated by a class of proteins called co-activators (25). The current model indicates that the binding of a ligand to the nuclear hormone receptor induces a conformational change in the ligand-binding domain (LBD) that allows interaction with the co-activator. For example, the steroid receptor coactivator 1 (SRC-1) binds to xPPARα in the presence of its ligand Wy14,643 but not in the presence of a nonligand such as β-estradiol. This type of semifunctional assay (CARLA, see Fig. 2A), has been successfully exploited to identify different fatty acids, eicosanoids, and hypolipidemic agents as ligands for PPARs (11).

FIG. 1. A, CAT reporter plasmid for transient transfection assays. The promoter consists of two copies of the CYP4A6 PPAR response element (in a palindromic configuration) upstream of the thymidine kinase minimal promoter. B, effect of 10 µM compounds on mPPARs. Graphs indicate the standardized CAT activity obtained for the for BLTR effectors (LTB₄, BAg1, BAntag1, and BAntag2), the hypolipidemic drug Wy14,643, and solvent control (Me₂SO (DMSO)), when HeLa cells are transfected with either vector pSG5 (open columns) or vector containing mPPARα (striped columns), β (dark shaded columns), or γ (light shaded columns). C, effect of increasing concentrations of BAg1, BAntag1, and LTB₄ on mPPARs activity. HeLa transient transfection experiments indicate an EC₅₀ of 0.5 µM for BAg1 (rectangles), 8 µM for BAntag1 (triangles), and >10 µM for LTB₄ (circles). Results above are a summary of experiments done at least in triplicate.

Studies have indicated that although PPAR response to natural compounds, such as fatty acids and eicosanoids, is conserved between species, response to xenobiotics can be species-specific (10). For this reason, GST fusion proteins with the different mouse PPAR LBDs were constructed (Fig. 2, B and C). Expression of soluble fusion protein of the mouse PPARα isotype was not as efficient as for PPARβ and PPARγ. However, all proteins were active, as confirmed by CARLA on known
CARLA was used to evaluate the three BLTR effectors as ligands of mPPARs (Fig. 3). At 10 μM concentrations, BAg1 and BAntag1 were ligands of mPPARα, but BAntag2 was not. Similar profiles were obtained with the Xenopus PPARα (data not shown). The amino acids of the different PPARs included in the GST fusion proteins are indicated in the schematic. C, soluble proteins were partially purified on glutathione-Sepharose beads, and the beads were analyzed on a 10% SDS-polyacrylamide gel electrophoresis Coomassie stain. The sizes of the Bio-Rad low molecular weight standards (M) are indicated (in thousands), and the proteins of interest are marked to the left by a filled circle. D, evaluation of fusion proteins by CARLA. Autoradiograms showing amount of radiolabeled SRC-1 pulled down by respective GST fusion proteins (either mPPARα, β, or γ). The solvent used was ethanol. Synthetic ligands (10 μM) used were Wy14,643 for mPPARα, Merck A for mPPARβ (40), and BRL 49643 for mPPARγ (41). Nonligands (10 μM) used were BRL 49643 for mPPARα and Wy14,643 for mPPARβ and mPPARγ. LTB₄, the ligand for mPPARα was used at 50 μM.}

