Cyclooxygenase-2 Inhibitor Parecoxib Was Disclosed as a PPAR-γ Agonist by In Silico and In Vitro Assay

Bin Xiao1,*, Dan-dan Li2,†, Ying Wang3, Eun La Kim1, Na Zhao1, Shang-Wu Jin3, Dong-Hao Bai1, Li-Dong Sun2,† and Jee H. Jung2,*

1Laboratory of Clinical Pharmacy, Ordos Central Hospital, Ordos School of Clinical Medicine, Inner Mongolia Medical University, Ordos 017000, China
2College of Pharmacy, Pusan National University, Busan 46241, Republic of Korea
3The Fourth People’s Hospital of Ordos, Ordos 017000, China

Abstract

In a search for effective PPAR-γ agonists, 110 clinical drugs were screened via molecular docking, and 9 drugs, including parecoxib, were selected for subsequent biological evaluation. Molecular docking of parecoxib to the ligand-binding domain of PPAR-γ showed high binding affinity and relevant binding conformation compared with the PPAR-γ ligand/antidiabetic drug rosiglitazone. Per the docking result, parecoxib showed the best PPAR-γ transactivation in Ac2F rat liver cells. Further docking simulation and a luciferase assay suggested parecoxib would be a selective (and partial) PPAR-γ agonist. PPAR-γ activation by parecoxib induced adipocyte differentiation in 3T3-L1 murine preadipocytes. Parecoxib promoted adipogenesis in a dose-dependent manner and enhanced the expression of adipogenesis transcription factors PPAR-γ, C/EBPα, and C/EBPβ. These data indicated that parecoxib might be utilized as a partial PPAR-γ agonist for drug repositioning study.

Key Words: Parecoxib, In silico screening, PPAR-γ agonist, Adipogenesis

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors and comprised three subtypes: PPAR-α, β/δ, and γ (Mangelsdorf et al., 1995; Berger and Moller, 2002; Evans et al., 2004). In particular, PPAR-γ is expressed in adipose tissue, colon, and macrophages and plays essential roles in the regulation of lipid metabolism, adipogenesis, glucose homeostasis, and insulin sensitization (Willson et al., 2001; Semple et al., 2006; Higgins and Mantzoros, 2008); hence, PPAR-γ is the target for drug discovery efforts for diseases such as type 2 diabetes mellitus (T2DM) (Higgins and Mantzoros, 2008). Although PPAR-γ agonists such as thiazolidinediones (TZDs; e.g., rosiglitazone and troglitazone, Fig. 1A) have been used to treat T2DM for many years in clinical practice and have been shown to lower blood glucose levels and improve insulin sensitivity (Elte and Blickle, 2007), the adverse effects including increased risk of heart attack, weight gain, edema, and fluid retention remain to be challenged. Therefore, new PPAR-γ ligands with less adverse effects are still in demand for T2DM treatment (Motoshima et al., 2011). In addition to TZDs (Petersen et al., 2011), a series of synthetic L-tyrosine analogs (e.g., farglitazar and muraglitazar, Fig. 1A) have been developed as PPAR-γ agonists and subjected to phase II clinical trials (Cobb et al., 1998; Collins et al., 1998; Henke et al., 1998). Linoleic acid, α-linolenic acid, and prostanoit 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) are known as putative endogenous ligands for PPAR-γ with relatively low affinities (Fig. 1B) (Forman et al., 1995; Krey et al. 1997; Berger and Moller, 2002). All known PPAR-γ ligands comprise three distinct substructures—a polar head, linker, and hydrophobic tail (Fig. 2).

In recent years, computational methods have become an integral approach to screen and design novel bioactive compounds. However, a drug repositioning study with a combination of molecular docking and biological evaluation of known pharmacophores will be a promising method to develop new
bioactive leads (Hu et al., 2017). In this study, we screened 110 clinically used drugs for binding affinity to the PPAR-γ ligand-binding domain (LBD) via molecular docking, and 9 drugs, including parecoxib, were selected for subsequent biological analyses. Among these, parecoxib showed the best PPAR-γ binding affinity (Table 1).

