The Potential of Peroxisome Proliferator-Activated Receptor γ (PPARγ) Ligands in the Treatment of Hematological Malignancies

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Abstract: PPARγ has emerged as a key regulator of cell growth and survival, whose activity is modulated by a number of synthetic and natural ligands. Here we shall review the activities of PPARγ ligands in the control of immune cell proliferation, differentiation and apoptosis and their potential therapeutic applications to hematological malignancies.

Key Words: PPARγ ligands, cancer, immune system, cell cycle, apoptosis, TZDs, myeloid neoplasms, lymphoid neoplasms.

1. PPARS: EXPRESSION, STRUCTURE AND FUNCTIONS

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of steroid, thyroid and retinoid nuclear receptors and were identified in the 1990s as mediators of peroxisome proliferators (PPs) [1]. PPARs exist in three isoforms, PPARα, PPARβ/δ and PPARγ, which are encoded by different genes and harbour specific expression patterns and functions. As members of the nuclear receptor family, transcriptional activity of PPARs is controlled principally by ligand binding, although post-translational modifications have been recently reported to affect the state of receptor activation depending on the isoform and cellular context. PPARs function as heterodimers with the retinoid X receptor (RXR). Upon ligand binding, PPAR-RXR complexes undergo conformational changes that are strictly ligand-dependent, leading to recruitment of either coactivators or corepressors, which results in opposing activities on the transcriptional state of target genes. Because PPAR-responsive elements are found in a wide array of genes involved in lipid metabolism, glucose homeostasis, cell proliferation and differentiation, as well as in inflammation and immunity, these receptors are key modulators of a number of biological function. Understanding how both natural and synthetic ligands control the activity of PPARs is likely not only to provide key information on how these molecules are regulated but also to result in the development of novel therapeutics.

1.1. Tissue Specificity of PPAR Expression

PPARs are expressed in several tissues of adult rodents [2]. PPARα, principally expressed in the central nervous system, liver, kidney, heart and digestive tract [2], plays an important role in regulation of fatty acid catabolism [3]. PPARβ/δ is abundantly and ubiquitously expressed in the adult rat and has been shown to regulate cholesterol trafficking and high density lipoprotein metabolism in macrophages [4]. PPARγ is expressed in the white adipose tissues as well as in the immune system [2]. PPARγ, initially shown to be required during adipocyte differentiation, is now known to enhance insulin sensitivity and suppress inflammatory responses [5].

In humans, PPARα is expressed at significant levels in liver, heart, kidney, skeletal muscle, intestine and pancreas and, to a lesser extent, in lung, placenta and adipose tissue, while PPARβ is expressed ubiquitously [6]. PPARγ is expressed in adipose tissue, skeletal muscle, liver, heart and hematopoietic cells [7, 8, 9]. Four human PPARγ mRNA species have been described, all of which transcribed from the same gene, resulting from alternative splicing and promoter usage. The PPARγ1, 3 and 4 transcripts encode the same widely expressed protein, while PPARγ2 encodes a protein specifically expressed in adipocytes containing 28 additional amino acids [10].

1.2. Structure and Regulation of PPARs

PPARs have a domain structure shared by most nuclear receptors. As summarized in Fig. (**1**), PPARs are composed of five different domains, which show some variation in length among the three isoforms: an N-terminal region (A/B domain) which contains the ligand-independent transcriptional activation function 1 (AF-1), a DNA binding domain (C domain), a hinge region (D domain) and a ligand binding region (E domain). The latter contains the ligand-dependent activation function 2 (AF-2) and is required for PPAR heterodimerization with retinoic X receptors (RXR). No function has been assigned to date to the C-terminal domain (F domain). Amino acid sequence comparison of human and mouse PPAR subtypes reveals a high degree of identity in the DNA binding and ligand-binding domains [7].

1.2.1. A/B Domain

The N-terminal A/B domain modulates PPAR activity in a ligand-indipendent manner through the AF-1 function, which is responsible for recruitment of transcriptional coactivators. A truncated version of PPARα lacking the A/B domain has indeed 60-70% lower transactivating function than the full-length protein both in the presence and in the absence of ligand [11]. The capacity of the A/B domain to recruit cofactors through the AF-1 function is retained even in the absence of the other PPAR domains, as recently shown for the interaction involving peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase [12].

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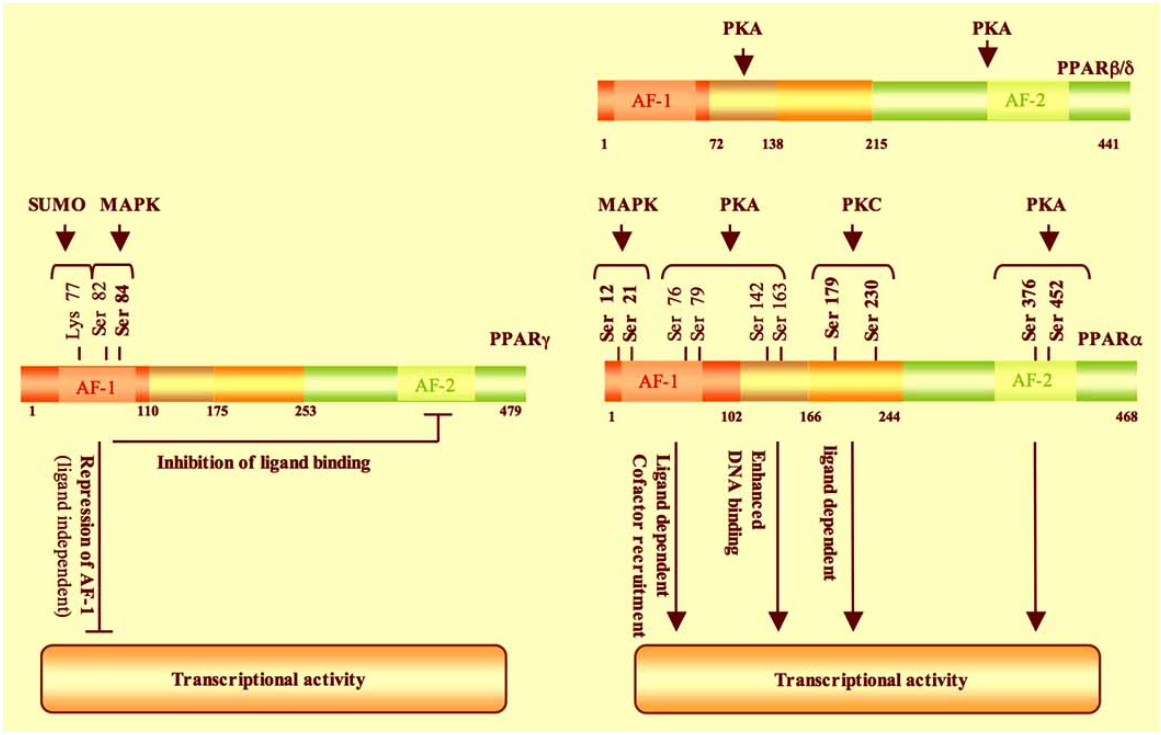


