The Role of Partial Peroxisome Proliferator-Activated Receptor ϒ Agonist (Ppar-4) in High-Fat Diet-Induced Obesity and Insulin Resistance.

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Abstract— The thiazolidinediones (TZDs) are a class of medications used for treatment and possibly the prevention of type 2 diabetes. We characterized the pharmacological profiles of PPAR-4 chemically known as (5Z)-5-[4-hydroxy-3-methoxyphenyl) methylene] thiazolidine-2, 4-dione), as a selective partial activator of PPARγ. PPAR-4 showed good in vivo pharmacokinetic profiles in C57BL/6J mice at 30 mg/kg oral dose with Cmax-26 μM, terminal elimination half-life— 2.5 h and bioavailability of 85%. Furthermore, PPAR-4 significantly improved hyperglycemia and insulin resistance in DIO animals when orally administered at a dose of 30 mg/kg/day for 45 days without significant weight gain. Overall, these studies suggest that PPAR-4 improves insulin resistance in such animal models through activation of PPARγ-mediated transcriptional activity and that it would be a new therapeutic candidate with potential for the treatment of type 2 diabetic patients.

Key words: Hypoglycemic, Metabolic syndrome, Insulin resistant, lowering triglycerides.

1 Introduction

Peroxisome proliferator activated receptors (PPARs) are ligand-activated nuclear transcription factors that belong to the nuclear receptor superfamily. Three isoforms of PPAR have been identified, α, δ and γ, which play distinct roles in the regulation of key metabolic processes, such as glucose and lipid redistribution. PPARα is expressed predominantly in the liver, kidney and heart, and is primarily involved in fatty acid oxidation. PPARγ is mainly associated with adipose tissue, where it controls adipocyte differentiation and insulin sensitivity. PPARδ is abundantly and ubiquitously expressed, but as yet its function has not been clearly defined. Activators of PPARγ (PPAR-4; recent studied drug and pioglitazone) have been used clinically for a number of years in the treatment of hyperlipidemia and to improve insulin sensitivity in diabetes.

A recent study by has suggested that macrophages pecific PPARγ activation also reduces insulin resistance in adipose tissue via differentiation of alternatively activated monocytes with an anti-inflammatory phenotype.[1]

Consistent with the PPARs being major regulators of FA metabolism, they are expressed at high levels in tissues that are most active in lipid metabolism. However, the three PPAR subtypes display highly distinct expression profiles and biochemical properties resulting in subtype selective activation of target genes. PPARγ is a major inducer of FA oxidation (FAO) and highly expressed in tissues with substantial mitochondrial and peroxisomal β-oxidation, such as brown adipose tissue (BAT), liver, kidney, and heart.[2] PPARγ exist in two isoforms, PPARγ1 and PPARγ2, which are encoded by the same gene by selective promoter usage and alternate splicing. They differ only in their extreme N-terminal end, where the NTD of PPARγ2 is 28 aa (30 aa in mice) longer than the NTD of PPARγ1 and has a slightly greater transactivation potential, at least in adipocytes.[3] The expression of PPARγ is almost exclusively adipocyte-specific, whereas PPARγ1 is more widely expressed.[2] PPARγ is a major activator of adipocyte differentiation and plays a general role in lipogenesis in many different cell types but associated with risk factor like obesity, dyslipidemia, hypertension, impaired fibrinolysis[4], and atherosclerosis. Looking at these side effect associated with Full ppar-y agonist we have initiated the search for some novel potential partial PPARγ agonist with lesser side effect. In an effort to search for novel PPARγ agonists, we screened a library of various 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 PPAR-4 as a lead compound for novel partial PPARγ agonists.

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2. MATERIALS AND METHODS

2.1 Compounds

PPAR-4 and pioglitazone were synthesized at Poona college ofpharmacy, Pune, India. The compounds were dissolved in dimethyl sulfoxide (DMSO) and and were suspended in 0.5% Tween-80+0.5% carboxy methyl cellulose solution for in vivo studies.

