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Improvement of dyslipidemia, insulin sensitivity, and energy balance by a peroxisome proliferator–activated receptor α agonist

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Abstract

Peroxisome proliferator–activated receptor α (PPARα) is a member of the nuclear receptor family of ligand-activated transcription factors. It plays an important role in the regulation of genes involved in lipid metabolism and transport. Compound A is a potent and orally active PPARα agonist that activated both human and rat PPARα receptors. The compound induced the expression of genes involved in fatty acid metabolism in a rodent hepatoma cell line and in the liver of *db/db* mouse. The ability of compound A to stimulate fatty acid β-oxidation was demonstrated in human hepatocytes and human skeletal muscle cells, which confirmed a functional activation of PPARα-mediated activities. Compound A was shown to be a more potent and efficacious antidyslipidemic agent in atherogenic rat and *db/db* mouse models as compared with fenofibrate. The increase in high-density lipoprotein cholesterol levels by compound A was at least partially due to an increase in serum apolipoprotein A-I protein concentrations in human PPARα transgenic mouse. The triglyceride-lowering effect was further confirmed in a higher species, obese dog models. In addition, compound A dose-dependently ameliorated hyperglycemia and hyperinsulinemia, and improved glucose tolerance in *db/db* mice. In a diet-induced obesity mouse model, compound A decreased body weight mainly by increasing energy expenditure and reducing fat deposition. In conclusion, the novel and potent PPARα agonist improves lipid profile, insulin sensitivity, and energy balance in animal models.

1. Introduction

Atherosclerotic cardiovascular disease is the leading cause of death worldwide. It has been shown that dyslipidemia, insulin-resistance state, and inflammation contribute to the development and progression of atherosclerosis [1-4]. Current therapeutic drugs for dyslipidemia include statins, fibrates, niacin, bile acid–reducing resins, and the cholesterol absorption inhibitor ezetimibe [5-7]. The benefit of fibrates, which are low-potency peroxisome proliferator–activated receptor α (PPARα) agonists [8], on cardiovascular risk factors are well established [9-11]. Peroxisome proliferator–activated receptor α is a member of the family of nuclear transcription factors that act as lipid sensors and regulate lipid metabolism [9,12]. Several clinical studies also suggest beneficial effects of fibrates (bezafibrate, clofibrate, and gemfibrozil) on glycemic control in type 2 diabetes mellitus, although glucose-lowering effects of these drugs are mild and interpretation of the data may be misled by the lack of PPARα selectivity or potency of some of these fibrates [13,14]. On the other hand, in some preclinical studies, more potent and selective PPARα agonists have been shown to prevent the development of diabetes/insulin resistance in rodent models of diabetes by reducing adiposity, improving peripheral insulin action, and exerting beneficial effects on pancreatic β-cells [15-17]. Therefore, novel PPARα

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agonists with increased potency and selectivity should improve efficacy in lipid and glycemic control in type 2 diabetes mellitus. The purpose of the present study was to characterize a potent and selective PPARα agonist and compare efficacy of the novel PPARα agonist with fenofibrate using dyslipidemia and diabetes animal models.

Compound A is an internally discovered and orally active PPARα agonist with a chemical structure of aminoindane ([18] compound 35). Our results have demonstrated that, in dyslipidemic animal models, compound A was a more potent lipid-lowering agent than fenofibrate, with positive effects on triglycerides (TG), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), and free fatty acids (FFA). In addition, compound A improved insulin sensitivity and increased energy expenditure in diabetes/insulin resistance and obesity animal models.