The cyclooxygenase-2 (COX-2) inhibitor parecoxib is clinically used to relieve pain, inflammation, central sensitization, and postoperative cognitive dysfunction (Bian et al., 2018; Huang et al., 2019; Wang et al., 2019). However, there was no report on the therapeutic potential of parecoxib through activation of PPAR-γ. Parecoxib is metabolized to the active metabolite valdecoxib in a biological system. The structure of parecoxib may be depicted as a collection of three distinct partial structures, i.e., a polar head, linker, and hydrophobic tail, similar to other typical PPAR-γ agonists (Fig. 2). Therefore, we explored the pharmacological potential of parecoxib as a PPAR-γ agonist with regard to drug reposition.

**Materials and Methods**

**Materials**

Rosiglitazone, cefazolin, omeprazole, furosemide, esomeprazole, parecoxib, ondansetron, and linezolid were purchased from Sigma-Aldrich (St. Louis, MO, USA) with more than 98% purity. All compounds were dissolved in dimethylsulfoxide (Sigma-Aldrich) at 20 mM concentration. 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin were obtained from Sigma-Aldrich and dissolved in 0.5 M KOH, 100% ethanol, and 0.02 M HCl, respectively.

**Computational methods**

For molecular docking, protein coordinates were downloaded from the Protein Data Bank (accession code: 2PRG) (Bernstein et al., 2000). Chain A was prepared for docking using the molecular modeling software package Chimera 1.5.3 (National Institutes of Health, Bethesda, MD, USA) (Pettersen et al., 2004). Polar hydrogen and setting grid box parameters were added using MGLTools 1.5.4 (The Scripps Research Institute, La Jolla, CA, USA) (Sanner, 1999; Morris et al., 2009). Docking calculations were performed using AutoDock Vina 1.1.2.

![Fig. 1. Structures of natural or synthetic PPAR-γ agonists.](image)

**Fig. 1.** Structures of natural or synthetic PPAR-γ agonists. (A) Representative synthetic PPAR-γ agonists; (B) putative endogenous PPAR-γ agonists.

**Table 1.** PPAR-γ docking simulation results of selected drugs

| Drug          | Bioactivity | Drug target | Binding affinity (kcal/mol) | H-bond                  |
|---------------|-------------|-------------|----------------------------|-------------------------|
| Rosiglitazone | Antidiabetic | PPAR-γ      | -9.0                       | Tyr<sup>147</sup>/Arg<sup>139</sup>/His<sup>200</sup>/Ser<sup>209</sup>/Gln<sup>296</sup> |
| Cefazolin     | Antibacterial| PBP<sup>a</sup> | -7.8                       | Tyr<sup>147</sup>/His<sup>200</sup>/Arg<sup>139</sup>/Glu<sup>296</sup> |
| Papaverine    | Smooth muscle relaxant| phosphodiesterase | -8.0                       | Ser<sup>142</sup>/Arg<sup>139</sup> |
| Omeprazole    | PPI         | H<sup>+</sup>/’K<sup>-</sup>/ATPase | -8.0                       | Ser<sup>142</sup>/Tyr<sup>271</sup> |
| Furosemide    | Diuretic    | NKCC2       | -7.4                       | Glu<sup>343</sup>/Gly<sup>344</sup>/Arg<sup>348</sup>/Leu<sup>340</sup> |
| Esomeprazole  | PPI         | H<sup>+</sup>/’K<sup>-</sup>/ATPase | -8.0                       | Ser<sup>142</sup>/Tyr<sup>271</sup> |
| Parecoxib     | Analgesic   | COX-2       | -9.4                       | Tyr<sup>271</sup>/Ser<sup>299</sup>/His<sup>213</sup> |
| Ondansetron   | Antiemetic  | 5-HT3       | -7.9                       | Ser<sup>242</sup> |
| Valdecoxib    | Analgesic   | COX-2       | -8.8                       | Tyr<sup>271</sup>/Ser<sup>299</sup>/Glu<sup>296</sup>/Tyr<sup>271</sup>/Arg<sup>298</sup> |
| Linezolid     | Antibacterial | 50S         | -8.2                       | |

<sup>a</sup>PPIs, proton pump inhibitors; <sup>b</sup>PBPs, penicillin-binding proteins; NKCC2, Na-K-Cl cotransporter 2; 50S, the larger subunit of the 70S ribosomes of prokaryotes; 5-HT3, 5-hydroxytryptamine receptor 3; COX-2, cyclooxygenase 2; PPAR-γ, peroxisome proliferator-activated receptor γ. Binding affinity is shown as kcal/mol. Omeprazole is a racemate, and esomeprazole is an (S)-isomer. However, tetrahedral configuration of the sulfoxide could not be generated by Chemdraw software, so the planar configuration, instead of a tetrahedral configuration, was employed for docking.