Fig. 2. A, schematic of CARLA. Fusion proteins of GST and the LBD of the nuclear hormone receptor were bacterially expressed and partially purified onto glutathione-Sepharose beads (Amersham Pharmacia Biotech). Beads were incubated with test compound (solvent control, ligand, or nonligand) and radiolabeled SRC-1 (produced in vitro using a coupled transcription-translation rabbit reticulocyte lysate system (Promega)). The reaction was incubated to equilibrium, and beads were recuperated by centrifugation. Beads were then washed and analyzed for interaction SRC-1 using SDS-polyacrylamide gel electrophoresis. Coomassie staining of GST-LBD fusions allows standardization between different reactions. The SRC-1 protein is visualized by autoradiography. Because a ligand enhances interaction between the LBD and SRC-1, the amount of SRC-1 pulled down in the presence of a ligand was higher than in the absence of ligand (solvent or nonligand compound). B, GST-mPPAR(LBD) fusions. The sizes of the Bio-Rad low molecular weight standards (M) are indicated (in thousands), and the proteins of interest are marked to the left by a filled circle.
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NIH PPARα stable cell lines were created by transduction of NIH 3T3 fibroblasts with packaged pBABE-mPPARα retroviral vector. Cells were cultured under conditions that permit adipogenesis and challenged with the various compounds. The effects of the compounds on cells were analyzed by staining of neutral lipids with Oil Red O or Northern blot analyses showing expression of the adipocyte fatty acid-binding protein, aP2. BAg1, the most potent inducer of lipid accumulation, also induced expression of this adipocyte fatty acid-binding protein.

Stabilizing LTB₄—The adipogenesis assay result with LTB₄ (see Fig. 5) can be improved significantly by either stabilizing the reactive triene system (BAg1) or by introducing a trifluoromethyl group at the ω-position (CF₃-LTB₄). In the LIC assay (8), complex formation between different samples. We have already seen above that BAg1 is more efficacious than LTB₄ in both transient transfections for mPPARα and LTB₄-mediated lipid accumulation than Wy14,643. The staining pattern with BAntag1 was positive, but less efficient than the standard Wy14,643. BAntag2 scored negative in this assay.

Depending on their potency in lipid accumulation, activators of PPARα can also trigger the adipogenic program in NIH PPARα cells (17). For instance, Wy14,643 will induce expression of adipogenic genes, whereas a weaker mPPARα activator will not. Analyses of NIH mPPARα cells treated with BLTR effectors were consistent with this (Fig. 4B). The weak inducer of lipid accumulation (BAntag1) did not significantly increase aP2 mRNA, whereas BAg1, the most potent inducer of lipid accumulation, also induced expression of this adipocyte fatty acid-binding protein.

FIG. 3. BLTR effectors as ligands for PPARs. CARLAs with GST-LBD fusion proteins of the three PPAR isotypes (α, β, and γ) were used to test BAg1, BAntag1, and BAntag2 as ligands for the different PPARαs. One μg of GST-PPAR (LBD) fusion protein was used for α, and 3 μg was used for β and γ. Compounds were used at concentration of 10 μM. Lanes with solvent alone (MeSO) show the background level of SRC-1 obtained in the assay. Graphical representation of the average intensity of the SRC-1 bands for each duplicate is shown below. Me2SO lanes (DMSO) are used as reference point for each protein (α, β, and γ). BAg1 and BAntag1 were the ligands for mPPARα. BAg1 also bound to mPPARβ. By this assay, none of the BLTR effectors were found to be ligands for mPPARγ.

FIG. 4. Biological assay. NIH PPARα cells were treated with 10 μM of the following compounds: either the BLTR effectors (LTB₄, BAg1, BAntag1, and BAntag2) or controls (MeSO (DMSO) solvent as a negative control and lipid lowering drug Wy14,643 as a positive control). After 7 days of treatment, they were evaluated for lipid accumulation and expression of the adipocyte fatty acid-binding protein, aP2. A. Oil Red O staining of neutral lipids. B. Northern blot analyses showing aP2 mRNA. The 36B4 message was used to standardize amounts of RNA between different samples.

aP2 mRNA, whereas BAg1, the most potent inducer of lipid accumulation, also induced expression of this adipocyte fatty acid-binding protein.