2. Materials and methods

2.1. In vitro assays

2.1.1. Human PPARs-GAL4 cotransfection reporter assay and full-length PPARα cotransfection reporter assay

HEK293 cells were cultured and PPAR ligand binding domains–GAL4 DNA binding domain cotransfection assays were performed as previously described [19]. Full-length PPARα (FL-PPARα) cotransfection assays were conducted using HEK293 cells cotransfected with DNA for receptor FL-PPARα-PCDNA3 and TK-luciferase reporter (Promega BioSciences, San Luis Obispo, CA). Briefly, the cells were incubated with the lipid-DNA complex solution (DMRIE-C reagent; Life Technologies, Rockville, MD) for 16 hours at 37°C in 5% CO_2 incubator. On the following day, the DNA-containing medium was replaced with 5% charcoal-treated fetal bovine serum growth medium. After 6 hours, the cells were seeded in 96-well plate and incubated overnight. Cells were then challenged with the compound solution (or vehicle, 0.1% dimethyl sulfoxide [DMSO]) and incubated for 24 hours at 37°C in 5% CO_2 incubator. Luciferase-assay was then performed as previously described [19].

2.1.2. 3-Enoyl-hydroxyacyl–coenzyme A dehydrogenase gene induction assay

3-Enoyl-hydroxyacyl–coenzyme A dehydrogenase (HD) gene induction was measured using branch DNA technique [20] (Panomics, Redwood City, CA), and the specific HD probe sequences were generated using software supplied by the manufacturer. H4llE cells, a rat hepatoma cell line obtained from American Type Culture Collection (Manassas, VA), were cultured in 96-well plates with culture medium containing 10% fetal bovine serum and 10% calf serum and maintained at 37°C and 5% CO_2 throughout study. The branch DNA assay was performed after 24-hour treatment of H4llE cells with vehicle (DMSO, 0.1%), compound A, or fibrates (comparators) according to the manufacturer's protocol.

2.1.3. Mouse gene expression quantification

Liver tissues were collected from female *db/db* mice after 11 days of oral treatment of compound for measuring hepatic target gene expression. Total RNA isolation, reverse transcriptase polymerase chain reaction conditions, as well as the quantification method were conducted as described previously [19].

Polymerase chain reaction primers and fluorescent probes (TaqMan probes) were designed using the software Primer-Design (Applied Biosystems, Foster City, CA).

Sequences of primers and TaqMan probes:

Mouse HD:
Forward primer: 5-ATATGCTCTGTGAAGCTGGGC
Reverse primer: 5-GAAAGCCAGGGATCAGGTTTG
TaqMan probe: 6 FAM-TATGACAAGCCACTGGGTC-GCATCC
Mouse fatty acid transporter protein (FATP):
Forward primer: 5-ACTGCAGCATTGCCAACATG
Reverse primer: 5-CCTTGACCAGACGGATGGG
TaqMan probe: 6 FAM-TGCGGCTTCAACAGCCGT-ATCCTC
Mouse mitochondrial medium-chain acyl–coenzyme A dehydrogenase (MCAD):
Forward primer: 5-GGGACGCCAAGATCTATCAGAT
Reverse primer: 5-TTTTATACTTTTCAATGTGC-TCACGA
TaqMan probe: 6 FAM-TGAAGGTACTGCACAAA-TTCAGAGGCTGATC
Mouse UCP2:
Forward primer: 5-TCACTGTGCCCTTACCATGCT
Reverse primer: 5-GAAAGGAAGGCATGAACCCC
TaqMan probe: 6 FAM-AGGAGGGACCCCGCGC-CTTCTA

2.1.4. Fatty acid β-oxidation assay

HepG2 cells purchased from American Type Culture Collection were cultured in Minimum Essential Media (MEM) supplemented with nonessential amino acids (GIBCO 10370-021, Rockville, MD), 10% fetal bovine serum, 1% glutamine, and 100 U penicillin/streptomycin. Primary human skeletal muscle cells (HSKMCs) were cultured in SKGM BulletKit medium (Cambrex, Walkersville, MD). For inducing differentiation into myotubes, HSKMCs were then cultured in fusion medium (Dulbecco modified Eagle medium–F12 supplemented with 2% horse serum and GA-1000 from Invitrogen, Carlsbad, CA) for approximately 20 days. Cells were treated with compounds or vehicle (DMSO, 0.1%) for 48 hours, with a medium change after the first 24 hours. The rate of cellular β-oxidation of [9,10-^{3}H]palmitic acid was measured as ^3H_2O release, as described previously [21,22].