https://doi.org/10.4062/biomolther.2021.008
software (The Scripps Research Institute) (Trott and Olson, 2010). The default settings and the Vina scoring function were applied. For ligand preparation, Chem3D Ultra 8.0 software (CambridgeSoft Corporation, Cambridge, MA, USA) was used to convert the 2D structures of candidates into 3D structural data. The analysis and visual investigation of ligand-protein interactions of docking poses were performed using PyMol v1.5 (Schrodinger LLC, New York, NY, USA).

Cell culture

Rat liver cells (AC2F), human hepatoma cells (HepG2), and preadipocytes cells (3T3-L1) were obtained from American Type Culture Collection (ATCC, VA, USA) and cultured in Dulbecco’s Modified Eagle’s medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, NY, USA) and 1% penicillin/streptomycin at 37°C containing 5% CO2.

Cell viability

AC2F and 3T3-L1 cells (1×10^4 cells/well) were seeded into a 96-well plate. After 12 h, the cells were treated with parecoxib at different concentrations (5, 10, 20, 40, and 80 μM) for 12 h or 48 h. After treatment, 0.5 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added into every well and incubated for another 4 h in the dark. After discarding the medium containing MTT, 150 μL DMSO was added to every well to dissolve the formazan crystals, and the optical density value was measured using a microplate reader (Elx 800, Bio-Tek, Winooski, VT, USA). The OD value was determined using iMark Microplate Absorbance Reader (Bio-Rad Laboratories, Hercules, CA, USA) at 549 nm.

Western blot assay

The AC2F cells and adipocytes (after differentiation) were seeded in 60 mm dishes overnight. The dishes were treated with parecoxib (15, 30, and 60 μM) and rosiglitazone (10 μM) and incubated for 48 h. After treatment, the total protein was extracted using radioimmunoprecipitation assay buffer containing a protease inhibitor mixture of 1% PMSF, 1% aprotinin, and 1% pepstatin. Nuclear protein was extracted using NE-PER® nuclear and cytoplasmatic extraction reagents (Thermo Scientific, Rockford, IL, USA) containing 1% PMSF, 1% aprotinin, and 1% pepstatin. BCA kit was used to confirm the protein concentration. The total and nuclear proteins were separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride membranes. The membranes were blocked by 5% milk at 25°C for 2 h and then incubated by PPAR-γ (Cell Signaling Technology, USA), C/EBPα (Cell Signaling Technology), C/EBPβ (Cell Signaling Technology) antibodies at 4°C overnight. On the second day, the membranes were washed using Tris buffered saline with Tween-20 and incubated with Anti-rabbit horseradish-linked IgG for 1 h. Enhanced chemiluminescent reagent and the ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories) were used to analyze the protein band.

Immunofluorescence staining

The Ac2F cells and adipocytes (after differentiation) were seeded into the confocal dishes overnight. The cells were treated with parecoxib (15, 30, and 60 μM) and rosiglitazone (10 μM) for 48 h. Then, the cells were fixed with formalin for 10 min. The permeabilization of cells was achieved by incubating the cells with 0.3% Triton X-100 for 15 min. Next, the cells were blocked using 10% FBS and incubated with a PPAR-γ antibody at 4°C overnight. The cells were incubated with anti-mouse Alexa 488 secondary antibodies (Cell Signaling Technology) for 30 min, and then PI (10 μg/mL) and RNase (10 μg/mL) reagents were added to the cells and incubated for 40 min. Finally, FluoView FV10i confocal microscope (Olympus, Tokyo, Japan) was used to analyze the stained cells.

