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Different binding and recognition modes of GL479, a dual agonist of Peroxisome Proliferator-Activated Receptor α/γ

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ABSTRACT

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-dependent transcription factors that control various functions in human organism, including the control of glucose and lipid metabolism. PPARγ is a target of TZD agonists, clinically used to improve insulin sensitivity whereas fibrates, PPARα ligands, lower serum triglyceride levels. We report here the structural studies of GL479, a synthetic dual PPARα/γ agonist, designed by a combination of clofibric acid skeleton and a phenyldiazenyl moiety, as bioisosteric replacement of stilbene group, in complex with both PPARα and PPARγ receptors. GL479 was previously reported as a partial agonist of PPARγ and a full agonist of PPARα with high affinity for both PPARs. Our structural studies reveal different binding modes of GL479 to PPARα and PPARγ, which may explain the distinct activation behaviors observed for each receptor. In both cases the ligand interacts with a Tyr located at helix 12 (H12), resulting in the receptor active conformation. In the complex with PPARα, GL479 occupies the same region of the ligand-binding pocket (LBP) observed for other full agonists, whereas GL479 bound to PPARγ displays a new binding mode. Our results indicate a novel region of PPARs LBP that may be explored for the design of partial agonists as well dual PPARα/γ agonists that combine, simultaneously, the therapeutic effects of the treatment of insulin resistance and dyslipidemia.

1. Introduction

The lifestyle of the 21st century, characterized by people sedentary routine and diets rich in fat and carbohydrates, has contributed to an increase in the occurrence of metabolic syndromes, including type 2 diabetes mellitus, dyslipidemia, obesity and other cardiovascular diseases (Eckel et al., 2005). Several of the metabolic pathways involved in these disorders are regulated by Peroxisome Proliferator-Activated Receptors (PPARs), which are members of the superfamily of the nuclear receptors and function as transcription factors activated by several synthetic and natural ligands (Forman et al., 1997). These receptors form heterodimers with the Retinoid X Receptor (RXR) and the heterodimerization is essential for the recruitment of coregulators and consequent initiation of transcription (Auwerx, 1999; Berger and Moller, 2002; Michalik et al., 2006; Rastinejad et al., 2015). There are three PPAR isotypes, encoded by distinct genes and designated as PPARα, PPARβ/δ and PPARγ. PPARα is expressed predominantly in metabolically active tissues, including liver, adipose tissue, kidney and heart. PPARβ/δ expression occurs in a wide variety of tissues and cells, with relatively higher levels in adipose tissue, brain, liver and skin. PPARγ expression is abundant in fatty tissues, liver and heart (Auwerx, 1999; Auwerx et al., 2003; Kota et al., 2005).

Among the three isotypes, PPARα and PPARγ have been the most widely studied, as they have important roles in regulating glucose, lipids and cholesterol metabolism as well as in the fatty acid β-oxidation and homeostasis. It makes these receptors important pharmacological targets of the drugs used for treatment of type 2 diabetes mellitus (T2DM) and dyslipidemia (Harmon et al., 2011; Mandard et al., 2004). The PPARα is a molecular target for fibrates, a class of hypolipidemic drugs used in the treatment of

Abbreviations: PPAR, Peroxisome Proliferator-Activated Receptor; LBD, ligand binding domain; DMSO, dimethyl sulfoxide; GL479, (*E*)-2-methyl-2-(4-(2-(4-(phenyldiazenyl)phenoxy)ethyl)phenoxy)propanoic acid; DTT, dithiothreitol; IPTG, Isopropyl β-D-1-thiogalactopyranoside; HDL, high density lipoprotein; LBP, ligand binding pocket.

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dyslipidemia in humans. PPARα activation by fibrates decreases triglyceride levels, while increasing HDL cholesterol via metabolism control of fatty acids and lipoproteins. Clinical studies have also demonstrated that fibrates reduce the incidence of atherosclerosis and cardiovascular events. Additionally, activation of PPARα produces an increased sensitivity to insulin and glucose tolerance in patients with T2DM. Fibrates are well tolerated by most patients, however some adverse effects have been reported, mostly gastrointestinal, but also anxiety, headache, dizziness, sleep disorder, rash and hives. Because fibrate metabolism and excretion are mainly performed by the kidneys, patients with kidney problems are advised not to use them (Barter and Rye, 2008). PPARγ is the molecular targets of several marketed drugs, mostly the thiazolidinediones (TZDs) that were developed mainly to have high affinity and full agonism towards this receptor subtype (Henke et al., 1998). These pharmaceutical molecules increase insulin sensitivity and are used clinically for the treatment of T2DM, which is associated with various metabolic disorders including obesity, hypertension and dyslipidemia. Albeit their antidiabetic effect, the TZDs present risks to the patients (Levin et al., 2015); troglitazone was withdrawn from the market for causing hepatotoxicity, while rosiglitazone has a restricted use because of the greater risk of death from cardiovascular events. Currently, only pioglitazone is in unrestricted use, however many adverse effects are attributed to the continued use of pioglitazone, such as increased incidence of bone fractures distal, fluid retention, weight gain and increased occurrence of heart failure (Inzucchi et al., 2015; Nathan et al., 2009). The undesirable side effects attributed to the TZDs appear to be linked to the full activation (full agonists) of gene expression in diverse tissues, which is related to a non-specificity of this class of ligands (Liu et al., 2015). Consistent with this notion, some of the non-TZD ligands, that are also full agonists with antidiabetic activity, exhibited similar side effects (Liu et al., 2015). Therefore, a promising approach for the development of PPARγ agonists, with an acceptable safety profile, is the search for agonists that partially modulate PPARγ target genes (Argmann et al., 2005; Cock et al., 2004; Liu et al., 2015). Despite weak receptor activation, partial PPARγ agonists may have a higher selectivity and fewer side effects (Choi et al., 2010). Structurally, full agonists generally make interactions with residues of H12, whereas partial agonists stabilize other regions of the ligand binding pocket (LBP), without direct contact with the H12 (Bruning et al., 2007).