2.2 In Vivo pharmacokinetic profile of PPAR-4

C57BL/6J mice (adult males, 20–25 g) fasted for 6 h were ad-

ministered PPAR-4 at 30 mg/kg for oral and 3 mg/kg for intravenous pharmacokinetic profile. Blood samples were collected at different time interval by retroorbital puncture and separated plasma was stored at −80 °C until used. Compound concentrations in plasma were determined by HPLC analysis and the pharmacokinetic parameters were calculated by a non-compartmental method with Win Nolin professional Version 4.1.

2.3 In Vivo efficacy of PPAR-4 in DIO mice

DIO mice were orally gavaged with the PPAR-4 (3, 10, 30 mg/kg, p.o.) and piogltazone— 30 mg/kg, p.o. for 45 days. Animals were randomized based on pretreatment fed blood glucose levels and body weights. An oral glucose tolerance test (OGTT) was performed at day 1 and day 42. Insulin tolerance test (ITT) was performed at day 28. Body weight, feed intake and ad lib fed blood glucose were taken every week. Animals were sacrificed at day 45 for plasma and tissue collection. Biochemical parameter was analyzed from day 45 plasma samples.[5]

2.4 Oral glucose tolerance test

An oral dose of vehicle or compounds were given in 6 h fasted DIO mice after fasting blood glucose (t=–30 min) measurement. The mice were then gavaged with an oral bolus of glucose (2 g/kg) after baseline blood glucose measurement. Subsequent blood glucose measured at 15, 30, 60 and 120 min. The test is performed on 6 h fasted DIO mice. The mice are injected with insulin (0.75 U/kg) in ~0.1 ml 0.9% NaCl intraperitoneally. A drop of blood (5 µl) is taken from the cut tail vein before the injection of insulin and after 15, 30, 60 and 120 min for the determination of blood glucose with a glucometer.

2.5 Biochemical assay

Total plasma cholesterol, triglyceride and non-esterified fatty acid (NEFA) levels were measured from frozen plasma using the Hitachi 717 clinical chemistry analyzer. Blood glucose levels were measured immediately following blood collection on a glucometer (Accuchek performa). Reagents for cholesterol and triglyceride measurements were purchased from Merck, USA). Reagents for NEFA measurement were obtained from Randox. Insulin levels were measured by using Mercodia ELISA assay kit.

3. RESULTS

3.1 In vivo pharmacokinetic profiles of PPAR-4 in C57BL/6J mice

In vivo pharmacokinetic profiles of PPAR-4, when C57BL/6J mice received an oral dose of 30 mg/kg of PPAR-4, the Cmax was 26 µM with a terminal elimination half-life of 2.5 h. Absolute bioavailability was 85%, showing a good pharmacokinetic profile (Figure 1). A summary of pharmacokinetic parameters is shown in Table 1.

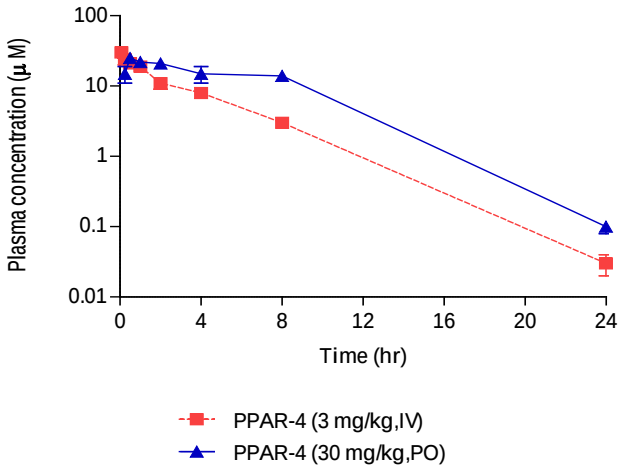

Figure 1. Plasma concentration–time profiles of PPAR-4 after i.v. (3 mg/kg) and p.o. (30 mg/kg) administration to male C57BL/6J mice (mean ± S.D., N = 6 animals/route of administration).

