Newer approaches to the discovery of glitazones

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Abstract

Thiazolidines (TZDs) or glitazones are one of the important classes of insulin sensitizers used in the management of type 2 diabetes mellitus. These agents, however, suffer from some serious side effects such as fluid retention, weight gain, congestive heart failure, bone fracture and possibly bladder cancer, which resulted in their withdrawal from clinical use. The TZDs that were withdrawn from the clinical use were developed at the time when enough scientific data were not available on the structure and the transcriptional mechanisms of peroxisome proliferator activated receptors (PPARs). Recent advances in the understanding the structure and function of PPARs, however, has led to more rationalized approaches to develop these agents. The present review discusses the various approaches that have been made to develop newer glitazones devoid of the problems associated with current TZDs. These approaches are based on the structural considerations of both the ligands and the receptors and also on the profile alterations of the ligands.

Key Words: Glitazones, Insulin sensitizers, Peroxisome proliferator activated receptors, Thiazolidinediones, Type 2 Diabetes Mellitus
INTRODUCTION

Thiazolidines (TZDs) or glitazones are one of the important classes of insulin sensitizers used in the management of type 2 diabetes mellitus (T2DM). TZDs are reported to reverse insulin resistance without stimulating the release of insulin from β-cells. They reduce hepatic glucose production and increase peripheral utilization of glucose thus reducing both preload and after load on β-cells. This action, therefore, enhances the effectiveness of endogenous insulin needed to maintain the given level of blood glucose by about 30%. In addition, unlike sulphonyl ureas these agents are devoid of mechanism based hypoglycemic side effects [1,2]. There is, thus, an excellent rationale for the use of TZDs in the management of T2DM. Earlier this laboratory was involved in the synthesis and evaluation of several such glitazones [3-7].

Historical

In early 1975, in an attempt to discover more potent fibrate hypolipidemic drugs, Takeda laboratories in Japan synthesized 71 analogs of clofibrate and tested them for their hypolipidemic activity. Interestingly some these compounds also showed hypoglycemic effects in diabetic mice. Later in the year 1982, extensive structure-activity relationship studies on the hit molecules led to the discovery of the first TZD, ciglitazone, which showed promising lipid and glucose lowering effects in animal models. This molecule was later discontinued due to its unacceptable liver toxicity [8,9]. In the year 1988, Sankyo Company discovered troglitazone, with potential for glucose lowering effect. In the year 1997, troglitazone became the first FDA approved TZD available for the management of T2DM. In the mean time SmithKline, UK, and Takeda laboratories, Japan, developed two potent insulin sensitizers, rosiglitazone and pioglitazone, respectively. In the year 1999, both these drugs were approved by FDA for the management of T2DM [8]. Unfortunately, within 6 weeks of its launch by Glaxo Wellcome, troglitazone was withdrawn from UK market as a result of rare but potentially fatal idiosyncratic hepatotoxicity. In March 2000, FDA, therefore, ordered the removal of troglitazone from the market [9,10]. However, both rosiglitazone and the pioglitazone were reported to be safe on the hepatic system. After its launch in May 1999, rosiglitazone, captured the major share of the diabetic market and become one of the top 25 selling brands in US. However, in July 1999, a prominent endocrinologist publicly raised concerns about the cardiovascular safety of rosiglitazone [11]. In March 2001, reports started suggesting that rosiglitazone may induce heart failure as a result of fluid retention [11]. In 2007, a meta analysis of controlled clinical trials once again found the cardiovascular risks associated with rosiglitazone [12,13]. In September 2011, the European Medicines Agency (EMA) recommended the suspension of rosiglitazone from the market. In May 2011, the US FDA placed several restrictions on the prescribing and use of rosiglitazone [14]. Pioglitazone, unlike rosiglitazone, did not attract the same degree of controversy with regard to cardiovascular risks. In fact, it is reported to have a modest cardioprotective role. Elevated risk of acute ischemic events with this agent has been not reported till date. However, recently concerns were raised on the apparent risk of bladder cancer with pioglitazone and as a result of which FDA has updated the label of pioglitazone. As per the updated label, pioglitazone should not be started in patients with active bladder cancer and should be used with caution in patients with prior history of bladder cancer [14]. The structures of these glitazones are given in Figure 1.
The molecular mechanisms of biological responses of TZDs are reported to be mediated through the modulation of peroxisome proliferator activated receptors (PPARs). In the year 1990, Issemann and Green identified these nuclear receptors in mouse [15]. These receptors are reported to be activated upon exposure to Peroxisome proliferators, such as hypolipidemic drugs, herbicides and industrial plasticizers and hence named as PPARs. In 1992, Walter Wahli et al., described the three isotypes of PPARs, namely, PPAR-α (NR1C1), PPAR-β/δ (NR1C2) and PPAR-γ (NR1C3) in xenopus [16]. PPARs are transcription factors that can be turned on or off by binding to small lipophilic compounds. These receptors are reported to act by coordinating the activities of multiple pathways involved in metabolism instead of acting through one major target (one enzyme or one pathway). This unique property of PPARs attracted considerable attention because of the hope that complex metabolic disorders such as T2DM which often requires a combination of symptomatic therapies, can possibly be treated effectively by the use of a single agent which can act by modulating PPARs [17]. PPARs are, therefore, one of the most promising targets in the management of metabolic syndrome.