Fig. (1). Schematic representation of the functional domains of PPARs. The A/B domain (amino acid residues 1-110, 1-72 and 1-102 in PPARγ1, PPAR βδ and PPARα, respectively) contains the activating function 1 (AF-1) which is ligand-independent. The domain C (amino acid residues 110-175, 72-136 and 102-166 in PPARγ1, PPAR βδ and PPARα, respectively) is implicated in DNA binding, the domain D (amino acid residues 175-253, 136-215 and 166-244 in PPARγ1, PPAR βδ and PPARα respectively) is a hinge region and the E domain (amino acid residues 253-479, 215-441 and 244-468 in PPARγ1, PPAR βδ and PPARα, respectively), which contains the activating function 2 (AF-2), is the ligand recognition module and is required for receptor dimerization with RXR. The amino acid residues which are targets of several intracellular signalling cascades, as well as the outcome of their post-translational modification on the activity or PPARs, are indicated (residues in human PPARs are indicated in bold).

Accumulating evidence underlines the role of A/B domain phosphorylation in the fine-tuning of PPAR activity. A major phosphorylation site for mitogen-activated protein kinase (MAPK) has been mapped to the A/B domain, which, intriguingly, appears to affect PPAR activity differentially in an isoform-and cell type-specific fashion. In adipocytes treated with mitogenic stimuli, the outcome of MAPK-dependent phosphorylation of the PPARγ A/B domain, which has been mapped by mutational analysis to a specific serine residue both in human and mouse (Ser82 in mouse PPARγ1, Ser112 in mouse PPARγ2, Ser84 in human PPARγ1), results in a decrease in the transcriptional activity of PPARγ [13-15]. In the case of mouse PPARγ1, this effect has been ascribed to a reduction in ligand binding affinity, due to intramolecular interactions between the A/B and E domains [16]. The role of Ser phosphorylation on PPARγ1 is however as yet controvertial, as PPARγ phosphorylation in response to insulin treatment has been reported to enhance its transcriptional activity in rat adipocytes and 3T3-L1 cells, independently of ligand binding [17]. A MAPK-dependent mechanism of positive regulation has also been observed for PPARα. Phosphorylation of the A/B domain on two conserved MAPK sites (Ser12 and Ser21) results indeed in enhancement of PPARα-dependent trascription in insulin-treated rat adipocytes [18, 19].

In addition to the MAPK-dependent mechanism of PPAR regulation, recent data have established a role for sumoylation in the control of PPAR activity. PPARγ1 sumoylation of lysine 77 (lysine 107 for PPARγ2) within the A/B domain inhibits indeed its transcriptional activity [20]. Furthermore, site-directed mutagenesis of PPARγ2, combined with gene reporter assays and sumoylation analyses, has demonstrated that sumoylation represses the ligand-independent transactivating function carried out by the A/B domain [21]. Interestingly, phosphorylation of Ser112 on mouse PPARγ2 enhances lysine 107 sumoylation [22], suggesting a cooperation between these two post-translational modifications in the negative control of PPARγ activity.

1.2.2. DNA Binding (C Domain) and Hinge (D Domain) Domains

The C domain or DNA binding domain (DBD) is highly conserved across the nuclear receptor superfamily and is required for PPRE binding. PPREs are specific DNA sequences in the promoter regions of target genes containing one or more copies of the hexameric DNA consensus sequence (AGGTCA) separated by one or two nucleotides [23]. The C domain, which is composed of approximately 70 amino acid residues (110-175, 72-138 and 102-166 in human PPARγ PPARβ/δ and PPARα, respectively [24]), folds into

two zinc finger-like structures,, where the first zinc finger contains a P-box (CEGCKG) that interacts directly with the specific DNA hexamers, and the second zing finger a D-box (CC), which is responsible for recognition of the spacing between the response element half-site [7].

Phosphorylation of a specific serine residue within the C domain has been shown to modulate PPAR binding to the PPRE. Indeed, phosphorylation of Ser142 and Ser163 of mouse PPARα by PKA results in enhanced target gene expression due to stabilization of nuclear receptor binding to the DNA [25]. Furthermore, phosphorylation by PKC of Ser179 and Ser230 within the D domain of human PPARα also contributes to enhance the transcriptional activity of PPARα, as pharmacological PKC inhibitors, as well as mutation of these serine residues, have been associated with impaired PPARα-dependent gene transcription [26].

1.2.3. Ligand Binding Domain (E-Domain)

PPARs regulate gene expression by binding to PPREs as heterodimers with RXRs. The E domain or LBD (ligand binding domain), corresponding to the region encompassing amino acids 253-479, 215-441 and 244-468 in human PPARγ, PPARδ and PPARα, respectively, is required for receptor dimerization with RXRs as well as for ligand-dependent activation through the AF-2 function. In its active state, the RXR-PPAR heterodimer is able to associate with coactivators at PPREs, thereby promoting target gene expression.