Statistics

Data were analyzed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The significance between groups was analyzed using one-way analysis of variance and tukey’s range test. All results are expressed as means ± standard error of the mean, and p<0.05 was considered significant.
RESULTS

Docking simulations of clinical drugs to PPAR-γ

One-by-one molecular docking was performed to gain insight into the interaction between ligand and PPAR-γ in silico screening. For in silico screening, compounds of molecular weight less than 500 amu (Lipinski et al., 1997) were selected from 110 clinical drugs used in Ordos Central Hospital. Nine clinical drugs (ceftazolin, papaverine, omeprazole, furosemide, esomeprazole, parecoxib, ondansetron, valdecoxib, and linezolid) with substantial binding affinities (ranging from −7.4 kcal/mol to −9.4 kcal/mol) and valid binding conformations (bound to the PPAR-γ LBD and formed H-bonds with key amino acid residues) were selected (Table 1). In particular, parecoxib showed a strong binding affinity (−9.4 kcal/mol) when compared with other clinical drugs and the standard PPAR-γ agonist rosiglitazone (−9.0 kcal/mol).

Rosiglitazone formed hydrogen bonds with key amino acids Ser289(H3), Arg288(H3), Gln286(H3), His323(H11), and Tyr473(H12) residues on helices H3, H11, and H12 in the PPAR-γ LBD (Fig. 3A). It is known that rosiglitazone stabilizes the dynamic H12 region through the aforementioned interactions and activates PPAR-γ. Parecoxib showed hydrogen bonding with Ser289 (H3), Tyr327(H5), His323(H11) residues on helices H3, H5, and H11 (Fig. 3B). The simulation data indicated that parecoxib skeleton might be suitable for PPAR-γ LBD (Fig. 3B); the sulfonamide moiety may serve as a head group forming H-bonds with key amino acids Ser289(H3), Tyr327(H5), and His323(H11) in the PPAR-γ LBD, whereas the phenol group serves as a linker, and the 5-methyl-3-phenylisoxazol-4-yl moiety serves as a hydrophobic tail positioned in the hydrophobic binding pocket. However, the binding conformation of parecoxib lacked the key hydrogen bonding with Tyr473(H12), which was observed for rosiglitazone. Instead, parecoxib seems to be positioned between the helix 3 (H3) and the α-sheet to stabilize this part, which is a typical binding profile of partial agonists, such as amorfrutin (Wang et al., 2014). Partial PPAR-γ agonists are proposed to be useful as antidiabetic agents because it would be free of side effects of full agonists (Kroeker and Bruning, 2015).

Parecoxib is metabolized to valdecoxib (amide moiety being hydrolyzed) after absorption into the human body, and finally, valdecoxib itself manifests COX-2 inhibition. Therefore, the pharmacologically active valdecoxib was also assessed for in silico PPAR-γ binding (Fig. 3C). Valdecoxib showed a lower binding affinity (−8.8 kcal/mol) than parecoxib (−9.4 kcal/mol). Parecoxib showed hydrogen bonding with Ser289, Tyr327, and His323 residues on helices H3, H5, and H11 (Fig. 3B). Furthermore, valdecoxib showed hydrogen bonding with only Tyr327 and Ser289. The interaction between ligand and Tyr473 is suggested to be essential for the activation of PPAR-γ by a full agonist such as rosiglitazone.

**Fig. 3.** Docking structures of ligand/PPAR-γ binding. (A) Zoomed view of the hydrogen bonding interactions (yellow dotted lines) between the rosiglitazone and surrounding amino acids Ser289(H3), Arg288(H3), Gln286(H3), His323(H11), and Tyr473(H12) on helices H3, H11, and H12. Helices of PPAR-γ are labeled in blue. (B) Zoomed view of the hydrogen bonding interactions between parecoxib and surrounding amino acids Ser289(H3), Tyr327(H5), and His323(H11) on helices H3, H5, and H11. (C) Zoomed view of the hydrogen bonding interactions between valdecoxib and surrounding amino acids Ser289(H3) and Tyr473(H5).

**Fig. 4.** PPAR-γ activation of nine clinical drugs and rosiglitazone. Ac2F cells were transfected with PcDNA, PPRE, and/or pFlag-PPAR-γ1, and then treated with nine clinical drugs (20 μM and 50 μM) and rosiglitazone (10 μM parecoxib and rosiglitazone in Ac2F cells) for 6 h. PPAR-γ activation was measured using luciferase expression assay. Rosi: rosiglitazone, cefa: cefazolin, papa: papaverine, omep: omeprazole, furo: furosemide, esom: esomeprazole, pare: parecoxib, onda: ondansetron, vald: valdecoxib, line: linezolid. Ac2F cells were transfected with PcDNA as the blank group. Control group cells were transfected with PPRE and pFlag-PPAR-1. Triplicate wells were used in every sample group and the experiments were repeated for three times. **p<0.01, ***p<0.001 vs. PPAR-γ.**
PPAR-γ transactivation by parecoxib