**PPARs**

The isotypes of PPARs, namely PPAR-α, PPAR-β/δ and PPAR-γ, are reported to regulate a number of genes involved in the metabolism and energy homeostasis [18]. The receptors are unique in their expression in various tissues, indicating their distinct physiological roles. PPAR-α is reported to be expressed in tissues with high rates of fatty acid catabolism such as the liver, heart, kidney, large intestine, skeletal muscle and atherosclerotic lesions [19]. It is reported to promote lipid metabolism by increasing fatty acid oxidation and thus plays a very important role in lipid homeostasis. It is reported to modulate the expression of various genes involved in lipid homeostasis. Some of these include, genes of carnitine palmitoltransferase-1, acetyl-CoA-synthase, lipoprotein lipase, etc [19,20]. Its activation is reported to prevent the proatherogenic effects of cholesterol in macrophages by stimulating cholesterol efflux. It also exerts pleiotropic antiinflammatory and antiproliferatory effects [21]. Mono and polyunsaturated fatty acids and various fatty acid derived eicosanoids are reported to be the natural ligands. The fibrates (clofibrate,
fenofibrate and gemfibrozil), the antihyperlipidemic agents, are one of the important class of synthetic ligands that act via these receptors [18].

PPAR-δ is expressed ubiquitously and is often at higher levels than PPAR-α and PPAR-γ. The higher degree of expression is seen in gastrointestinal and placental tissues [18]. Initially it was thought to play an important role in housekeeping function. Only recently its role in normal adipocyte development and lipid homeostasis was elucidated. It is reported to improve insulin sensitivity and increase fatty acid oxidation. Fatty acids and eicosanoids are the natural ligands. Many synthetic PPAR-δ ligands are in the development phase for the potential treatment of metabolic disorders [18].

PPAR-γ is reported to be abundantly expressed in adipose tissues and tissues which show lower levels of expression like skeletal muscle, heart and liver. It is reported to influence the expression of various genes involved in the metabolism. It is also reported to have diverse effects on the adipocytes and its secretions. It regulates the adipocyte differentiation, influx of FFA into the mature adipocytes and secretion of adipokines. These effects on the fat cells are reported to mediate the insulin sensitizing actions of PPAR-γ. The over activation of PPAR-γ by its ligands (TZDs) is reported to increase body weight through its adipocyte differentiation effects and also because of its ability to cause fluid retention [18, 22]. It is also reported to prevent atherosclerosis formation by promoting lipid efflux in macrophages. Its ability to control the expression of many proinflammatory mediators such as TNF-α, iNOS, MMP9, NF-κB, etc, has made it one of the important targets in the management of inflammation. It has been implicated in both tumor suppression and promotion activities. Fatty acids, eicosanoids, components of oxidized low-density lipoproteins, lysophosphatidic acid and nitrolinoleic acid are the reported natural ligands and the glitazone class of drugs are the important synthetic ligands which are used in the management of T2DM [18, 22].