The crystal structure of human PPARβ, PPARγ and PPARα has been solved by X-ray crystallography [27-31]. These structures reveal a bundle of 13 α-helices (H1-H13) and a small four-stranded β-sheet arranged in three layers to form an anti-parallel α-helical sandwich [32]. Repositioning of helixes H11-H12, which contain the core residues of the AF-2 transactivation domain, is the main difference between the unliganded (apo-) and liganded (holo-) receptor LBD. The latter structure is characterized by the presence of a large hydrophobic cavity having a volume of 1300 Å, known as ligand binding pocket (LBP) [7]. The large LBP of PPARs allows these receptors to bind to several ligands [33]. The affinity of the ligand is however an important determinant in the stability of the holoreceptor. The PPARγ LBD, which is a very dynamic structure in solution, is stabilized into a more rigid conformation following ligand binding. The aminoacid residues essential for stabilization have been mapped by site-directed mutagenesis to helixes H1 and H8 [34]. Ligands with different affinities affect differentially the stability of the holoreceptors. Specifically, full agonists stabilize the PPAR LBD more than partial agonists or antagonists [35]. Furthermore, the AF-2 transactivation domain becomes fully functional only in the presence of strong agonists [36]. Interestingly, AF-2 may also be regulated by PKA-dependent phosphorylation, which has been reported for Ser376 and Ser452 on PPARα. This issue is however as yet not fully clarified, as the A/B and C domains are better PKA substrates than the LBD domain [37].

Collectively, the structure-function studies of PPARs indicate that PPARs regulate gene expression in a ligand-dependent fashion. In the unbound state, the heterodimer PPAR-RXR is sequestred away from its promoter by corepressors, and transcription is inhibited. Upon ligand binding a conformational change occurs, which results in release of corepressors and recruitment of coactivators, and hence in initiation of target gene transcription Fig. (**2**). The extent of PPAR stabilization following ligand binding, which is dependent on ligand affinity, may account at least in part for the differential transcriptional modulation of PPAR target genes by different ligands. In addition to ligand-dependent activation, the activity of PPARs is finely tuned by their phosphorylation status, which results from integration of several intracellular signalling cascades involving MAPK, PKA and PKC.

2. PPARγ LIGANDS: STRUCTURE AND ACTIVITIES ON THE CELLULAR COMPONENTS OF THE IMMUNE SYSTEM

PPARγ is activated by several compounds, including both synthetic and natural molecules. Most known synthetic PPAR ligands are characterized by a hydrophilic head group, a central hydrophobic part and a flexible linker to the tail. All PPAR ligands share a similar binding mode to the LBD, characterized by interaction of the head group with the AF-2 helix, formation of hydrophobic interactions through the central ring and extention of the tail toward the lower or upper distal cavity [31]. The structure of PPARγ-ligand complexes has revealed that full PPARγ agonists form conserved H-bonds with the AF-2 helix which, in turns, leads to recruitment of coactivators [38, 30].

The anti-diabetic drugs thiazolidinediones (TZDs) were the first compounds reported as high affinity PPARγ agonists, and troglitazone, pioglitazone and rosiglitazone have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of type 2 diabetes. However troglitazone, the first of the thiazolidinediones, has been removed from the market in March 2000 due to increased risk of idiosyncratic hepatotoxicity [39]. The TZDs head group forms specific H-bonding interactions with PPARs, among which of crucial importance is the H-bond with Tyr473, which is localized in the AF-2 helix [40]. In addition, TZDs forms H-bonds with His323 on PPARγ. This residue is the major determinant of ligand selectivity, as shown by the finding that farglitazar, a full PPARγ agonist, is significantly less potent on a PPARγ mutant lacking H323 [38]. Using PPARγ binding assays it has been shown that only the (*S*)-enantiomers of TZDs bind to the receptor, indicating that only 50% of the drug is biologically active. Recently a series of non-thiazolidinedione L-tyrosine-based PPARγ agonists, which include GW-7845 and GW1929 [41], as well as partial agonist, CDDO, have been synthesized [42]. The (*S*)-enantiomers of L-tyrosine compounds showed higher selectivity and binding affinity for PPARγ compared to the other PPAR isoforms. In addition, diindolymethanes (DIM) and several ring-substituted DIM derivatives have been shown to transactivate PPARγ[43]. The principal synthetic PPARγ ligands are shown in Table **1**.

PPARγ is expressed in a number of normal and transformed hematopoietic cells, including dendritic cells, eosinophils, macrophages and lymphocytes [2,9,44]. Strong indications of the potential role of PPARγ agonists in the modulation of immune cell functions have been provided by

