Antidiabetic effect of SN158 through PPARα/γ dual activation in ob/ob mice

Yujung Jung a,1, Yongkai Cao b, c,1, Suresh Paudel c, Goo Yoon d, Seung Hoon Cheon c, Gyu-Un Bae e, Li Tai Jin f, Yong Kee Kim c,*, Su-Nam Kim a, **

a Natural Products Research Institute, Korea Institute of Science and Technology, Gangneung, Gangwon-do 25451, Republic of Korea
b Integrated Chinese and Western Medicine Postdoctoral Research Station, Jinan University, Guangzhou 510632, China
c College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 61186, Republic of Korea
d College of Pharmacy, Sookmyung Women’s University, Seoul 04310, Republic of Korea
e School of Pharmaceutical Sciences, Key Laboratory of Biotechnology Pharmaceutical Engineering, Wenzhou Medical University, Wenzhou 325000, China
f College of Pharmacy, Mokpo National University, Muan 58554, Republic of Korea

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A B S T R A C T

In this study, we aimed to demonstrate the antidiabetic potential of (E)-N-(4-(3-(5-bromo-4-hydroxy-2-methoxyphenyl)acryloyl)phenyl)-4-tert-butylbenzamide (SN158) through peroxisome proliferator-activated receptor (PPAR)–α/γ dual activation. SN158 interacted with both PPARα and PPARγ, and increased their transcriptional activities. Simultaneously, SN158 treatment led to an increase in adipogenic differentiation of 3T3-L1 preadipocytes and fatty acid oxidation in hepatocytes. In addition, glucose uptake in myotubes was significantly increased by SN158 treatment. Finally, SN158 significantly lowered the plasma levels of glucose, triglycerides, and free fatty acids in ob/ob mice without severe weight gain and hepatomegaly. These results suggest that SN158 can be useful as a potential therapeutic agent against type 2 diabetes and related metabolic disorders by alleviating glucose and lipid abnormalities.

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) regulate the various metabolic pathways involved in glucose and lipid metabolism, and any dysregulation of these pathways can lead to abnormalities in glucose and lipid metabolism, obesity, and other cardiovascular diseases [1]. The PPAR family comprises three subtypes: PPARα, PPARβ/δ, and PPARγ [2]. PPARα and PPARγ are the most extensively studied PPARs, as they have critical roles in regulating glucose and lipid metabolism as well as in fatty acid β-oxidation [3,4]. These receptors are considered important pharmacological targets for the treatment of type 2 diabetes mellitus (T2DM) or dyslipidemia. PPARα activation results in decreased triglyceride levels, while enhancing the high-density lipoprotein (HDL) cholesterol levels via stimulation of intracellular fatty acid oxidation, cellular fatty acid uptake, and lipoprotein metabolism [5]. In other studies, PPARα activation was found to increase insulin sensitivity and ameliorate impaired glucose tolerance in T2DM patients [6,7]. PPARγ is the molecular target of thiazolidinediones (TZDs), which reverse insulin resistance and are used in the clinical treatment of T2DM [8]. Despite their beneficial antidiabetic potential, TZDs have several side effects. Troglitazone was withdrawn from clinical use because of hepatotoxicity, and it was replaced by rosiglitazone and pioglitazone. However, rosiglitazone appears to have an increased risk of cardiovascular events; pioglitazone showed several adverse effects such as bone fractures, edema, weight gain, and increased incidence of heart failure [9–12]. These undesirable side effects of TZDs appear to be associated with the full activation of the PPARγ target genes in diverse tissues, which is related to the non-specificity of this class of ligands [13]. This notion is consistent with the observation that some non-TZD ligands,
which are also full agonists and exhibit antidiabetic activity, showed similar side effects [13]. Therefore, the development of novel PPARγ agonists that partially modulate its target genes is considered as one of the most promising approaches for the development of antidiabetic agents [13–16]. Despite the weak activation of receptors, partial PPARγ agonists may have higher selectivity and fewer side effects [17–19].