MECHANISM OF ACTION OF TZDs

Binding of TZDs to PPAR-γ results in either transactivation or transrepression of the target genes. In transactivation, a DNA dependent process, binding of TZDs results in heterodimerization of PPAR-γ with retinoid X receptor (RXR) and recognition of DNA response elements (PPAR response elements [PPRE]) in the promoter region of target genes. In addition, the ligand mediated conformational changes in PPARs results in recruitment of cofactors and coactivators. The coactivators interact with the nuclear receptors in a ligand dependent way and thereby influence the set of genes transcribed (Figure 2) [1, 23]. In transrepression, PPARs can repress the target gene expression by negatively influencing the other signal transduction pathways, such as the nuclear factor κB (NF-κB) signaling pathway in a DNA binding independent manner (Figure 2) [1,23]. TZDs are reported to exert their insulin sensitizing actions either directly (the fatty acid steal hypothesis) or indirectly by means of altered adipokine release [1, 22]. According to the “fatty acid steal” theory, TZDs are reported to promote fatty acid uptake and storage in adipose tissue. As a result of this it decreases the fat accumulation in the non-adipose tissues such as liver, muscle and pancreas, and thereby protects these tissues from the harmful metabolic effects of high concentration of free fatty acids. The target genes which are involved and regulated by PPAR-γ for this process include lipoprotein lipase, fatty acid transport protein and oxidized LDL receptor-1. All these favour movement of fatty acids in to the
adipocytes. In addition, regulation of phosphoenolpyruvate carboxykinase, glycerol kinase and glycerol transporter aquaporin-7 genes promote recycling rather than export of intracellular fatty acids (Figure 3) [22].

![Figure 2. Molecular mechanisms of biological responses of TZDs](image)

STAT: Signal transducers and activators of transcription; ISGF-RE interferon-stimulated gene factor responsive element; TRE TPA: responsive element, where TPA is a phorbol ester (Source: N Engl J Med, 2004; 351: 1106)

Although TZDs enhance insulin sensitivity through their direct effects by keeping the fat where it belongs, a lot of evidence is available to support the role of indirect mechanisms in the regulation of insulin sensitivity by TZDs, especially in the liver and muscle tissues. The existence of indirect mechanisms is supported by the fact that mice lacking adipose tissue or adipose PPAR-γ are reported to be refractory to the insulin sensitizing effects of TZDs [24]. Also, TZDs are reported to retain their glucose lowering capacity in PPAR-γ knockout models of liver and muscle [25, 26]. It is apparent, therefore, that the action TZDs must also alter the communication between adipose tissue and the muscle and liver, the main insulin sensitive organs. This indirect effect is reported to be mediated through modulation effect of TZDs on the adipokines gene expression. TZDs are reported to increase the gene expression of insulin sensitizing adipokines, such as adiponectin (adipocyte complement related protein-30 [Acrp-30]) and decrease the gene expression of adipokines which are involved in induction of insulin resistance, such as TNF-α, resistin and 11-β-hydroxysteroid dehydrogenase-1 (11βHSD1) (Figure 3) [27]. Unfortunately, the over activation of PPAR-γ by its ligands (TZDs) is reported to increase body weight through its adipocyte differentiation effects and also because of its ability to cause fluid retention by inducing Na transporter, ENaC, expression in the collecting duct of the kidney [28-30]. These effects promote cardiac risks in the susceptible individuals that resulted in the withdrawal of some of these agents from the clinical use.