The PPAR-γ transactivation activity of nine clinical drugs was evaluated using Ac2F rat liver cells. Consistent with molecular docking, luciferase expression assay demonstrated that parecoxib has the best effect on PPAR-γ activation, though it was not as potent as rosiglitazone (Fig. 4). The effect of parecoxib on PPAR-γ transactivation was further examined at various concentrations. Prior to the PPAR-γ transactivation assay using Ac2F cells, the cytotoxicity of parecoxib to Ac2F cells was measured using MTT assay (Fig. 5A). The concentrations for the PPAR-γ transactivation assay were chosen from five non-cytotoxic concentrations. Parecoxib activated PPAR-γ in a dose-dependent manner (Fig. 5D). The effect of parecoxib on the PPAR isoforms PPAR-α and PPAR-β/δ was also investigated in comparison to PPAR-α agonist (WY-14643) and PPAR-β/δ agonist (GW501516), respectively (Fig. 5B, 5C). Parecoxib showed lesser activity to PPAR-α than rosiglitazone, and was non-active to PPAR-β/δ.

Once the PPAR-γ ligand binds to the receptor, the activated PPAR-γ will transfer to the cell nucleus and bind to PPRE to regulate subsequent gene expression (Su et al., 2017). Therefore, the level of endonuclear PPAR-γ was detected using western blot and immunofluorescence staining assay. As shown in Fig. 6A, parecoxib significantly increased the endonuclear PPAR-γ concentration in Ac2F cells, similar to rosiglitazone. A significant level of PPAR-γ protein was detected as green fluorescence in the nuclei of parecoxib (60 μM)-treated and rosiglitazone (10 μM)-treated groups (Fig. 6B). This indicated that parecoxib activated PPAR-γ and promoted endonuclear PPAR-γ translocation. Thus, parecoxib was defined as a potential PPAR-γ activator.

Adipocyte differentiation in 3T3-L1 cells

In this study, the adipogenic activity of parecoxib was examined using murine preadipocytes (3T3-L1). 3T3-L1 cells were treated with IBMX, dexamethasone, insulin plus rosiglitazone, or different parecoxib concentrations for 8 days. Lipid droplets accumulated by adipogenesis can be visualized through red color by Oil Red O staining. The Oil Red O staining showed that parecoxib stimulated adipogenesis in a dose-dependent manner.
The preadipocytes were well-differentiated into mature adipocytes following rosiglitazone or parecoxib treatment (shown as red lipid in Fig. 7A), and this microscopic observation was well correlated with the concentration-dependent increase in total lipid accumulation in adipocytes (Fig. 7B).

Subsequently, the expression of C/EBPα and C/EBPβ and the protein level of PPAR-γ in the nucleus were compared using western blot and immunofluorescence staining assay. As shown in Fig. 8A and 8B, parecoxib increased the expression level of C/EBPα and C/EBPβ in 3T3-L1 cells. Additionally, the endonuclear PPAR-γ protein level was increased when treated with parecoxib and rosiglitazone (Fig. 8C, 8D). This result demonstrated that parecoxib might promote endonuclear translocation of PPAR-γ. Consistent with the western blot, immunofluorescence staining assay also showed that parecoxib enhances endonuclear PPAR-γ protein level in 3T3-L1 cells (Fig. 9). Thus, parecoxib worked as a PPAR-γ agonist and promoted endonuclear PPAR-γ translocation in Ac2F cells and 3T3-L1 cells.
DISCUSSION

In this preliminary study on drug repositioning, a COX-2 inhibitor parecoxib was selected as a tentative PPAR-γ agonist via in silico analysis and in vitro evaluation of clinically used drugs. In docking simulations, parecoxib showed higher binding affinity than rosiglitazone, but its hydrogen bonding network was distinct from that of rosiglitazone. As for rosiglitazone, the hydrogen bonding with Tyr323 was suggested to be essential to induce H12 into active conformation and thereby activate PPAR-γ (Waku et al., 2009). However, parecoxib did not show direct hydrogen bonding with Tyr in the helix H12. Instead, it formed an alternative hydrogen bond with Tyr327 on the helix H5 and was positioned between H3 and β-sheet, stabilizing this region similar to the typical partial agonist amorglitazone.