There have been intensive research efforts to develop PPARα/γ dual agonists because the activation of both PPARα and PPARγ with a single drug can simultaneously normalize glucose and lipid impairment. Nevertheless, the dual activation of PPARα/γ still offers an attractive therapeutic option, particularly if the agonists can partially modulate the expression of their target genes [20]. Recently, (E)-N-(4-(3-(5-bromo-4-hydroxy-2-methoxyphenyl)acryloyl) phenyl)-4-tert-butylibenzamide (SN158) has been reported as a PTP1B inhibitor [21]. In this study, we further characterized the pharmacological properties of SN158 as a partial PPARα/γ dual agonist and antidiabetic agent.

2. Materials and methods

2.1. Chemicals and cell culture

(E)-N-(4-(3-(5-bromo-4-hydroxy-2-methoxyphenyl)acryloyl) phenyl)-4-tert-butylibenzamide (SN158) (Fig. 1A) was synthesized by Professor S. H. Cheon [21]. Troglitazone, pioglitazone, rosiglitazone, GW6471, and WY14643 were purchased from Sigma-Aldrich Co. (St Louis, MO). CV-1, 3T3-L1, and C2C12 cells were obtained from ATCC and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT) and 1% penicillin/streptomycin (Invitrogen). HepG2 cells were purchased from ATCC and cultured in Minimum Essential Medium with Earle’s Balanced Salts (MEM/EBSS), supplemented with 10% FBS, 1% penicillin/streptomycin, 1× non-essential amino acid (WelGENE, Daegu, Korea), and 1 mM sodium pyruvate (WelGENE) at 37 °C with 5% CO2.

2.2. Ligand binding assay

The LanthaScreen™ TR-PRET PPAR competitive binding assay (Invitrogen) was performed according to the manufacturer’s guidelines [22].

2.3. 2-NBDG glucose uptake assay

C2C12 myoblasts were differentiated into myotubes, and 2-NBDG glucose uptake assay was performed according to previous report [23].

2.4. Adipocyte differentiation

Adipocyte differentiation was induced by treating the cells with a differentiation mixture (DM) containing 10 μg/mL insulin, 1 μM dexamethasone, and 0.5 mM 3-Isobutyl-1-methylxanthine with 10% FBS in DMEM for 48 h before the cells were switched to a maintenance medium with 10% FBS and 10 μg/mL insulin. After treatment for additional 48 h, the medium was changed with 10% FBS-DMEM. Thereafter, the medium was changed every 2 days. The test drugs were administered at the initiation of differentiation and with every medium change for 8 days. The lipid accumulation in the cells was detected by Oil Red O staining.

2.5. Gene expression analysis

Total RNA was isolated from 3T3-L1 adipocytes using the TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. cDNA synthesis and quantitative real-time PCR (Q-PCR) analyses were performed according to previous report [23]. The primer sets for mC/EBPβ were 5′-AGGTCTGGAGGTAGCAGCT-3′ and 5′-CAGCCTAGAGATCCCGAGC-3′ (NM_00768); mAdiponectin, 5′-AGCTGCGAGGACGGCTTAT-3′ and 5′-TGCCCTGATGCTGAGCAGC-3′ (NM_008084); hHSP22, 5′-TTGTGCCATCAAGACCCC-3′ and 5′-GGGCTAAATTCTTCTGACG-3′ (NM_001876); hACOX2, 5′-GGCGGATGCTACTCAAACG-3′ and 5′-GAGCTG-CACCTTAGAGCCCTG-3′ (NM_003500.1); and hGAPDH, 5′-AGACGGTGGAACAGAGGCTGAAG-3′ and 5′-TGAGCACAACAAATGGTAGTGCTGACG-3′ (NM_003500.1); and hGAPDH, 5′-ACCA- CAGTCTAGCCCATCAC-3′ and 5′-TCCACCACCTGGTGGCTGA-3′ (NM_002046).

