BIOCHEMICAL PHARMACOLOGY 72 (2006) 446–454

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/biochempharm

KR-62980: A novel peroxisome proliferator-activated receptor γ agonist with weak adipogenic effects

Kwang Rok Kim [a], *Jeong Hyung Lee* [b], *Seung Jun Kim* [b], *Sang Dal Rhee* [a], *Won Hoon Jung* [a], *Sung-Don Yang* [a], *Sung Soo Kim* [a], *Jin Hee Ahn* [a], *Hyae Gyeong Cheon* [a,*]

[a] *Medicinal Science Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Jang-Dong 100, Yuseong-Gu, Daejeon 305-343, South Korea*

[b] *Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yuseong-Gu, Daejeon 305-600, South Korea*

ARTICLE INFO

Article history:
Received 23 February 2006
Accepted 3 May 2006

Keywords:
PPARγ
Adipogenesis
High fat diet-induced model
Glucose-lowering
Weight gain
Pharmacokinetics

Abbreviations:
AF-2, activation function-2
LBD, ligand binding domain
PPARγ, peroxisome proliferator-activated receptor γ
RT-PCR, reverse transcriptase-polymerase chain reaction

ABSTRACT

The nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) is the target for the anti-diabetic drugs including thiazolidinediones. We report here the identification and characterization of a novel PPARγ agonist KR-62980. KR-62980 acted as a selective PPARγ agonist in transactivation assay with an EC_{50} of 15 nM. In fully differentiated 3T3-L1 adipocytes, KR-62980 induced [^{3}H]-deoxyglucose uptake in a concentration-dependent manner in the presence of insulin. KR-62980 was weakly adipogenic with little induction of aP2 mRNA, and was able to antagonize the adipogenic effects of rosiglitazone in C3H10T1/2 cells. In vivo pharmacokinetic profile of KR-62980 revealed that the compound exhibited good oral bioavailability of 65% with a terminal elimination half-life of 2.5 h in the rat. Treatment of high fat diet-induced C57BL/6J mice with KR-62980 for 14 days reduced plasma glucose levels with little side effects with regard to weight gain, cardiac hypertrophy and hepatotoxicity. These results suggest that KR-62980 acts as a selective PPARγ modulator with anti-hyperglycemic activity, and that the mechanism of actions of KR-62980 appears to be different from that of rosiglitazone with improved side effect profiles.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are a member of nuclear receptor superfamily that acts as a transcription factor upon activation [1,2]. PPARs regulate the expression of genes by heterodimerization with another nuclear receptor retinoid X receptor (RXR) and by binding to the PPAR responsive element (PPRE) regions of the target gene promoter [3,4]. Of the three PPAR isoforms identified so far (PPARα, δ/β, γ), PPARγ, predominantly expressed in adipose tissues [5], has been an attractive target for anti-diabetic thiazolidinediones, such as rosiglitazone and pioglitazone, by regulating glucose and lipid homeostasis.

The activation of PPARγ is known to induce insulin sensitization [6,7] as well as the differentiation of pluripotent cell lines into mature adipocytes [8,9]. While insulin sensitiza-

* *Corresponding author*. Tel.: +82 42 860 7542; fax: +82 42 861 0770.
E-mail address: hgcheon@krict.re.kr (H.G. Cheon).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.05.005

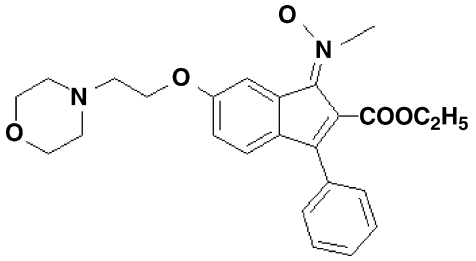

Fig. 1 – Chemical structure of KR-62980, 1-(trans-methylimino-*N*-oxy)-6-(2-morpholinoethoxy)-3-phenyl-1H-indene-2-carboxylic acid ethyl ester.

tion is responsible for the anti-diabetic efficacy of PPARγ agonists, the adipogenic activity may result in undesirable effects such as obesity. In addition, adverse effects, such as cardiac hypertrophy, edema, hemotoxicity and hepatotoxicity, have been reported with existing PPARγ agonists either in animal models or in humans [10,11]. Therefore, the development of safer and efficacious PPARγ agonists is necessary for better anti-diabetic therapy. Recently, novel PPARγ ligands, such as GW0072 [12], *N*-(9-fluorenyl)methoxycarbonyl (FMOC)-L-leucine [13], PAT5A [14] and nTZDpa [15], were shown to inhibit adipocyte differentiation with partial agonistic activity in transactivation assays.