Table 1: Pharmacokinetic parameters of PPAR-4

Parameters	I.V. (3 mg/kg)	P.O. (30 mg/kg)
Cmax (µM)	30	25
Tmax (h)	0.08	0.5
AUC (0-24h) (µM.h)	98	245
Vss (L/Kg)	0.4	NA
CL (ml/min/Kg)	1.0	NA
$T_{1/2}$ (h)	2.4	2.1
F%	NA	65

Pharmacokinetic parameters of PPAR-4 after i.v. (3 mg/kg) and p.o. (30 mg/kg) administration to male C57BL/6J mice (mean ± S.D., N = 6 animals/route of administration).

3.2. In vivo efficacy of PPAR-4 in DIO mice

PPAR-4 treatment results in dose dependent lowering of both fed Blood glucose (Figure 2) and fasted plasma glucose (Table 2). PPAR-4 (30 mg/kg, p.o.) showed significant decrease in fed blood glucose starting from day 21 to day 42. PPAR-4 (30 mg/kg, p.o.) showed significant decrease in fasted plasma glucose and insulin while no significant change in triglyceride, Total cholesterol and NEFA levels on day 45 (Table 2). Amelioration of hyperglycemia in the presence of reduced plasma insulin levels suggests that insulin sensitivity has been improved in PPAR-4-treated DIO mice. Pioglitazone at 30 mg/kg,p.o. showed significant increase in body weight (On day 42) and feed intake (On day 21,28,35 and 42), while PPAR-4-treated mice did not showed significant change in body weight and feed intake in comparison with the vehicle-treated mice (Figure 3 and 4). Pioglitazone at 30 mg/kg, p.o. showed

significant increase in subcutaneous fat while PPAR-4-treated mice did not showed significant change in all fat pad in comparison with the vehicle-treated mice which correlates with body weight changes.

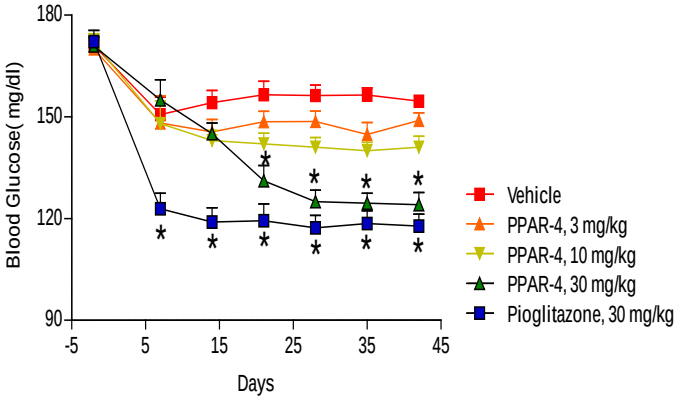

Figure 2. Effect of PPAR-4 on fed blood glucose in DIO mice. Data are presented as means ± SEM; * P < 0.05 versus control.

Table 2: Effect of Biochemical Parameter and Tissue weight in DIO mice

Parameter		Effect of PPAR-4			Pioglitazone
	Vehicle	3mg/kg	10mg/kg	30mg/kg	(30mg/kg)
Fasting Plasma Glucose (mg/dl)	160.4±5.2	158.2±5.5	125.8±3.1*	104.5±2.1*	102.13±4.1
Fasting Plasma Insulin (ng/ml)	6.7±0.12	5.7±0.4	4.5±0.32	3.13±0.11	2.96±0.3
TG (mg/dl)	158±4.2	155.2±7.1	135.8±4.2	145.1±4.3	148±2.33
TC (mg/dl)	178±5.2	176±4.2	167±7.7	172±4.2	171±3.7
NEFA (mmol/l)	1.3±0.2	1.24±0.15	1.31±0.22	1.32±0.14	1.29±0.3
Epididymal Fat (g)	0.102±0.02	0.101±0.02	0.114±0.03	0.116±0.03	0.117±0.02
Subcutaneous Fat (g)	1.51±0.23	1.56±0.18	1.41±0.19	1.58±0.21	2.42±0.2*
Peri renal Fat (g)	0.298±0.03	0.311±0.04	0.319±0.03	0.283±0.01	0.302±0.04

