PPARγ- Novel Target in Antidiabetic Drug Design

Lalit V. Sonawane* and Sanjaykumar B. Bari

ABSTRACT: The nuclear receptors constitute one of the largest groups of transcription factors known today. The peroxisome proliferator-activated receptors (PPAR) are members of the nuclear receptor supergene family and are considered as key sensors of both lipid and glucose homeostasis. The role of the PPAR γ isoform in glucose metabolism is illustrated by the fact that antidiabetic thiazolidinediones have been shown to be bonafide PPARγ ligands. The present review has established the PPARs as molecular target for the design and development of antidiabetic drugs.

KEYWORDS: Diabetes; Peroxisome proliferator-activated receptors; Thiazolidinediones

Introduction

The nuclear receptors constitute one of the largest groups of transcription factors known today. The nuclear receptors are transcription factors that upon binding of ligand regulate both target gene expression and repression. The most conserved domains of this protein family are the centrally located DNA binding domain and the C-terminal part of the receptor, which encompasses the ligand binding domain (LBD).

The PPAR(s) (peroxisome proliferator-activated receptor) is a subgroup of the nuclear receptor gene family that has been suggested to play important role in both lipid and glucose homeostasis. The Peroxisome Proliferator-Activated Receptors (PPARs), which were cloned a decade ago as orphan receptors, belong to the thyroid hormone-like subfamily of NRs. Today three PPAR genes (α, β/δ or FAAR, and γ) have been identified, and recent studies have shown that the different PPAR isoforms appear to have distinct but overlapping ligand binding specificities.

The perhaps unfortunate name “peroxisome proliferator activated receptors (PPARs)” was initially based upon the prior observation that certain “industrial compounds” when given to rodents, could induce enlargement, or proliferation, of peroxisomes, a subcellular organelle.

The term “peroxisome proliferator-activated receptors” evolved, although evidence for a peroxisomal proliferative response in humans has never been seen. The distribution of the PPARs in different body parts is given in the Table.

Like other nuclear hormone receptors (e.g., the estrogen receptor, the thyroid-hormone receptor, and the retinoic acid receptor), PPARs are ligand-activated transcription factors. Like these other nuclear receptor super family members, PPARs contain both a ligand-binding domain, as well as a DNA-binding domain, in addition to other structural motifs. All nuclear hormone response elements can be loosely categorized by the nature of the response elements, consisting either of inverted repeats, or direct repeats, with certain number of intervening base pairs at the center of the response element. Thus, PPREs fall into the Direct Repeat-1 (DR-1) category of nuclear receptor transcription factors.

Table 1 Example of different tissue distribution of PPARs.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Liver</th>
<th>Intestine</th>
<th>Spleen</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>δ</td>
<td>++</td>
<td>+++</td>
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<td>-</td>
</tr>
<tr>
<td>γ</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

A list of various PPAR gamma and alpha exogenous as well as endogenous ligands is given in Table 2.

Table 2 PPAR ligands.

<table>
<thead>
<tr>
<th>Exogenous PPAR ligands</th>
<th>Endogenous PPAR ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>PPARγ</td>
</tr>
<tr>
<td>Wy-14643</td>
<td>Indomethacin</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Gemfibrotil</td>
<td>Piroxican</td>
</tr>
<tr>
<td>Nafenopin</td>
<td>Pioglitazone</td>
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<tr>
<td>Bezafibrate</td>
<td>Ciglitazone</td>
</tr>
<tr>
<td></td>
<td>Englitatez</td>
</tr>
<tr>
<td></td>
<td>BRL-49653</td>
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**PPARγ**

PPARγ is the most extensively studied of the three PPAR subtypes to date. The receptor has been cloned from a number of species, including salmon, mice, hamsters, frogs, pigs, rhesus monkeys and humans. The human PPARγ protein is homologous to the murine PPARγ protein, with 95% identity at the amino acid level. In fact, the PPARγ protein shows a remarkable conservation across all the species from which it has been cloned, in contrast to that found thus far for PPARα and PPARδ. This high level of conservation may reflect the pivotal role that PPARγ plays as a regulator of glucose and lipid homeostasis, essential functions across all species. The human PPARγ gene has nine exons that extend over more than 100 kb of genomic DNA and has been mapped to human chromosome 3p25 by somatic cell hybridization and linkage analysis. Agonist binding to the PPAR LBD triggers a cascade of events including heterodimerization with the retinoid X receptor (RXR) and co-activator recruitment, which events lead to the activation of DNA transcription.

**Crystal Structure of PPARγ**

The structure of apo-PPARγ-LBD reveals a new variation on the nuclear receptor fold, where the predominantly α-helical LBD core is conserved (Fig. 2). There are a total of 12 helices and a small b-sheet of four strands (Fig. 3). The helices are numbered in agreement with RXRα-LBD, although helices 10 and 11 are in fact one continuous helix in PPARγ. Among the LBD structures available in the Protein Data Bank, apo-PPARγ-LBD is most similar to RARγ-LBD bound to all-trans retinoic acid, and the two structures can be aligned with a root mean square deviation of 1.45 Å for 200 α-carbons.