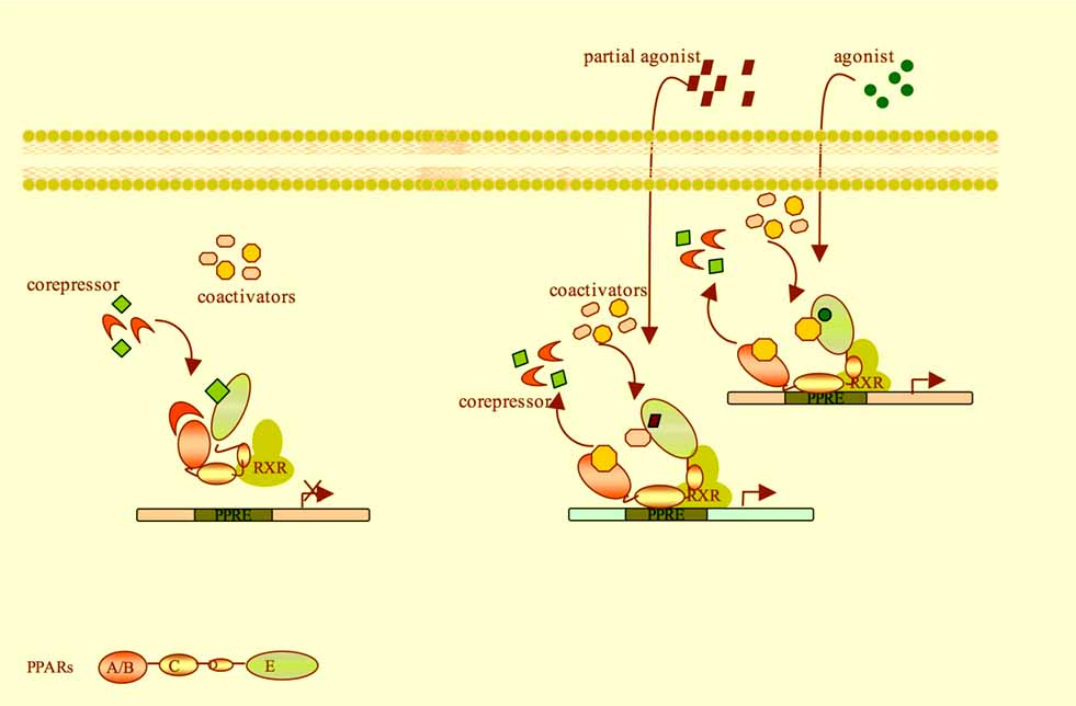


Fig. (2). Mechanism of action of PPARs and PPAR agonists and partial agonists. In the absence of ligand, the PPAR-RXR heterodimer forms high affinity complexes with nuclear corepressor proteins which prevent transcriptional activation. Receptor activation generally occurs after ligand binding to the E domain. The activity of activated PPARs is believed to be finely tuned by the phosphorylation status of the receptor. The E domain of PPAR is a dynamic structure which is stabilized into a more rigid conformation following ligand binding. Agonist and partial agonist binding to the receptor differentially influences the stability of the holoreceptor. Agonistic ligands induce a conformational change in which the E domain is firmly stabilized into an active conformation which is necessary for coactivator recruitment. In presence of partial agonists the holoform of the receptor is less stable, such that the active conformation may be adopted only transiently. The biological activity of such ligands might be dependent on the concentration or type of coactivator recruited.

several independent studies where the effects of natural and synthetic PPARγ ligands were assessed both on primary cells and on myeloid and lymphoid cells lines (Table **1**). PPARγ ligands were shown indeed to cause a general reduction of proliferation, cytokine production, expression of costimulatory and adhesion molecules and cell migration, as well as to promote cell apoptosis. These findings have highlighted the potential use of PPARγ ligands both as immunomodulators as in the treatment of lymphoproliferative disease, as underlined by ongoing clinical trials [Phase I study of CDDO in solid tumors and lymphomas: clinical trials. gov identifier : NCT00352040].

2.1. Synthetic PPARγ Ligands

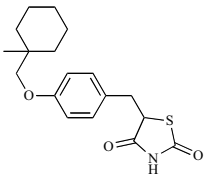
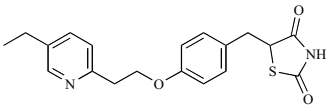
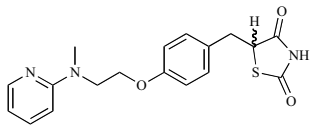
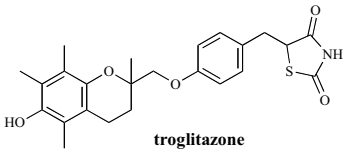
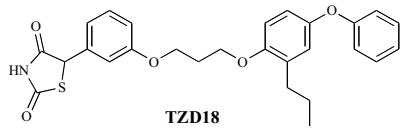
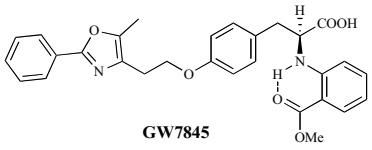
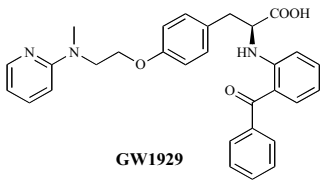
2.1.1. Thiazolidinediones (TZDs)

A number of TZDs, including ciglitazone, rosiglitazone, troglitazone and pioglitazone have been shown to inhibit proliferation of the human monoblastic leukemia cell line, U937. The mechanism appears somewhat different for different TZDs. For example, ciglitazone inhibits cell proliferation by causing an arrest in the G2/M phase of cell cycle [45], while rosiglitazone induces cell growth arrest and apoptosis [46]. A marked suppression of proliferation following rosiglitazone treatment, which has been associated to an increase in differentiation and lipogenesis, has also been observed in human promyelocytic leukaemia cells (NB4) [47].

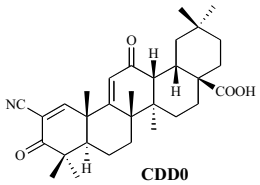
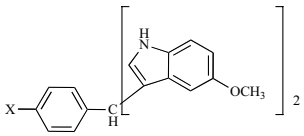
Inhibitory effects of TZDs have been observed not only on myeloid, but also on lymphoid cells. Troglitazone inhibits IL-2 secretion and proliferation in human peripheral blood T-cells. This effect has been shown to result from inhibition of the transcription factor NF-AT, which is required for IL-2 gene transcription, due to its physical interaction with ligand-activated PPARγ [48]. The related drug, pioglitazone, induces G1 cell cycle arrest in lymphoblastic leukemia cell lines [49]. Ciglitazone also attenuates the immunological alterations in a murine model of allergic asthma, which is characterized by a predominant type-2 helper T-cell response to airborn allergens. Accordingly, T-cells from ciglitazone-treated mice secreted less IFNγ, IL-4 and IL-2 upon *in vitro* restimulation with allergens [50, 51]. Inhibition of both the proliferative response and inflammatory cytokine secretion by $CD4^+$ T-cells results from inhibition of the transcription factors AP-1 and NFκB by ciglitazone-activated PPARγ[52].