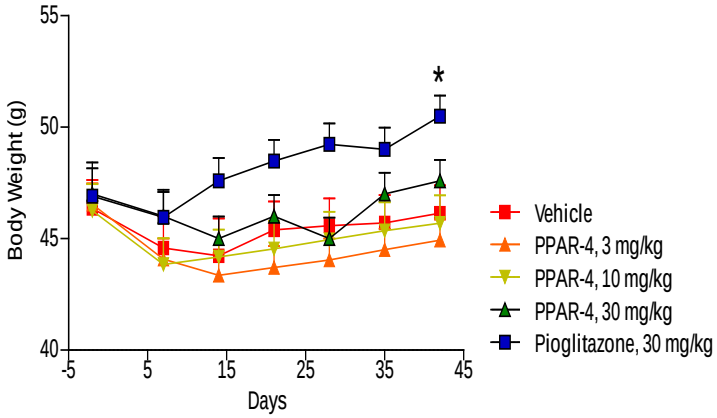

Figure 3. Effect of PPAR-4 on body weight in DIO mice.

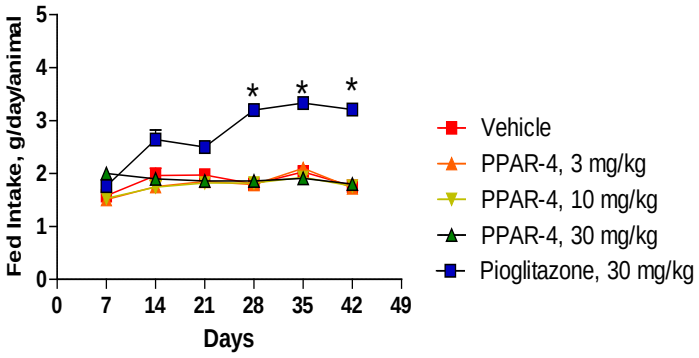

Figure 4. Effect of PPAR-4 on feed intake in DIO mice. Data are presented as means ± SEM; * P < 0.05 versus control.

3.3 Effect of PPAR-4 on glucose tolerance test in DIO mice

Oral glucose tolerance test was performed on day 42 of treatment period in DIO mice. On day 42 after chronic treatment, when challenged with an oral bolus of glucose, PPAR-4 (30 mg/kg, p.o.) treated animals exhibit a reduced glucose excursion (indicating increased tolerance to glucose) compared with vehicle-treated animals (Figure 5 and 6).

This indicates that insulin sensitivity has been improved in PPAR-4 treated mice. Pioglitazone (30 mg/kg, p.o.) treatment also showed improvement in insulin sensitivity comparable with PPAR-4.

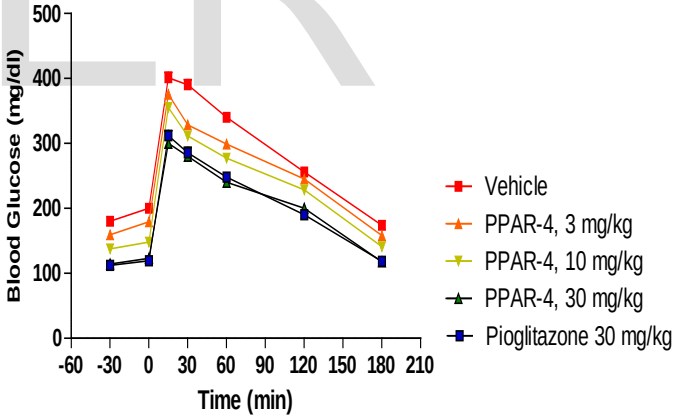

Figure 5. Effect of PPAR-4 in oral glucose tolerance test in DIO mice. Blood glucose on day 42. Data are presented as means ± SEM; * P < 0.05 versus control.