We have already seen above that BAg1 is more efficacious than LTB₄ in both transient transfections for mPPARα (EC₅₀ of 0.5 versus >10 μM) and in adipogenesis assays using NIH mPPARα cells. We evaluated whether making LTB₄ stable at ω-oxidation induces a better response in different assay systems (Fig. 6). In transient transfection assays, the EC₅₀ for mPPARα shifted from >10 μM LTB₄ to 0.8 μM CF₃-LTB₄ (Fig. 6B). Results from the ligand detection assays are consistent with this (Fig. 6, C and D). In the LIC assay (8), complex
formation was observed at 50 μM LTB₄, and stabilizing against ω-oxidation resulted in almost a 100-fold less concentration of CF₃-LTB₄ required for LIC detection (Fig. 6C). The complexes observed are ligand-specific and do not occur in the presence of 50 or 100 μM of non-PPARα ligands, such as β-estradiol or the eicosanoid and LTB₄ precursor 5S-HETE. Consistent with previous reports (8), the PPAR-RR complex did not occur at limiting concentrations of mPPARα but could be induced in the presence of 1 μM Wy14,643. The apparent affinity of mPPARα for CF₃-LTB₄ as detected by CARLA (Fig. 6D) is consistent with that observed in the LIC assay (0.5 μM). Finally, we evaluated CF₃-LTB₄ in the adipogenesis assay. When NIH mPPARα cells were induced with as little as 1 μM CF₃-LTB₄ the result was significant lipid accumulation (Fig. 6E). Thus, effectively increasing the lifetime of LTB₄, by stabilizing against ω-oxidation, resulted in the expected responses in the different assay systems.

DISCUSSION

Following its isolation, leukotriene B₄ was recognized as a potent chemoattractant and aggregatory agent in leukocytes (29, 21). The realization of LTB₄ as a proinflammatory signal triggered many drug discovery programs for anti-inflammatory agents, including the synthesis of banks of compounds as effectors of LTB₄-mediated processes. With the help of these chemical probes, rapid progress has been made in understanding the biology of LTB₄.

It is now clear that the action of LTB₄ is tightly regulated at many levels (for review, see Ref. 30). Below, we outline some key features of regulation of LTB₄ biosynthesis, specificity of response, and inactivation by catabolism (Fig. 7). LTB₄ is a downstream product of the 5-lipoxygenase (5-LO) pathway (31, 32). Accumulating evidence suggests that when activated, cytosolic and intranuclear pools of 5-LO translocate to the nuclear membrane, where they channel arachidonic acid to leukotriene biosynthesis (33). This activity is facilitated by FLAP, a nuclear envelope protein that binds the arachidonate released from the nuclear envelope phospholipids (34, 35). The translocation of the FLAP/5-LO complex to the nuclear membrane conceivably results in two pools of leukotrienes. The nuclear pool predicts both target(s) and functions for leukotrienes. Consistent with this is the identification of PPARα as a nuclear receptor for LTB₄ (6). Large quantities of LTB₄ are also released to the extracellular milieu to recruit circulating leukocytes (36). For many years, chemotaxis of the immune cells has been postulated to be mediated by a seven-membrane-spanning receptor. Recently, the full-length cDNA of BLTR from human HL-60 monocyte cell line has been isolated as a G protein-coupled cell surface receptor, which is functionally confirmed by its ability to render Chinese hamster ovary cells responsive to LTB₄ (22). One potential route for the inactivation of LTB₄ is through catabolism via the fatty acid ω- and β-oxidation pathways (16). This process can be up-regulated by exposure to polyunsaturated fatty acids; to xenobiotics, such as the hypolipidemic drug clofibrate; and also to LTB₂ itself. These structurally unrelated compounds have recently been shown to be activators and ligands of the nuclear receptor PPARα (for review, see Ref. 5).

Synthesis, uptake, and response to leukotriene B₄ are cell-type dependent (for review, see Ref. 37). For instance, polymorphonuclear leukocytes respond to LTB₄ by chemotaxis and hyperadhesion, and once activated, they can both produce and catabolize the eicosanoid. Hepatocytes, on the other hand, are efficient at clearing LTB₄ from the system via uptake and catabolism but they are unable to synthesize LTB₄ from arachidonic acid.