A challenge to activate both PPARα and PPARγ with a single drug, thus simultaneously normalizing glucose and lipids levels, has led to intensive research efforts. Several PPARα/γ dual agonists, such as muraglitazar, ragaglitazar, tesaglitazar, and aleglitazar, have been synthesized and tested in clinical phase 2 or 3 (Fiévet et al., 2006). Most of these tests have been canceled because of the pronounced side effects of the tested dual agonists. This situation might arise from the fact that all cited glitazars have significantly higher affinity to PPARγ than to PPARα and some of them can be considered as pure PPARγ agonists. When fenofibric acid and rosiglitazone were used as controls of PPARα and PPARγ activity, respectively, some glitazars (muraglitazar and farglitazar, for example) were significantly more potent to PPARγ than a rosiglitazone, when administered in clinically prescribed doses (Fiévet et al., 2006). Nevertheless, better dual agonists, developed to increase insulin sensitivity and concurrently prevent diabetic cardiovascular complications, still offer a very attractive therapeutic option, particularly if the compounds are able to combine an intermediate to higher PPARα affinity with a selective PPARγ-modulating capacity (Fiévet et al., 2006). Aiming to contribute to better understanding of a structural basis of full and partial activation of PPARs, here we present the structures of the ligand binding domain (LBD) of PPARα and PPARγ complexed with GL479, a PPARα/γ dual agonist (Fig. 1A). This compound was synthesized by Giampietro and colleagues in the search for novel PPAR ligands based on a combination of two key pharmacophores: the clofibric acid skeleton and natural products as stilbene, chalcone, and their bioisosters (Giampietro et al., 2012). The aim of this project was to improve the pharmacological activity of classical fibrates by introducing the antioxidant, antilipidemic and antiplatelet properties of natural scaffolds. The introduction of a diazenyl function spaced by a three atom linker from clofibric acid resulted in GL479, a good PPAR α/γ agonist (PPARα EC_{50} = 0.6 μM, PPARγ EC_{50} = 1.4 μM). GL479 was also able to influence the gene expression of CPT1A, an enzyme involved in lipid metabolism in liver, related with long-chain fatty acid transport into hepatocyte mitochondria (Giampietro et al., 2012). Our present crystallographic studies revealed that GL479 interacts with ligand binding pocket (LBP) of both PPARα and PPARγ but displays different binding modes. Considering the fact that GL479 acts as a full agonist of PPARα and partial agonist of PPARγ, we advocate that the observed differences in the binding modes are directly related to the efficacy of the PPARs activation by the ligand. Additionally, our structural analysis offers clues for amelioration of the ligands design for pharmaceutical applications, aimed to simultaneously activate more than one PPAR isoform.

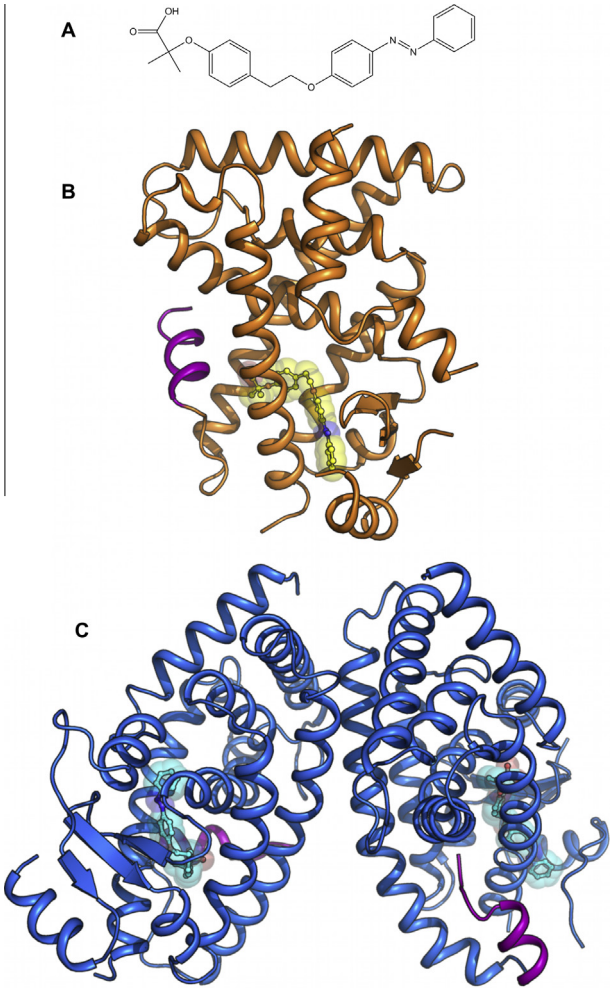

Fig. 1. Structures of PPARα and PPARγ LBDs bound to GL479. (A) Chemical structure of GL479 dual PPARα/γ agonist. (B) An overall schematic representation of the PPARα-LBD structure. The ligand GL479 is shown as a ball and stick model in yellow. (C) The PPARγ-LBD crystallized as an asymmetric unit containing active and inactive forms of the receptor. In both models the H12 is shown in purple.