In addition to inhibiting T-cell proliferation, ciglitazone is also a potent promoter of apoptosis, as shown in Jurkat T-cells, where ciglitazone-dependent apoptosis is associated with impairment of mitochondrial integrity and downregulation of c-myc expression [53]. Activation of PPARγ by ciglitazone also induces B-cell apoptosis by causing dissipation

Table 1. Structure and Activities of Synthetic PPARγ Ligands on the Cellular Components of the Immune System

Synthetic PPARγ ligands	Cells	Biological Effects	Molecular Mechanisms	Ref.[a]
 **ciglitazone**	U937	↓proliferation	G2/M phase arrest	[45]
	T lymphocytes	↓Inflammation proliferation ↑Apoptosis	inhibition of AP-1, NFκB ↓IL-4, IFNγ, IL-2 ↓c-myc	[50-53]
	B lymphocytes	↑Apoptosis	activation of caspase 3, 9 activation of NFκB	[53-55]
 **pioglitazone**	NB4 cell	↓proliferation		[47]
	U937, THP-1	↓proliferation migration	↓IL-8, CXCL10 inhibition of NFκB	[56-58]
	BV173, SD1, SupB-15 SupB-15	↓growth	G1 phase arrest	[49]
	human DC	↓costimulatory adhesion molecules secretion of cytokines	inhibition of MAPK, NFκB	[59]
 **rosiglitazone**	U937, HL-60 B, T lymphocytes	↑Apoptosis		[46]
	human DC	↓costimulatory adhesion molecules secretion of cytokines	inhibition of MAPK, NFκB	[59]
 **troglitazone**	T lymphocytes	↓proliferation	Inhibition of NF-AT binding to DNA ↓IL-2	[48]
	THP-1	↓proliferation migration	↓CCR2 mRNA	[56]
	human DC	↓costimulatory adhesion molecules secretion of cytokines	inhibition of MAPK, NFκB	[59, 60]
 **TZD18**	SupB-15 BV173, SD1	↓growth ↑Apoptosis	Inhibition of NFκB binding to DNA ↓c-myc, CDK2, CDK4 cyclin E2,D2 ↑bax, activation of caspase 9	[49]
 **GW7845**	WEHI-231, BU-11	↑Apoptosis	activation of NFκB	[55, 66]
 **GW1929**	T lymphocytes	↓IL-4, IFNγ		[50]

(Table 1. Contd….)

Synthetic PPARγ ligands	Cells	Biological Effects	Molecular Mechanisms	Ref.[a]
 **CDD0**	U937, HL-60 B, T lymphocytes	↑Apoptosis	activation of caspase 3, 9 mithocondrial membrane depolarization	[46]
 **DIM#34**	AML cells	↑Apoptosis	inhibition of MAPK release of CytC activation of caspase 3,9	[71, 72]

Ref.[a] number refers to the numbered reference in the text.

of mitochondrial transmembrane potential and activation of caspases 3 and 9 both in mouse B-lymphoma (WEH1-231) and pre-B (BU-11) cells and in normal mouse splenic B-cells. This activity is mediated by NF-κB activation, as a pharmacological NF-κB inhibitor prevents apoptosis [54, 55]. Rosiglitazone has also been shown to be potent inducer of differentiation and apoptosis in a number of lymphoid cell lines, as well as in primary chronic lymphocytic leukemia cells [46].

In addition to their anti-proliferative and pro-apoptotic activities on immune cells, TZDs prevent initiation of adaptive immunity, as well as effector function of cells of both the innate and adaptive immune compartments, by interfering with chemoattraction and cell adhesion. Both troglitazone and pioglitazone inhibit THP-1 monocyte/macrophage chemotaxis by suppressing transcription of the genes encoding the chemotactic receptor CCR2 and the chemokine CXCL10, respectively [56, 57]. Furthermore, pioglitazone suppresses expression in U937 cells of the neutrophil and mast cell chemoattractant, IL-8, through a mechanism involving inhibition of NF-κB [58]. TZDs also inhibit T-cell recruitment and activation by affecting maturation of dendritic cells (DC). Rosiglitazone, pioglitazone and troglitazone have indeed been shown to decrease expression of IL-12 and CXCL10 by DC, thereby antagonizing both Th1 differentiation and Th1 cell chemotaxis. Down-regulation of costimulatory molecules such as CD80 has also been described following treatment of human DC with TZDs [59,60]. This activity appears to result from inhibition of MAPK and NF-κB [60].

Collectively, these findings highlight TZDs as negative regulators of the activation, proliferation and migration of both monocytes/macrophages and lymphocytes and suggest a potential use of these drugs in therapeutic regimens for the treatment of leukemias. Several new TZDs are in fact currently being developed. Among these, the dual PPARα/γ ligand TZD18 appears particularly promising. TZD18 induces G1 cell cycle arrest in chronic myeloid leukemia cells at least in part through upregulation of $p27^{kip1}$ and downregulation of CDK-2/4, c-myc and cyclins E and D2. Of note, the finding that neither PPARα or PPARγ antagonists are able to antagonize the effects of TZD18 suggests that the activity of this drug may be independent of PPARs [49].

Interestingly, the notion that some of the activities of PPAR ligands may be PPAR independent is emerging as a common feature of these drugs. Inhibition of cell proliferation, resulting from inhibition of protein synthesis, has indeed been observed in $PPAR\gamma^{-/-}$ mouse embryonic stem cells treated with TZDs [61]. Furthermore, TZDs modified by the introduction of a double bond adiacent to thiazolidinedione ring that abrogates PPARγ activity retained their ability to induce apoptosis in prostate cancer cell lines [62] and to repress cyclin D1 in MCF-7 breast cancer cells [63], suggesting that the antiproliferative effects of TZDs may be at least in part PPAR-independent.