At a simplistic level, one can correlate the tissue expression patterns of the two LTB₄ receptors with what is known about their function thus far. The cell surface receptor, BLTR responds to subnanomolar concentrations of LTB₄, and is expressed primarily in leukocytes, where it mediates chemotaxis and hyperadhesiveness (21). The nuclear receptor PPARα has a 1000-fold lower affinity for LTB₄ and also exhibits a less restricted expression pattern (e.g. liver, kidney, brown adipose tissue, and immune system). Both characteristics of PPARα reflect a broader role in adaptive responses of the organism, including lipid homeostasis and detoxification of xenobiotics and lipid mediators (38). The different affinities of the two receptors for LTB₄, as well as the overlap in expression patterns, potentially result in complex cell contexts. For instance, polymorphonuclear leukocytes express both cell surface and nuclear receptors for LTB₄. This poses an interesting problem because polymophonuclear leukocytes are capable of all aspects of LTB₄ processes (production, response, and catabolism), and the balance of these activities is critical to the final biological outcome of inflammation (Fig. 7). To evaluate the mechanisms of cross-talk, we need tools that will differentially modulate activities of the two LTB₄ receptors.

BLTR Effectors—The structures of the BLTR effectors used are depicted in Fig. 5. Two compounds, the BLTR agonist ZK151657 (BAg1) and BLTR antagonist ZK158252 (BAnag1) are from libraries based on the structure of leukotriene B₄. For example, BAg1 is a stable BLTR agonist resulting from replacement of the reactive conjugated triene system in LTB₄ with a substituted pyridine ring. The third compound, ZK183838 (BAnag2), can be described as an acetylphenone type derivative or aryl-carboxylic acid derivative. It was isolated from a random compound library, and its structure is not obviously related to LTB₄.

BLTR and PPARα Are Pharmacologically Distinct—The three synthetic compounds (Fig. 5 and Table II) cover an interesting spectrum of activity on the two LTB₄ receptors. ZK151657 (BAg1) is an efficacious activator of both nuclear and cell surface receptors. Compared with the natural agonist and ligand LTB₄, the compound is more potent on PPARα activity. This is reflected in both the transient transfection assay (EC₅₀ of 0.5 μM) and the ability to accumulate lipid droplets in the biological assay. Even though ZK151657 is only a partial agonist of mPPARα, the adipogenesis assay indicates that it is
potent enough to induce expression of the aP2 gene, which codes for the adipocyte fatty acid-binding protein. ZK158252 (BAntag1) has an interesting profile with opposite effects on the cell surface and nuclear receptors. It is a partial agonist of PPARα, with a relatively high EC50 of 8 μM. Evaluation by the biological assay indicates that it is potent enough to induce lipid droplet accumulation, but not to the extent where cells markedly increase expression of the adipocyte-specific genes. ZK183838 (BAntag2) is an antagonist of BLTR that does not induce PPARα activity in transfection experiments. Consequently, it does not induce either coactivator interaction or lipid accumulation in NIH mPPARα cells.

We are only beginning to understand the significance of the sharing of ligands as a means of cross-talk between cell surface and nuclear receptors. In such a scenario, understanding the response of a cell to the shared ligand requires tools that differentiate or uncouple the different receptors. An earlier report by Regnato et al. (39) studies the role of PPARγ as a nuclear receptor in cross-talk of signaling by two different eicosanoids. The focus of this study is BLTR and PPARα as receptors for a common eicosanoid ligand, LTB4. The three
synthetic ligands identified are promising tools to probe for detailed mechanisms of the interplay between nuclear and cell surface LTB₄ receptors. Given the importance and success of BLTR and PPARs ligands as therapeutic targets, this information will no doubt aid in drug discovery for disorders associated with disturbance of lipid homeostasis, including inflammatory disorders, diabetes, obesity, and cardiovascular disease.

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