2.6. Cell-based transactivation assay

CV-1 cells were seeded into 24-well plates and cultured for 24 h. The reporter gene activities for PPARs were measured using the Dual-Luciferase® Reporter Assay System (Promega) according to previous report [19].

2.7. Animal studies

All the experiments were performed according to the procedures approved by the KIST’s Institutional Animal Care and Use Committee (IRB code No. AP201335). Six-week-old male C57BL/6JHamSlc-ob/ob mice were purchased from Shizuoka Laboratory Animal Center (Japan). The mice were housed under conditions of controlled temperature (23 ± 2 °C), humidity (55 ± 5%), and standard light cycles (12 h light/dark). The mice were orally administered 30 mg/kg pioglitazone or 30, 100 mg/kg SN158 daily for 3 weeks for the analysis of biomarkers in blood. For glucose-tolerance tests, 6-week-old mice were orally administered 30 mg/kg pioglitazone or 30, 100 mg/kg SN158 daily for 3 weeks and fasted overnight before the oral administration of 2 g/kg L-glucose. The glucose levels were measured in tail vein blood at the indicated time intervals using an Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN). At the end of the experimental period, the liver/blood weight ratios were measured, and blood samples were obtained from the abdominal aorta to determine the plasma biomarker concentrations. The triglyceride and free fatty acid (FFA) levels were measured using SICDIA L TG reagent (Eiken Chemical Co., Japan) and NEFAZyme-S (Eiken Chemical Co.), respectively.

2.8. Statistics

The data are expressed as the mean ± standard deviation (S.D.) of three independent experiments. The differences between the mean values in the two groups were analyzed using one-way analysis of variance (ANOVA). P < 0.05 was considered statistically significant.

3. Results

3.1. SN158 is a partial PPARα/γ dual agonist

To investigate the pharmacological properties of SN158 (Fig. 1A), we examined the effects of SN158 on the transcriptional activity of PPARs. SN158 treatment led to an increase in the transcriptional activities of both PPARα and PPARγ in a dose-dependent manner; however, their efficacies were much weaker than those of the
positive controls (Fig. 1C and D). In addition, SN158 had minimal effect on PPARα transactivation and no detectable effect on retinoid X receptor alpha (RXRa) activity (data not shown). We next examined the binding affinities of SN158 to PPARα and PPARγ by using the LanthaScreen competitive binding assay. SN158 could bind to both PPARα and PPARγ (Fig. 1B). The binding affinity of SN158 to PPARα was much weaker than that of the positive control GW6471, which is because of the weak ability of SN158 to activate PPARα transcription in comparison to that of the positive control GW6471, which is because of the weak ability of SN158 to activate PPARα transcription (Fig. 1B). However, despite the weak PPARγ transactivation potential of SN158, its binding affinity to PPARγ (IC50 = 70 nM) was much stronger than that of pioglitazone (IC50 = 1629 nM), indicating that SN158 functions as a partial agonist of PPARγ receptor. To confirm that SN158 act as a partial agonist, we examined the effect of SN158 on the PPARγ-reporter gene activity in the presence of PPARγ-full agonists. The reporter gene activation by PPARγ-full agonists was significantly suppressed by SN158 treatment (Fig. 1E), supporting that SN158 acts as a partial agonist. These findings indicate that SN158 acts as a partial PPARα/γ dual agonist by binding to PPARα and PPARγ.