2.1.2. L-Tyrosine Based Compounds

Notwithstanding the clinical application of TZDs in the treatment of type 2 diabetes, these drugs cause significant side effects, including enhanced risk of heart failure, edema and cardiac hypertrophy [64, 65]. Intensive ongoing research is therefore aimed at the discovery of novel PPAR ligands with improved therapeutic profiles. A series of L-tyrosine-based PPARγ ligands has been designed by replacing the thiazolidinedione ring with carboxylic acid and introducing an amine function on the adiacent carbon [41]. As opposed to TZDs, these compounds do not undergo racemization under physiological conditions. Two of these L-tyrosine-based PPARγ ligands, GW7845 and GW1929, are among the most potent PPARγ agonists [41]. GW7845 treatment of early B lymphocytes results in rapid apoptosis mediated by NFκB activation [55]. This effect can be abrogated by pharmacological inhibition of the stress-activated kinases p38 and JNK, suggesting that the mechanism of GW7845-induced B-cell apoptosis involves activation of these kinases [66]. On the other hand, GW1929 has been demonstrated to significantly reduce airway inflammation during allergic asthma induction, suggesting a potential use of this compound not only as antidiabetic, but also as immunomodulator [50].

2.1.3. CDDO

A novel synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), previously reported to have potent differentiating, antiproliferative and antiinflammatory activities in many tumor cell lines, has been recently identified as a partial agonist for PPARγ with nanomolar affinity [42]. The activities of CDDO have been compared to full PPARγ agonists, such as the TZD rosiglitazone. The results show that CDDO induces apoptosis of primary chronic lymphocytic leukemia cells by mithocondrial depolarization and caspase activation with significantly greater potency than rosiglitazone [46]. CDDO also induces apoptosis of acute myeloid leukemia cells by promoting release of cytochrome-*c* and activation of caspases 8 and 3 [67]. A possible explanation of the potency of this drug notwithstanding its classification as partial agonist is that the target genes of PPAR-CDDO complex may be different that those controlled by full agonists. Indeed, while less effective than the full agonist rosiglitazone in recruiting the known PPAR coactivators, CDDO can effectively promote the release of corepressors. Hence CDDO, and perhaps other partial agonists, may control transcription of a set of genes important for cell survival, different than the ones controlled by full agonists, by promoting the recruitment of specific coactivators to PPARγ [68]. Whichever the mechanism, partial agonists are the focus of intensive research as they might elicit milder side effects than those caused by full agonists [66].

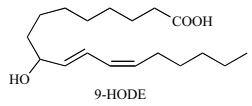
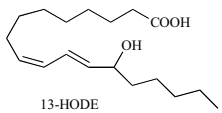
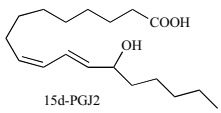
2.1.4. Diindolymethanes (DIM)

Diindolymethane (DIM) has been shown to cause cell cycle arrest and apoptosis in several human cancer cell lines including breast [69], prostate [70] and colon [71]. Recent studies have demonstred that DIM analogues transactivate PPARγ [43]. Furthermore, the novel ring-substituted DIM derivative, DIM#34, has proapoptotic activity in acute myelogenous leukemia (AML) cells, suggesting a potential use of these compounds in the treatment of hemetopoietic malignancies. DIM#34 activates the intrinsic apoptosis pathway, as shown by the fact that AML cell apoptosis occurs through dissipation of mitochondrial transmembrane potential, release of cytochrome-*c*, caspase activation and inhibition of the MAPK pathway [72]. A key feature of this drug is its low toxicity, as it appears to selectively kills cancer cells [72].

2.2. Natural PPARγ Ligands

PPARγ is the target of a number of naturally occuring compounds such as 15-deoxy-$\Delta^{12,14}$- prostaglandin J_2 (15d-PGJ_2) and 9-and 13-hydroxyoctadecadienoic acids (9- and 13-HODE) [49] (Table **2**). 15d-PGJ_2 activates PPARγ at micromolar concentrations. This compound has been shown to inhibit DC activation by inducing downregulation of costimulatory and adhesion molecules, as well as cytokine secretion. Similarly to the synthetic ligand troglitazone, this activity involves inhibition of NF-κB and MAPK signaling [60]. 15d-PGJ_2 also inhibits monocyte proliferation and migration, the latter activity resulting from suppression of expression of the chemokine receptor CCR2 [56]. The effects of 15d-PGJ_2 on lymphoid cells have been compared the ones elicited by several synthetic PPARγ agonists. The results indicate that 15d-PGJ_2 share significant similiarities with TZDs in terms of antiproliferative and cytotoxic effects, characterized by reduction in cytokine production through inhibition of NF-AT and NF-κB, and induction of apoptosis [46, 48, 51, 53, 54, 73]. On the other hand, Ji *et al*. [74] demonstrated that, as opposed to TZDs, 15-PGJ_2 block IL-10-induced STAT3 activation in human monocytes and macro-

Table 2. Structure and Activities of Natural PPARγ Ligands on the Cellular Components of the Immune System

Natural PPARγ ligands	Cells	Biological Effects	Molecular Mechanisms	Ref.[a]
 9-HODE	U937	↑Apoptosis	G1 phase arrest	45
 13-HODE	U937 T lymphocytes	growth arrest ↓IL-2	S phase arrest inhibition of NF-AT, NFκB	45 75
 15d-PGJ2	human DC THP-1, U937, HL-60, T, B lymphocytes	↓costimulatory adhesion molecules secretion of cytokines ↓migration ↓proliferation	inhibition of MAPK, NFκB PPARγ ind. NFkB inhibition ↓CCR2 mRNA inhibition of NF-AT, NFκB activation of caspase 3, 9 ↓c-myc	[60] [56, 58, 75] [48, 46] [3, 51] [54, 73]

phages, suggesting that some biological activities of this PPARγ ligand may be unique.