Human apo-PPAR-γ LBD has a sizable binding pocket, and rosiglitazone occupies roughly 40% of the ligand-binding site in a U-shaped conformation (Nolte et al., 1998). Full agonist rosiglitazone forms hydrogen bonds with three key amino acid residues (His323, His484, and Tyr573) to stabilize the AF-2 surface (Liu et al., 2015). The lack of these key interactions of parecoxib and valdecoxib may explain why they showed lower PPAR-γ transactivation activity than rosiglitazone and indicate they might be PPAR-γ partial agonists. Partial agonists have lower efficacy than full agonists and occupy a partial cavity of the binding pocket and interact with partial amino acid residues. In addition, the in vitro assay demonstrated that parecoxib was a more potent PPAR-γ agonist than valdecoxib (the in vivo active form of parecoxib). Therefore, degradation-resistant parecoxib analog may be devised by modifying the hydrolysis-susceptible amide moiety of parecoxib. For example, branched alkyl chain or cyclic alkyl group may be introduced in place of the ethyl chain, to increase steric hindrance against enzymatic hydrolysis.

Cyclooxygenase-2 (COX-2) is up-regulated in diabetes mellitus, and selective inhibition of COX-2 could enhance the insulin secretion (Bagi et al., 2006; Fujita et al., 2007). Parecoxib, as a selective COX-2 inhibitor and PPAR-γ agonist, would serve as a lead for potential antidiabetic drug. Adipose tissues play a vital role in metabolic regulation and excess lipid storage (Camp et al., 2002). However, in type 2 diabetes, the ability of subcutaneous adipose tissue to recruit and differentiate precursor cells into adipocytes is reduced (Smith and Kahn, 2016). It is well-known that the PPAR-γ gene, which is abundant in adipose tissue, regulates adipogenesis and lipid metabolism (Chao et al., 2000; Wang et al., 2017). PPAR-γ stimulates the production of small insulin-sensitive adipocytes. Adipose tissue produces several cytokines that regulate energy homeostasis, lipid, and glucose metabolism. In addition to its importance in adipogenesis, PPAR-γ plays an essential role in regulating lipid metabolism in mature adipocytes by increasing fatty acid trapping (Leonardini et al., 2009). PPAR-γ agonistic drugs TZDs, such as rosiglitazone and troglitazone, show antidiabetic effects through targeting adipose tissue (Chao et al., 2000). The anti-inflammatory drug parecoxib showed PPAR-γ transactivation in a concentration-dependent mode in Ac2F rat liver cells. As a PPAR-γ agonist, parecoxib was exploited as a potential antidiabetic agent through in vitro evaluations, similar to rosiglitazone.

Adipocyte differentiation is a complex process that is regulated by a set of transcription factors such as PPAR-γ and CCAAT-enhancer-binding protein α/β (C/EBPα/β) (Jiang et al., 2019). PPAR-γ is a master regulator to induce adipogenesis. The protein C/EBPα belongs to a family of basic region/leucine zipper (bZIP) transcription factors (Khanha-Gupta et al., 2012) which participate in adipogenesis along with PPAR-γ. Although the protein C/EBPβ is expressed in the early stage of adipocyte differentiation to activate the expression of PPAR-γ and C/EBPα, C/EBPβ together with PPAR-γ and C/EBPα then induce lipid accumulation or glucose uptake (Younce et al., 2009; Jiang et al., 2019). In this study, we found that parecoxib promoted differentiation of adipocytes in a concentration-dependent manner, stimulated the expression of adipogenesis transcription factors CEBPα and C/EBPβ, and promoted translocation of PPAR-γ into the nucleus in Ac2F and 3T3-L1 cells. Though the potency of parecoxib did not reach that of rosiglitazone, possibly because of its partially agonistic nature, a substantial activity was obtained at higher concentrations. According to our preliminary study, parecoxib may be eligible for drug repositioning study and further pharmacological study as a PPAR-γ agonist.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This research was supported by a 2-year grant from Pusan National University. We thank Hye Lim Byun and Sunwoo Yu for their sincere assistance in molecular docking calculations.

REFERENCES

Bagi, Z., Erdei, N., Papp, Z., Esdes, I. and Koller, A. (2006) Up-regulation of vascular cyclooxygenase-2 in diabetes mellitus. Pharmacol. Rep. 58, 52-56.