Specifically, the PPAR LBD exhibits a very similar fold to the other NRs structures comprising 12 α-helices arranged in three layers to form an anti-parallel ‘helical sandwich’. Within the central core of the PPAR’s LBD is a T-shaped binding cavity flanked by helices 3, 5, 7, 11, and 12 with a volume of about 1300 Å³. This cavity is two to three times larger than those observed in other NRs. Most of the known PPAR agonists which occupy only a fraction of the T-shaped binding cavity, share a hydrophilic head group involved in key hydrogen bonds (H bonds) with several protein side chains, a central hydrophobic part and a flexible linker to the tail (Scheme 1). One of these key H bonds involves a tyrosine of the C terminal helix, helix 12, which upon agonist binding, adopts a conformation, favoring co-activator recruitment. In crystal structures of agonist–PPAR complexes, helix 12 folds back over the ligand binding pocket, whereas helix 12 of PPARγ - apo structures can either extend away from the ligand binding pocket or fold back over this pocket.

Hence, agonist binding locks helix 12 in an active conformation. On the other hand, recent crystallographic data indicate that antagonist binding to PPARα prevents helix 12 from adopting the active position.

Despite the therapeutic potential of ligands with different PPAR subtype selectivity profiles, few studies have dealt with the structural determinants of receptor subtype selectivity. Recently, comparison of the LBD crystal structures of the three human PPAR subtypes together with site-directed mutagenesis has revealed that mutation of a single amino acid was sufficient to affect the subtype selectivity of several chemical classes of ligands.

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**Fig. 1** A stereo image of apo-PPARγ-LBD. The protein adopts a conformation for helix 12 similar to RARγ and PR, both of which have ligands bound. The figure was made with Molscript.
Role of PPARγ in diabetes

The most extensively studied therapeutic utility for PPARγ agonists has been in the treatment of type 2 diabetes. TZD PPARγ agonists have been shown to enhance the sensitivity of target tissues to insulin and to reduce plasma glucose, lipid, and insulin levels in animal models of type 2 diabetes as well as in humans. Pioglitazone (ACTOS), Rosiglitazone (Avandia), Ciglitazone (ADD-3878), En格litazone (CP-68722), Darglitazone (CP-86325), Isaglitazone (MCC-555), KRP-297, Troglitazone (Rezulin) and Ragaglitazar have been approved by the FDA and are currently marketed agents for the treatment of type 2 diabetes. These drugs represent molecules that are secreted by adipocytes, but direct action can occur by adipocyte-independent pathways. Although PPARγ levels are 10-100-fold higher in adipose than in muscle or liver, the receptor is expressed in these latter tissues. Thus, stimulation of PPARγ in these non-adipose tissues could contribute to altered gene expression and a reduction in insulin resistance and improved glucose disposal. PPARγ may also exert direct effects on genes involved in glucose homeostasis, although surprisingly little is known about this topic. One potential target gene for PPARγ that is central to improving muscle glucose disposal is the insulin-dependent glucose transporter GLUT4. TZD activation of PPARγ has been shown to increase the expression of this gene in adipocytes, but direct regulation of its expression in muscle has not been reported. UCP2, a mitochondrial uncoupling protein related to UCP1, has been shown to be expressed in a number of tissues including skeletal muscle and adipose tissue and appears to function as an important modulator of energy usage. Expression of UCP2 was up-regulated by TZD activation of PPARγ in cell lines derived from skeletal muscle, WAT, and brown adipose tissue (BAT).

Finally, PPARγ agonists may regulate the storage or release adipocyte-derived signaling factors that affect insulin sensitivity in muscle. Fatty acids are key mediators of this process. It is well-established that increased fatty acid concentrations decrease glucose metabolism in muscle. While PPARγ agonists induce LPL, FATP-1, and ACS in adipose tissue, their expression is apparently unchanged in muscle. This may allow for an increase in fatty acid clearance into adipose tissue with concomitant decrease in uptake of fatty acids into muscle, potentially improving insulin sensitivity. In support of this hypothesis, TZD treatment is reported to reduce the triglyceride content of muscle in diabetic rats. The mechanism of these effects may be related to the observation that PPARγ regulates expression of the fatty acid transporter CD36 which was has been implicated in the control of insulin sensitivity in rats. PPARγ-induced differentiation in WAT is also associated with a marked decrease in the levels of TNFR and leptin, two signaling molecules that are secreted by adipocytes. Elevated levels of TNFR can cause insulin resistance, and TZDs have been shown to block TNFR-induced insulin resistance in both cell culture and animals. The second apparent paradox lies in the fact that obesity is a major risk factor in the development of type 2 diabetes. How then is a receptor that promotes adipogenesis still able to enhance insulin sensitivity? Evidence suggests that PPARγ-mediated differentiation of white adipocytes in rodents produces an increased number of small adipocytes while decreasing the number of large adipocytes. These smaller adipose cells are usually more sensitive to insulin and would be expected to provide greater insulin-dependent glucose uptake. The smaller adipose cells should also have lower rates of lipolysis relative to larger adipose cells. Since high levels of free fatty acids have been linked to the induction of insulin resistance, the decreased amount of circulating free fatty acid levels would be expected to have beneficial effects on insulin sensitivity. TZDs also induce the differentiation of BAT in rodents and induce the expression of the mitochondrial uncoupling protein gene UCP1 in human primary adipocytes. These effects may also contribute to increased energy consumption and decreased glucose and lipid levels.

Conclusion

Intensive study of PPARs during recent years has revealed that these nuclear receptors play an important role both in normal physiology and the pathology of various tissues. They participate in the regulation of lipid metabolism, they play an important role during rodent hepatocarcinogenesis, inflammation and development of atherosclerosis and/or diabetes, and they also have an important role in the regulation of growth and differentiation of cancer cells. It
has been suggested that PPAR ligands with agonistic effects may have potent antidiabetic as well as anticancer potential and may serve as a rational basis for therapy of type 2 diabetes mellitus. We can expect many promising results in this field in the near future.

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References