The TZDs which are withdrawn from the clinical use are developed at the time when not much scientific data were available on the structure and the transcriptional mechanisms of PPARs. Recent advances in the understanding of the structure and function of PPARs, however, has no doubt given us more rationalized ways to develop this class of agents. The various approaches employed to develop newer agents devoid of the problems associated with current TZDs based on strong scientific knowledge are discussed below. These newer approaches are based on the structural considerations of both the ligands as well as the receptors and also on the profile alterations of the ligands.
STRUCTURAL CONSIDERATIONS

Based on the protein-ligand interaction studies the topological features of a typical PPAR agonist is defined. These features include an acidic head group, two linker groups, an aromatic centre (spacer ring) and a cyclic tail (Figure 4) [31].

The acidic head group is reported to form up to 4-hydrogen bonding interactions with the Arm-I of the Ligand binding domain (LBD) [32-34]. For a full agonist the acidic head group should form hydrogen bonding interactions with the conserved residues of the Arm-I. These residues in PPAR-γ include Ser 289 (H3), His323 (H5), His449 (H11) and Tyr473 (H12 or AF-2). Among these the hydrogen bonding with the Tyr473 residue is reported to play a vital role in the stabilization of AF-2 helix which is essential for the recruitment of coactivators necessary for transcriptional activation [32-34]. The ligands which fail to form hydrogen bonding with the Arm-I residues, especially with Tyr473, would fail in stabilizing the AF-2 helix and results in the destabilization of the LBD. These agents, therefore, are reported to act as either partial agonists or as antagonists [32-34]. Recently this laboratory was involved in the synthesis and evaluation of several (5Z)-5-[4-(3-phenoxypropoxy)benzylidene]-1,3-thiazolidine-2,4-dione derivatives [35]. The general structure of these molecules is given in Figure 5. The interactions of a full and a partial agonists are shown in Figure 6 as examples. The ligand receptor interaction studies show that effective PPAR-γ agonists should show a low transactivation activity (a minimal binding at Arm-I) but a high binding affinity (through hydrophobic interactions at the Arm-II and at the entrance and hydrogen bond at the entrance with Ser342) to inhibit phosphorylation at ser273. Inhibition of Ser273 phosphorylation is reported to prevent the unregulated
expression of some of the genes including adipsin and adiponectin [36]. The spacer ring, linker and the tailpiece portions of the ligand are reported to interact with the hydrophobic portion of the LBD (Arm-II and entrance). In addition the part of the tailpiece is reported to be solvent exposed and, therefore, polar and other diverse substitutions on this portion of the ligand are well tolerated [31]. Although PPARs are highly homologous, the LBD exhibits specific differences that convey isotype selectivity. In case of PPAR-δ the binding site near AF-2 is narrower than the other two isotypes. In addition, in AF-2 region the hydrogen bonding to His 323 of PPAR-γ is found to be less sterically demanding when compared to the corresponding Tyr314 residue in PPAR-α [18].

The modifications carried out on the 5 regions is reported to have resulted in a number of molecules with varied degrees of activities [34]. In brief, substituted oxazoles and pyridyl groups in the tail portion is reported to result in potent compounds. The preferred length of the carbon chain of the linker between the cyclic tail and the spacer ring was found to be two carbon ether or N-alkylethyleneoxy group. A one carbon methylene group was preferred as a linker between the acidic head and the spacer ring. A bivalent phenylene was preferred as the spacer ring. So far, carboxylic acid and thiazolidines-2,4-dione groups are reported as the acidic head groups. Further, it is observed that replacing TZD head group with rhodanine or 2-iminothiazolidinediones resuls in weaker molecules. N-methylated derivatives are found to be totally inactive, suggesting the importance of the acidic proton at position 3 of the TZD ring. Both unsaturated (benzylidene thiazolidine-2,4-dione derivatives)and saturated (benzyl thiazolidine-2,4-dione derivatives) TZD derivatives are reported to show no difference in their glucose lowering activities. However, some variations in their lipid lowering activities have been observed [34].