Barger, J. and Moller, D. E. (2002) The mechanisms of action of PPARs. Annu. Rev. Med. 53, 409-435.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000) The protein data bank. Nucleic Acids Res. 28, 235-242.

Blan, Y. Y., Wang, L. C., Qian, W. W., Lin, J., Jin, J., Peng, H. M. and Weng, X. S. (2018) Role of parecoxib sodium in the multimodal analgesia after total knee arthroplasty: a randomized double-blinded controlled trial. Orthop. Surg. 10, 321-327.

Camp, H. S., Ren, D. and Leff, T. (2002) Adipogenesis and fat-cell function in obesity and diabetes. Trends Mol. Med. 8, 442-447.

Chao, L., Marcus-Samuels, B., Mason, M. M., Molitra, J., Vinson, C., Arioglu, E., Gavrlova, O. and Reitman, M. L. (2000) Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. J. Clin. Invest. 106, 1221-1228.

Cobb, J. E., Blanchard, S. G., Boswell, E. G., Brown, K. K., Charifson, P. S., Cooper, J. P., Collins, J. L., Dezube, M., Henke, B. R., Hull-Ryde, E. A., Lake, D. H., Lenhard, J. M., Oliver, W. Jr., Oplinger, J., Pentti, M., Parks, D. J., Plunkett, K. D. and Tong, W. Q. (1998) N-(2-benzoylphenyl)-l-tyrosine PPAR-γ agonists. 3. Structure-activity relationship and optimization of the N-aryl substituent. J. Med. Chem. 41, 5055-5069.

Collins, J. L., Blanchard, S. G., Boswell, E. G., Charifson, P. S., Cobb, J. E., Henke, B. R., Hull-Ryde, E. A., Kazmierski, W. M., Lake, D. H., Leesnitzer, L. M., Lehnard, J., Lenhard, J. M., Orband-Miller, L. A., Gray-Nunez, Y., Parks, D. J., Plunkett, K. D. and Tong, W. Q. (1998) N-(2-benzoylphenyl)-l-tyrosine PPAR-γ agonists. 2. Struc-
ture-activity relationship and optimization of the phenyl alkyl ether moiety. J. Med. Chem. 41, 5037-5045.

Elte, J. W. F. and Bickler, J. F. (2007) Thiazolidinediones for the treatment of type 2 diabetes. Eur. J. Intern. Med. 18, 18-25.

Eom, S. H., Liu, S., Su, M., Noh, T. H., Hong, J., Kim, N. D., Chung, H. Y., Yang, M. H. and Jung, H. J. (2016) Synthesis of thialalidinam derivatives as potential PPARγ ligands. Mar. Drugs 14, 112.

Evans, R. M., Barish, G. D. and Wang, Y. X. (2004) PPARs and the complex journey to obesity. Nat. Med. 10, 355-361.

Forman, B. M., Tontonoz, P., Chen, J. R., Brun, R. P., Spiegelman, B. M. and Evans, R. M. (1995) 15-Deoxy-D12, 14-prostaglandin J2 is a ligand for the adipocyte differentiation factor PPARγ. Cell 83, 803-812.

Fujita, H., Kakei, M., Fujishima, H., Morii, T., Yamada, Y., Qi, Z. and Breyer, M. D. (2007) Effect of selective cyclooxygenase-2 (cox-2) inhibitor treatment on glucose-stimulated insulin secretion in c57bl/6 mice. Biochem. Biophys. Res. Commun. 363, 37-43.

Henke, B. R., Blanchard, S. G., Brackeen, M. F., Brown, K. K., Cobb, J. E., Collins, J. L., Harrington, W. W., Jr., Hashim, M. A., Hull-Ryde, E. A., Kaldor, I., Kliewer, S. A., Lake, D. H., Leesnitzer, L. M., Lehmann, J. M., Lenhard, J. M., Orband-Miller, L. A., Miller, J. F., Moor, R. A., Jr., Noble, S. A., Oliver, W., Jr., Parks, D. J., Plunket, K. D., Szewczyk, J. R. and Willson, T. M. (1998) N-(2-benzoylphenyl)-L-tyrosine PPARγ agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. J. Med. Chem. 41, 5020-5036.

Higgins, L. S. and Mantzoros, C. S. (2008) The development of INT131 as a selective PPARγ modulator: approach to a safer insulin sensitizer. PPAR Res. 2008, 936906.