3.2. SN158 induces both adipocyte differentiation and fatty acid oxidation

It has been demonstrated that PPARγ agonists enhance the differentiation of various preadipocytes and stem cells into mature adipocytes, indicating that PPARγ plays a key role in adipocyte differentiation. The adipogenic potential of SN158 was examined using a 3T3-L1 preadipocyte system. Treatment with the PPARγ agonist pioglitazone led to a dramatic increase in the adipogenic differentiation of 3T3-L1 cells as evidenced by Oil Red O staining (Fig. 2A and B). This observation was in accordance with the result that SN158 causes lipid droplet accumulation in adipocytes (Fig. 2A). Simultaneously, adipogenic differentiation was strongly enhanced by SN158 treatment, which was comparable to that observed with pioglitazone (Fig. 2A and B). We next examined the effect of SN158 on the expression of adipogenic transcription factor CCAAT/enhancer binding protein α (C/EBPα) and the marker gene adiponectin in differentiated adipocytes. After the induction of adipocyte differentiation by SN158, the mRNA levels of C/EBPα and
adiponectin were measured using Q-PCR. The mRNA levels of C/EBPα and adiponectin increased in the SN158-treated adipocytes when compared to those in the control group (Fig. 2C). The increase in C/EBPα levels by SN158 was quite similar to that of the pioglitazone-treated group; however, the adiponectin levels were much lower, indicating that SN158 partially modulates PPARγ target gene expression. To confirm the PPARα agonistic effect of SN158, we next determined its effect on fatty acid β-oxidation. The expression levels of PPARα target genes involved in β-oxidation were measured in PPARα-transfected HepG2 cells. The expression levels of carnitine-palmitoyl transferase 1α (CPT1α) and acyl-CoA oxidase 2 (ACOX2) were significantly increased by SN158 treatment (Fig. 2D). These results strongly support the hypothesis that SN158 enhances fatty acid β-oxidation via PPARα activation.

3.3. SN158 enhances glucose uptake in C2C12 myotubes

To ascertain the beneficial effects of SN158 on insulin sensitivity further, we examined the glucose uptake in C2C12 myotubes. Under normal conditions, the glucose uptake in C2C12 myotubes was dramatically enhanced by insulin treatment (Fig. 3A). SN158 treatment significantly enhanced glucose uptake in the absence or presence of insulin (Fig. 3A). Next, we investigated the effects of SN158 on glucose uptake under palmitate-induced insulin-resistant conditions. Glucose uptake in C2C12 myotubes under insulin-resistant conditions was dramatically enhanced by SN158 treatment (Fig. 3B). SN158-mediated increases in glucose uptake are quite similar with pioglitazone’s effects in both normal and insulin-resistant conditions. All these results support that SN158 improves insulin sensitivity and enhances glucose uptake in myotubes.

3.4. SN158 alleviates glucose and lipid impairment in ob/ob mice

To evaluate the antidiabetic effects of SN158 in vivo, we administered SN158 to obese and diabetic ob/ob mice and monitored the plasma glucose levels. SN158 treatment led to a significant decrease in plasma glucose levels (Fig. 4A). The glucose lowering effect at high dose (100 mg/kg) group was comparable to that observed in pioglitazone treated group (Fig. 4A). However, unlike pioglitazone that causes significant body weight gain, SN158 treatment did not promote considerable body-weight gain (Fig. 4C). Notably, we did not observe increase in liver weight in the SN158-treated group; however, the liver weight in pioglitazone-treated animals was significantly increased (Fig. 4D). In addition, glucose tolerance was significantly alleviated by SN158 treatment (Fig. 4B), even though the effect was weaker than that of pioglitazone. Taken together, these results strongly suggest that SN158 can efficiently alleviate glucose impairment without the side effects of significant weight gain and hepatomegaly. Next, we examined whether SN158 is capable of alleviating lipid abnormalities. The plasma levels of triglycerides and FFAs significantly decreased in SN158-treated mice, which was comparable to the effects observed following pioglitazone treatment (Fig. 4E).