In an effort to search for novel PPARγ agonists, we screened a library of 70,000 structurally diverse synthetic compounds. Among active compounds identified, a compound with indene structure was chosen based on the novelty and ease of derivatives synthesis, and chemical modification of this molecule lead to the KR-62980 as a lead compound for novel PPARγ agonists (Fig. 1). In the present study, we focused on the characterization of KR-62980, a novel non-thiazolidinedione PPARγ agonist by using various biochemical and pharmacological assays, and reported that KR-62980 acts as a selective PPARγ modulator with different activity profiles from rosiglitazone. The adipogenic potency is weak compared with rosiglitazone, possibly resulting from the differential binding mode and co-activator recruitments. Moreover, in vivo study shows that the compound exhibits an anti-hyperglycemic activity in high fat diet-induced C57BL/6 mice, suggesting its possible utility for the development of novel anti-diabetic agents.

2. Materials and methods

2.1. Materials

Rosiglitazone and KR-62980 were synthesized in Korea Research Institute of Chemical Technology. 2-Deoxy-[^{3}H]-glucose, [^{3}H]-glucose and liquid scintillation cocktail were obtained from Perkin-Elmer Life Sciences (Boston, MA, USA). Cell culture reagents were obtained from Life Technologies, Inc. (Gaithersburg, MD, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA). The 3T3-L1 cells and C3H10T1/2 cells were obtained from American Type Culture Collection (Rockville, MD, USA). The RNeasy mini kit was obtained from Qiagen (Valencia, CA, USA). Reverse transcription system was obtained from Promega Corp. (Madison, WI, USA). ExTaq polymerase kit was purchased from Takara Korea (Seoul, Korea).

2.2. Transactivation assay

The ligand binding domains (LBDs) of hPPARα (amino acids 167–468), hPPARδ (amino acids 167–441) and hPPARγ (amino acids 163–477) were generated by PCR amplification using Pfu polymerase (Stratagene, La Jolla, CA, USA) and gene specific primers flanked with restriction enzymes *Bam*HI and *Xba*I. The LBDs were subcloned in-frame into the pFA-CMV vector (Stratagene) to prepare pFA-Gal4-PPARα-LBD, -PPARδ-LBD and -PPARγ-LBD. At 75–90% confluence, NIH3T3 cells were transiently co-transfected with one of the expression vectors for pFA-Gal4-PPAR-LBDs together with pFR-Luc and pRL-CMV (Promega) using Lipofectamine plus reagent according to the instructions of manufacturer (Invitrogen, Carlsbad, CA, USA). Following 24 h incubation, the cells were treated with various concentrations of KR-62980 and incubated for 16 h. Luciferase assay was performed using dual-luciferase reporter assay system according to the instructions of the manufacturer (Promega), and the activity was determined in Microlumat Plus Luminometer (EG&G Berthold, Bad Wildbad, Germany) by measuring light emission for 10 s. The results were normalized to the activity of renilla expressed by co-transfected *Rluc* gene under the control of a constitutive promoter. To examine the effect of KR-62980 on the transactivation activity by rosiglitazone, various concentrations of KR-62980 were co-incubated with 5 μM of rosiglitazone for 24 h, and the activity was determined.

2.3. Glucose uptake assay

2-Deoxyglucose uptake was carried out as previously described with some modifications [16]. 3T3-L1 preadipocytes were differentiated with dexamethasone, insulin and isobutylmethylxanthine. Fully differentiated cells were incubated for 48 h with various concentrations of the compounds. Before measurement, the cells were replaced by serum free medium for 3 h and rinsed with KRB buffer (118 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM Na_2HPO_4, 2% BSA, 0.5 mM glucose, 25 mM $NaHCO_3$, pH 7.4). The cells were incubated with [^{3}H]-deoxyglucose (specific activity 6 Ci/mmol, 1 μCi/well) in the presence or absence of various concentrations of either KR-62980 or rosiglitazone. After 30 min incubation and washing with cold PBS, radioactivity of the cell lysates was determined by liquid scintillation counting.

2.4. Adipogenesis assay

The adipogenic potency of KR-62980 was determined as described previously [17]. Briefly, C3H10T1/2 pluripotent stem cells were grown in DMEM supplemented with 10% fetal calf serum. Confluent cells were incubated with various concentrations of KR-62980 or rosiglitazone in the presence of insulin (200 nM) with medium change every 2–3 days. After 7–9 days of differentiation, the cells were fixed and stained with Oil Red O for 1 h. Oil Red O was prepared by diluting a stock solution (0.5 g/10 mL isopropanol) with water (6:4).