A recent comparative study of 9- and 13-HODE in U937 cells showed that these two natural PPARγ ligands display different biological activities. While both inhibit cell proliferation, growth arrest occurs at different phases of the cell cycle (G0/G1 arrest for 9-HODE, S-phase arrest for 13-HODE). Furthermore, only 9-HODE induces apoptosis, an effect which was found to be independent of PPARγ [45]. Interestingly, 13-HODE, similarly to TZDs, inhibits IL-2 production by human T lymphocytes through inhibition of NF-AT and NF-κB [75].

Hence natural PPARγ ligands act not only as modulators of cell growth and apoptosis but also as negative regulators of both macrophage and lymphocyte functions, which identifies these compounds as a new class of antineoplastic and immunomodulatory molecules.

3. PPARγ LIGANDS IN THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES

The antiproliferative and proapoptotic activities of PPARγ ligands on both myeloid and lymphoid cell lines identify these compounds as novel class of drugs potentially valuable in the treatment of hematological malignancies. Significant effort has accordingly been invested in assessing their activities in neoplastic cells from patients with myeloid and lymphoid leukemias. In this section we shall summarize the most recent findings, which suggest that both synthetic and natural PPARγ ligands, particularly when used in combination therapies, may hold a promise for the treatment of these neoplasias.

3.1. PPARγ Ligands and Myeloid Malignancies

3.1.1. Chronic Myeloid Leukemia

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder whose hallmark is a chromosomal translocation between chromosomes 9 and 22, detected cytologically as the presence of a small chromosome known as the Philadelphia chromosome (Ph). This translocation results in the production of the oncogenic BCR-ABL fusion protein, a constitutively active tyrosine kinase which activates the Ras signalling pathway, leading to uncontrolled cell proliferation and enhanced cell survival. Imatinib, a specific tyrosine kinase inhibitor, selectively suppresses the proliferation of cells expressing BCR-ABL and as such represents the elective therapy for CML [76]. Persistence of BCR-ABL positive cells or secondary resistance have however been documented [76]. Significant effort is therefore currently being invested in the search of additional molecular targets for CML treatment. Several new molecules, including PPARγ ligands, are now being tested either alone or in combination with Imatinib to overcome the drug resistance problems. Liu *et al.* have shown that TZD18, a synthetic dual PPARα/PPARγ ligand discussed in section 2.1.1, has a profound inhibitory activity on the growth of Ph+ lymphocytic leukemia cell lines. In addition, they found that TZD18 promotes apoptosis of these cells. These effects appear PPAR-independent, as they cannot be reversed by PPARα or PPARγ antagonists. Remarkably, TZD18 synergistically enhanced the effects of Imatinib [49]. Recently this group investigated the effect of this compound, either alone or in combination with Imatinib, in human CML myeloid blast crisis cell lines. Their findings strongly indicate that TZD18 treatment may be beneficial both for Imatinib-sensitive and Imatinib-resistant CML [77].

3.1.2. Acute Myeloid Leukemia

Acute myeloid leukaemia (AML) is characterized by impaired apoptosis which correlates with an increase in the levels of Bcl-2. The Bcl-2 protein family controls apoptosis through a finely tuned balance of anti-apoptotic members, such as Bcl-2 and Bcl-xL, and proapoptotic members, such as Bax, Bid and Bad [78]. Bcl-2 is a mitochondrial protein which antagonises apoptosis by blocking cytochrome-*c* release, thereby preventing initiation of the caspase cascade. Since in AML impaired apoptosis correlates to resistance to chemiotherapy, compounds which activate apoptosis may be useful for treatment of drug-refractory AML. The PPARγ ligand CDDO has been shown to promote mitochondria-mediated AML cell apoptosis by inducing cytochrome-*c* release and activation of caspases 8 and 3 [77], suggesting a potential use of this ligand in the treatment of drug-resistant AML. Of note, the novel C-28 imidazole derivative of CCDO, CDDO-*Im*, appears significantly more potent than CDDO both *in vitro* and *in vivo*, as assessed in a mouse leukemia model [79]. Another novel PPARγ agonist, DIM#34, has been recently demonstrated to be effective in promoting AML cell apoptosis through both PPARγ-dependent and PPARγ-independent mechanisms. DIM#34 selectively induces apoptosis of AML cells by modulating Bcl-2 phosphorylation by Erk1/2, an event which results in inhibition of its antiapoptotic activity [80]. Furthermore, troglitazone and 15d-PGJ_2 have been demonstrated to strongly induce apoptosis in two human myeloid leukaemia cell lines through upregulation of Bax as well as downregulation of Bcl-2 expression [81].

The therapeutic potential of PPARγ ligands in the treatment of multiple myeloma has also been investigated. Based on results obtained on myeloma cell lines *in vitro,* several reports support a role for the TZDs ciglitazone, troglitazone and pioglitazone, as well as for the natural ligand 15d-PGJ_2, in the induction of apoptosis [82-84].

Hence, despite the absence of conclusive information on the role of PPARγ itself in the biological activities of PPARγ ligands, their documented ability to induce apoptosis of leukemic cells strongly supports the notion that these drugs may serve as potential therapeutics for the treatment of both acute and chronic myeloid leukaemia.