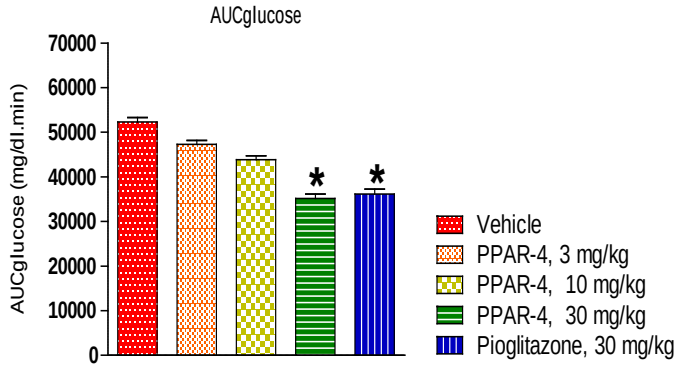

Figure 6. Effect of PPAR-4 in oral glucose tolerance test in DIO mice. AUC glucose on day 42. Data are presented as means ± SEM; * P < 0.05 versus control.

4. DISCUSSION

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors, and when activated by their ligands, they induce perixosome proliferation. Three receptors have been identified: PPAR γ, PPAR δ, and PPAR α, all with different tissue expression. PPAR γ is predominantly expressed in adipose tissue and regulates the formation of fat cells and their function. The effect of PPAR γ activation is to enhance the action of insulin in insulin-sensitive tissue by increasing peripheral glucose disposal and decreasing hepatic glucose production. The thiazolidinediones (TZDs) are a class of medications used for treatment and possibly the prevention of type 2 diabetes, which are potent agonists for the PPAR γ receptor. Because the thiazolidinediones target insulin resistance, these agents may improve many of the risk factors associated with obesity and insulin resistance including dyslipidemia, hypertension, impaired fibrinolysis, and atherosclerosis.[6]

Despite the proven benefits of targeting PPARs, adverse effects, have been reportedwith PPAR agonists both in preclinical and clinical studies.[7] PPARγ agonists such as rosiglitazone and pioglitazone have an unattractive side effect profile that includes weight gain, edema, neutropenia and hemodilution.[8] Recent studies have indicated that partial PPARγ agonist exhibit improved safety margins compared to full PPARγ agonists and consequently much effort has been put in promoting these partial PPARγ agonists for clinical development.[9]

The PPARγ partial agonist activity of PPAR-4 may become a distinct advantage for this compound because a number of studies have shown that PPARγ partial agonists including selective PPAR modulators have improved side effect profiles compared with full agonists.[10,11,12] PPARγ activation is well known to increase body weight and feed intake. Two major mechanisms of PPARγ-mediated body weight gain are increase of adipocyte differentiation and fluid retention.[13]

These reports are consistent with our findings in DIO mice, which demonstrate that PPAR-4 has similar antidiabetic efficacy with the less weight gain and suggest that at equivalent glucose-lowering doses, PPAR-4 administration would lead to less weight gain compared with pioglitazone.

In the present study, the antihyperglycemic activity of PPAR-4 was tested in an established animal model of type 2 diabetes, i.e., using DIO mice. The in vivo data demonstrate that PPAR-4 is a potent and efficacious antidiabetic agent in DIO mice. After chronic treatment with PPAR-4 lowers both fasted and fed glucose levels comparable with lean normal mice. In addition, PPAR-4 treatment reduces fasted insulin levels. Also, glucose clearance was significantly increased in mice treated with PPAR-4 and the finding was confirmed by AUC analysis.

In conclusion, the present study demonstrates that PPARY-4 showed good pharmackinetic profile and Chronic treatment with a novel partial PPARγ agonist blunted the development of diabetes in DIO mainly by improving the glucose tolerance and insulin sensitivity without demonstrating the adverse effects on body weight gain typically seen with PPARγ agonists.

5. ACKNOWLEDGMENT

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