2.2. In vivo studies

2.2.1. Animals

Female *db/db* mice (Jackson Laboratories, Bar Harbor, ME), at about 7 weeks of age when study was initiated, were

fed certified National Institutes of Health rodent diets #5K52 (PMI Nutrition International, St Louis, MO). Diet-induced obesity (DIO) mice were generated from male C57Bl/6j mice (Jackson Laboratories) that were fed a high-fat diet (catalog number D12492; Research Diets, New Brunswick, NJ) for 4 to 5 months. Human apolipoprotein (apo) A-I transgenic mice (Jackson Laboratories), about 8 to 9 weeks of age, were fed a standard mouse chow (PMI Nutrition International). An atherogenic rat model was generated using male Sprague-Dawley rats (ACE Animals, Boyertown, PA) at 6 to 7 weeks of age when study was initiated. The rats were fed a high-cholesterol diet (catalog number C13002, Research Diets) for 6 days before receiving drug treatment, and the diet was continued during the 8 days of drug treatment. Food and water were supplied ad libitum to all rodents unless specified. Female obese beagle dogs (retired breeders) (Marshall Bioresources, North Rose, NY) were maintained on a regular canine diet, and food was supplied once daily. For all the animal housing, room temperature was maintained at 68°F to 72°F and humidity at 50% to 65%. Room lighting was on a 12-hour light/12-hour dark cycle. All of the procedures for animal studies were approved by the J&J Institutional Animal Care and Use Committee.

2.2.2. In vivo experiments

All animals were treated by oral gavage (the vehicle in all cases was 0.5% Methocel, formulated in-house, Johnson & Johnson), and serum parameters were determined under fed conditions unless stated otherwise. At approximately 18 hours (unless specified) after the final dose following multiple days of treatment, the rats and mice were bled by retroorbital sinus puncture under CO_2/O_2 (70%:30%) anesthesia. The dogs were bled via venipuncture at 6 hours after the last dose. For hepatic gene expression analysis, liver tissues were removed and snapped frozen in liquid nitrogen. Hepatic gene expression analysis was described above ("In vitro assays"). Serum levels of apo A-I were measured using human apo A-I kit provided by Sigma (St Louis, MO). Serum glucose, lipid, and insulin analysis and oral glucose tolerance test (OGTT) were performed as described previously [19], unless specified in the text.

Table 1
Effect of compound A in human FL-PPARα and PPARα, δ, and γ-GAL4 cotransfection reporter assays

Compound	EC_{50} (μmol/L)			
	hPPARα FL	hPPARα GAL4	hPPARγ GAL4[a]	hPPARδ GAL4[a]
Compound A	0.30 ± 0.059	0.88 ± 0.010	>10	2.11
Ciprofibrate	ND	20.1	>100	NA

Each value represents the mean ± standard error of 3 values. hPPARα FL indicates full-length PPARα receptor; hPPAR GAL4, human PPAR receptor ligand binding GAL4 chimeric constructs; ND, not determined; NA, no activity at 300 μmol/L.

[a] n = 1.

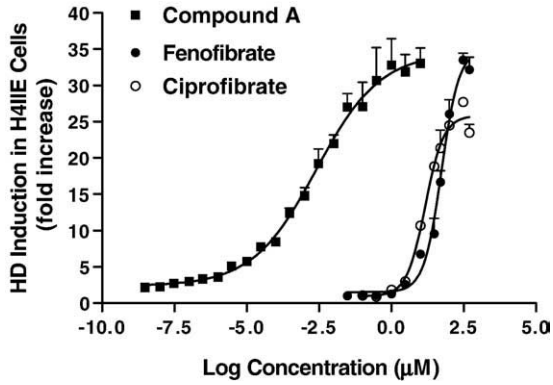

Fig. 1. Dose response of compound A in HD gene induction assay using rat H4IIE hepatocytes. Data represent fold increase over vehicle, mean ± SEM. Dose-response curves were generated from triplicate measurements at each concentration. ■ indicates compound A; ●, fenofibrate; ○, ciprofibrate.

2.2.3. Statistical analysis

Statistical analysis was performed using the Prism program (Graphpad, Monrovia, CA) and 1-way analysis of variance with a Dunnett multiple comparison test.