3.2. Lymphoid Malignancies

3.2.1. B-ALL

B-cell acute lymphoblastic leukaemia (B-ALL) is a lymphoid neoplasm frequently associated with a t(14;18) chromosomal translocation which results in c-myc overexpression and correlates with poor prognosis [85]. *c-myc* is a gene centrally implicated in the control of cell survival, a function which is likely to underlie the apoptotic defects observed in ALL B-cells. Resistance to apoptosis has been established as

one of the causative mechanisms for the failure of therapeutic approaches in this hematopoietic malignancy. Recent studies have investigated the effect of PPARγ ligands in B-ALL cell lines. Treatment of these cells with the TZDs pioglitazone and troglitazone, or with the natural PPARγ ligand 15d-PGJ_2, resulted in dose-dependent growth inhibition. Growth arrest was associated with G1 cell cycle arrest and apoptosis. Troglitazone induced-apoptosis correlated with downregulation of both c-myc mRNA and c-myc protein expression [86]. In pioglitazone- or 15d-PGJ_2-treated cells apoptosis was partially caspase-independent, as caspase inhibitors could not reverse this effect [87]. The apoptotic mechanisms regulated by 15d-PGJ_2, but not by ciglitazone, may be related to enhanced production of reactive oxygen species [88]. The effects of these compounds both on cell cycle arrest and apoptosis of B-ALL cells appear to be PPARγ-independent, as neither irreversible PPAR antagonists nor a dominant-negative PPARγmutant prevented ciglitazone or 15d-PGJ_2-induced B-cell apoptosis [88].

3.2.2. Diffuse Large B-Cell Lymphoma and B-CLL

Two chronic lymphoid malignancies, diffuse large B-cell lymphoma (DLBCL) and B-cell chronic lymphocytic leukemia (B-CLL) are also characterized by apoptosis defects. In B-CLL impaired apoptosis has been correlated to enhanced Bcl-2 expression, due either to promoter hypomethylation or, more frequently, to chromosomal deletion 13q14, which encodes two natural Bcl-2 antisense RNAs [89, 90]. Diffuse large B-cell lymphoma is the most common non-Hodgkin lymphoma characterized by clinical and biologic heterogeneity [91], which has been classified by DNA microarray analysis in two molecularly distinct forms, the germinal center and activated B-cell subtypes [92]. Poor prognosis correlates with Bcl-2 overexpression which, in the activated B-cell subtype, may be responsible for the impaired apoptotic response to chemotherapy [93, 94].

The potential use of proapoptotic PPARγ ligands for the treatment of these neoplasias is currently being evaluated. The triterpenoid CDDO has been shown to inhibit proliferation and induce apoptosis of human DLBCL cells of both subtypes in a PPARγ-independent manner [95]. Interestingly, exposure of these cells to CDDO resulted in NF-κB activation. Combined treatment with CDDO and an NFκB inhibitor enhanced DBLCL cell apoptosis, suggesting that NFκB may antagonise the proapoptotic effects of CDDO by triggering a survival pathway [95].

The effects of CDDO have also been tested on CLL B-cells. In these cells CDDO acts as a potent inducer of apoptosis through the intrinsic apoptosis pathway. This activity may be PPARγ-independent as CDDO, a partial agonist, is more potent than a full agonist such as troglitazone [96]. The results suggest that this PPARγ ligand may be effective in chemo-refractory B-CLL patients, whose neoplastic B-cells are characterized by defects in the intrinsic apoptosis pathway.

4. PERSPECTIVES

A large body of evidence highlights the potential use of PPARγ ligands as anti-neoplastic and immunomodulatory drugs. In this respect, the synergism of PPARγ ligands with currently used anti-tumoral compounds, such as Imatinib, opens a promising novel avenue to the treatment of hematological malignancies. Fully understanding the mechanism of action of these drugs has therefore become a priority, as this may result in the development of second-generation rationally designed PPARγ ligands with equal or enhanced potency compared to the lead compounds, but with reduced side effects. This is particularly compelling if we consider the paradigm of CDDO which, while acting as a partial PPARγ agonist, is a more potent inducer of apoptosis than full agonists. One obvious direction is to investigate how the structure of ligand-bound LBD affects PPARγ activity, a task that has become feasable now that a sensitive probe of LBD conformation is available [36]. Furthermore, identification of the specific cofactors which are recruited to distinct ligand-PPARγ complexes is likely to provide an important breakthrough in understanding the activities of the different PPARγ ligands. The contribution of phosphorylation to the fine-tuning of PPAR activity needs also to be systematically addressed, particularly as it appears to differentially affect PPARs in a isoform- and cell type-specific fashion. A second aim is to understand to which extent PPARγ is responsible for the different activities and relative potencies of PPARγ ligands. Convincing evidence shows that at least part of the activities ascribed to specific PPARγ ligands are solely due to the drug. Identifying molecular targets of these drugs other than PPARs will provide novel clues for the development of more effective and safer therapeutics.

ABBREVIATIONS

HODE	=	Hydroxyoctadecadienoic acid
15dPGJ2	=	15-deoxy-$\Delta^{12,14}$- prostaglandin J_2
AF	=	Activation function
ALL	=	Acute lymphoblastic leukaemia
AML	=	Acute myeloid leukaemia
CCR	=	CC chemokine receptor
CDDO	=	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid
CDK	=	Cyclin-dependent kinase
CLL	=	Chronic lymphocytic leukaemia
CML	=	Chronic myeloid leukaemia
CXCL	=	CXC chemokine ligand
DBD	=	DNA binding domain
DC	=	Dendritic cell
DIM	=	Diindolymethane
DLBCL	=	Diffuse large B-cell lymphoma
GST	=	Glutathione-S-transferase
IL	=	Interleukin
JNK	=	Jun NH_2-terminal kinase
LBD	=	Ligand binding domain
LBP	=	Ligand binding pocket

MAPK = Mitogen activated protein kinase

MCP-1 = Monocyte chemoattractant protein -1

MDS = Myelodysplastic syndrome

NFκB = Nuclear factor κB

NFAT = Nuclear factor of activated T cells

PKA = Protein kinase A

PKC = Protein kinase C

PP = Peroxisome proliferator

PPAR = Peroxisome proliferator-activated receptor

PPRE = PPAR response element

ROS = Reactive oxygen species

RXR = Retinoid X receptor

STAT = Signal transducer and activator of transcription

SUMO = Small ubiquitin-like modifier

TZD = Thiazolidinedione

TLR = Toll-like receptor

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