Figure 5. General structure of (5Z)-5-[4-(3-phenoxypropoxy)benzylidene]-1,3-thiazolidine-2,4-dione derivatives (R₁= H,C₃H₇; Br, F; R₂= H,NO₂,Br,F; R₃= H, C₃H₇; Br, F, Cl; R₄= H, F; R₅= H, C₃H₇)

Figure 6. (a) The acidic head group of a thiazolidine derivative showing full agonist type of interaction with the key residues of LBD of the PPAR-γ (PDB ID: 2PRG); (b) The acidic head group of a thiazolidine derivative showing partial agonist or antagonist type of interaction [Note the absence of H-bond interaction with Tyr473 residue of LBD of the PPAR-γ (PBD ID: 2PRG)]
PROFILE ALTERATIONS

**PPAR-α/γ dual agonists**

In recent years an intense search has been made for molecules which possess both PPAR-α/γ dual agonistic activities. Such molecules have been claimed to achieve a broad spectrum of metabolic effects by improving insulin resistance, hyperglycemia and atherosclerotic dyslipidemia. These molecules are most beneficial in those T2DM patients with coexisting dyslipidemia, most common in T2DM patients. In addition, PPAR-α promotes lipid oxidation and decreases adiposity and hence neutralize the PPAR-γ induced weight gain as a result of increased adipogenesis [37-39]. Many structurally diverse PPAR-α/γ dual agonists have been evaluated and many of these have reached advanced stages of clinical trials [37].

**PPAR-δ/γ dual agonists**

PPAR-δ activation is reported to improve insulin sensitivity and increase fatty acid oxidation [18]. The molecules with dual PPAR- δ/γ activities may, therefore, provide similar kind therapeutic benefits as that of PPAR-α/γ dual agonists. A very few such compounds with dual PPAR-δ/γ activities have been reported so far [37].

**PPARpan agonists**

The benefits of PPAR dual agonists concept was realized with the development of ligands for both PPAR-α/γ and PPAR- δ/γ dual agonists. As a result of this, a new concept of PPARpan agonists has recently emerged. Pan agonists can target all three isotypes of PPARs and may have much broader metabolic effects than the dual agonists. PPARpan agonists are still in early stages of development and very few molecules have reached the stage of clinical trials [18, 37].

**Selective PPAR-γ modulators and partial agonists**

Recent findings of ligand receptor interaction studies have proposed an alternative mechanism to explain the agonistic mechanisms of PPAR ligands. This mechanism is completely independent of the classical transactivation phenomenon. As per this mechanism, an ideal PPAR-γ agonists should show a low transactivation activity (a minimal binding at Arm-I) but a high binding affinity (through hydrophobic interactions at the Arm-II and at the entrance, and hydrogen bond at the entrance with Ser342) to inhibit phosphorylation at ser273. Inhibition of Ser273 phosphorylation is reported to prevent the unregulated expression of some genes including adipsin and adiponectin [36].

With this new knowledge, the focus today has changed towards the development of selective PPAR-γ modulators (SPPARγM) or partial agonists. According to this theory some of the problematic side effects of PPAR-γ full agonists, such as weight gain or fluid retention, may occur through classical agonist action and that a substantial portion of the therapeutic benefits of full and partial PPAR-γ agonists occurs through the inhibition of the PPAR-γ phosphorylation at Ser273. Thus, an effective partial agonist of PPAR-γ would have a weak transactivation activity
and high phosphorylation inhibitory activity on PPAR-γ at Ser273. These compounds could, therefore, provide the same therapeutic benefits without the associated side effects [18, 36, 37]. Many such SPPARγM are in the pipeline and many have shown promising activities without the side effects related to PPAR-γ activation [37].

CONCLUSION

The withdrawal of glitazones from the clinical use has created a vacuum in the insulin sensitizer class of antidiabetic agents. The recent understanding of structure and function of PPARs has resulted in a rationale to develop newer TZDs. This new knowledge has led research into developing of dual and Pan PPAR agonists, and partial agonists or selective PPAR modulators. The new research direction has, therefore, created possibilities of developing novel TZDs that are devoid of or show minimal unwanted side effects.
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