4. Discussion

In this study, we demonstrated the antidiabetic potential of SN158 as a novel partial PPARα/γ dual agonist, which alleviates glucose and lipid impairment in ob/ob mice. The essential role of PPARs in various metabolic diseases has been well demonstrated by several functional studies: TZDs drugs improve insulin resistance by PPARγ activation, and PPARα agonists decrease the circulating
lipid levels. Although many dual PPAR agonists have been synthesized and studied, to date there are no dual PPAR agonists available for clinical use because of their serious side effects. However, balanced dual PPAR\(\alpha\)/\(\gamma\) agonists could potentially benefit patients with diabetes and metabolic disorders [24]. In addition, when compared to full agonists, some partial agonists may selectively modulate PPAR\(\gamma\) activity through a different binding mode in the ligand binding pocket and by the selective recruitment of
transcriptional coactivators [25]. Notably, partial or weak agonists have been shown to have similar efficacy as full agonist TZDs, but they exhibit an improved side effect profile.

A few partial agonists have entered clinical development [26]. Here, we showed that SN158 has dual agonistic activities for both PPARα and PPARγ; however, the binding affinity of SN158 to PPARα (980 nM) is weaker than that of the positive control GW6471 (232.7 nM); its binding affinity (70 nM) to PPARγ is quite stronger than that of pioglitazone (1629 nM) (Fig. 1). Unexpectedly, despite the higher binding affinity of SN158 to PPARγ, its effect on PPARγ transcriptional activity is much weaker than that of pioglitazone, indicating that SN158 acts as a partial agonist. This assumption was further strengthened by the observation that SN158 treatment significantly suppressed the PPARγ-reporter gene activation by TZDs (Fig. 1E). In addition, our data show that the adipogenic potential of SN158 is quite similar to pioglitazone (Fig. 2A). Furthermore, our observation that SN158 efficiently stimulates PPARα activity supports the hypothesis that SN158 may primarily govern lipid metabolism by fatty acid oxidation (Fig. 2D). In addition, SN158 exhibits partial agonistic function to PPARα and its target genes, suggesting the enhancement of lipid catabolism. Treatment with SN158 led to an improvement in insulin sensitivity, resulting in enhanced glucose uptake in myotubes (Fig. 3). So far, we have not been able to describe the precise mechanism by which a weak partial agonist SN158 enhances adipogenic differentiation to a similar extent as that of the full agonist pioglitazone. Recent reports showed that the phosphorylation of the nuclear receptor PPARγ, a dominant regulator of adipogenesis and fat cell gene expression, leads to the dysregulation of a large number of genes, including adiponectin [17,27]. In addition, PPARγ phosphorylation is highly associated with glucose tolerance and insulin sensitivity [28]. Therefore, it is possible that SN158 has an effect on the phosphorylation of PPARγ. To confirm this hypothesis, we tested PPARγ phosphorylation status with phospho-specific antibody (phospho-S273 and phospho-S112, LifeSpan BioSciences, Seattle, WA), but could not ascertain this right now due to the technical limitations.

PPARγ agonists such as rosiglitazone and pioglitazone show severe side effects including hepatomegaly, body-weight gain, and fluid retention, in both humans and mice [29–31]. Increased body and liver weights were observed in mice treated with pioglitazone for 3 weeks (Fig. 4). However, our data showed that SN158 significantly improved glucose impairment without hepatomegaly and body-weight gain in ob/ob mice. The lipolysis of adipose tissue leads to the hydrolysis of triglycerides and release of FFAs [32]. The circulating levels of FFAs are usually elevated in obesity and type 2 diabetes [33]. The PPAR partial agonist SN158 may have a distinct advantage for its pharmacological application, because a number of studies have shown that PPARγ partial agonists including selective PPAR modulators have decreased side effect profiles when compared with those of full agonists [34–36].

5. Conclusion

In summary, it was revealed that SN158 has beneficial effects in the improvement of glucose and lipid metabolism disorders by selectively activating both PPARα and PPARγ; SN158 treatment is not associated with severe adverse reactions such as weight gain and hepatomegaly that have been observed for other PPAR agonists. Therefore, SN158 has a strong potential for further development as antidiabetic agents to correct glucose and lipid abnormalities as well as insulin resistance.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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