Huang, S., Hu, H., Gai, Y.-H. and Hua, F. (2019) Effect of parecoxib in the treatment of postoperative cognitive dysfunction: a systematic review and meta-analysis. Medicine 98, e13812.

Hu, Y. Stumpe, D. and Bajorath, J. (2017) Recent advances in scaffold hopping. J. Med. Chem. 60, 1238-1246.

Jiang, T., Shi, X., Yan, Z., Wang, X. and Sun, G. (2019) Isoimperatorin enhances 3T3-L1 preadipocyte differentiation by regulating PPARγ and C/EBPα through the Akt signaling pathway. Exp. Ther. Med. 18, 2160-2166.

Khanna-Gupta, A., Abayasekara, N., Levine, M., Sun, H., Virgilio, M., Cai, Y.-H. and Hua, F. (2019) Effect of parecoxib in the treatment of postoperative cognitive dysfunction: a systematic review and meta-analysis. Medicine 98, e13812.

Krey, G., Braissant, O., L’Horset, F., Kalkhoven, E., Perroud, M., Rico-rakowa, R., Rosenfeld, M. G., Willson, T. M., Glass, C. K. and Willson, M. V. (1998) Ligand binding and co-activator assembly in the peroxisome proliferator-activated receptor-γ. Nature 395, 137-143.

Petersen, R. K., Christensen, K. B., Assimoupolou, A. N., Fretté, T., Papageorgiou, V. P., Kristiansen, K. and Kouskounekaki, I. (2011) Pharmacophore-driven identification of PPARγ agonists from natural sources. J. Comput. Aided Mol. Des. 25, 107-116.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblat, D. M., Meng, E. C. and Ferrin, T. E. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605-1612.

Sanner, M. F. (1999) Python: a programming language for software integration and development. J. Mol. Graph. Model. 17, 57-61.

Semple, R. K., Chatterjee, V. K. K. and O’Rahilly, S. (2006) PPARγ and human metabolic disease. J. Clin. Invest. 116, 581-589.

Smith, U. and Kahn, B. B. (2016) Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J. Intern. Med. 280, 465-475.

Su, M., Cao, J., Huang, J., Liu, S., Zou, J.-W. and Jung, J. H. (2017) The in vitro and in vivo anti-inflammatory effects of a thalidomide PPARγ agonist. Mar. Drugs 15, 7.

Trot, O. and Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31, 455-461.

Waku, T., Shira, T., Oyama, T. and Morikawa, K. (2009) Atomic structure of mutant PPARγ LBD complexed with 15d-PGJ2: novel modulation mechanism of PPAR/RXR function by covalently bound ligands. FEBS Lett. 583, 320-324.

Wang, L., Wangenberger, B., Pferschy-Wenzig, E.-M., Blunder, M., Liu, X., Malainer, C., Blazevic, T., Schweiger, S., Rollinger, J. M., Heiss, E. H., Schuster, D., Kopp, B., Bauer, R., Stuppner, H., Dirsch, V. M. and Atanassov, A. G. (2014) Natural product agonists of peroxisome proliferator-activated receptor γ (PPARγ): a review. Biochem. Pharmacol. 92, 73-89.

Wang, Q., Imam, M. U., Yida, Z. and Wang, F. (2017) Peroxisome proliferator-activated receptor gamma (PPARγ) as a target for concurrent management of diabetes and obesity-related cancer. Curr. Pharm. Des. 23, 3677-3688.

Wang, Y., Chen, Z., Li, J. and Shi, J. (2019) Parecoxib improves the cognitive function of POCD rats via attenuating COX-2. Eur. Rev. Med. Pharmacol. Sci. 23, 4971-4979.

Willson, T. M., Lambert, M. H. and Kliewer, S. A. (2001) Peroxisome proliferator-activated receptor γ and metabolic disease. Annu. Rev. Biochem. 70, 341-367.

Yonce, C. W., Azfer, A. and Kolattukudy, P. E. (2009) MCP-1 (monocyte chemotactic protein-1)-induced protein, a recently identified zinc finger protein, induces adipogenesis in 3T3-L1 pre-adipocytes without peroxisome proliferator-activated receptor γ. J. Biol. Chem. 284, 27620-27628.

https://doi.org/10.4062/biomolther.2021.008

526