3. Results

3.1. In vitro pharmacologic effects

3.1.1. Activation of PPARα and receptor selectivity

The ability of compound A to activate human PPARα, γ, and δ receptors was assessed using either full-length PPARα receptor, or PPARα, γ, and δ receptor ligand-binding–GAL4 chimeric constructs cotransfected with the appropriate promoter-luciferase reporter constructs. Compound A activated PPARα receptor activity in both models, with EC_{50} of 0.3 μmol/L at full-length PPARα receptor and 0.88 μmol/L in PPARα-GAL4 cotransfection reporter assay (Table 1). Compound A was a much more potent PPARα agonist than ciprofibrate, with an EC_{50} of 20 μmol/L. Compound A had no PPARγ activity when tested up to 10 μmol/L, and only weak PPARδ activity.

3.1.2. Induction of the expression of a key enzyme (HD) involved in fatty acid β-oxidation in the rat hepatoma cell line H4IIE

The major target tissue of PPARα is the liver; and key genes include the enzymes involved in the β-oxidation of fatty acids (acyl–coenzyme A oxidase, carnitine palmitoyl transferase 1 [CPT-1], HD, etc), the liver FATP, and apo A-I and A-II [23-25]. To quickly identify PPARα agonists in a cell-based assay, we used target gene as a surrogate marker. The gene induction response of HD to PPAR agonists was the most robust in H4IIE cells. Therefore, HD was selected as a target in the assay. Branched DNA technology, a technique that permits rapid measurement of RNA levels in cells without RNA purification, was used to determine the activity of compounds on HD induction. As shown in Fig. 1,

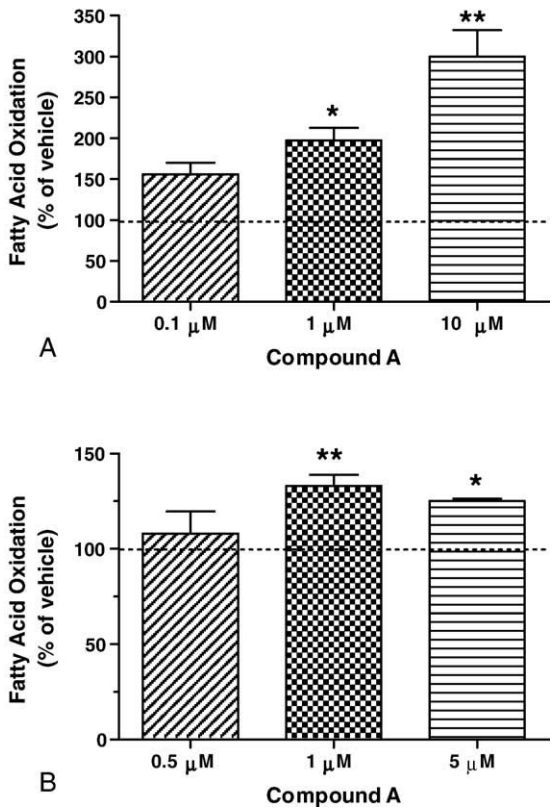

Fig. 2. Effect of compound A on fatty acid β-oxidation in HSKMCs and HepG2 cells. Data are expressed as percentage vehicle (the vehicle response is indicated by the dash line), mean ± SEM. *$P < .05$ and **$P < .01$, compound A vs vehicle.

the expression of HD messenger RNA was dose-dependently induced by compound A, with an EC_{50} of 2 nmol/L. The activity of compound A was compared with that of ciprofibrate and fenofibrate. Compound A was much more potent than ciprofibrate and fenofibrate, as shown with the left-shift dose-response curve. The efficacy of compound A and the fibrates was similar in HD induction.

Compound A also increased messenger RNA levels of CPT-1 and ABC1 in primary HSKMCs and increased CPT-1 gene expression in a human hepatoma cell line (data not shown).

3.1.3. Stimulation of FFA β-oxidation

The induction of genes involved in fatty acid oxidation by compound A should result in increased functional activity of this pathway in treated cells. Primary HSKMCs and HepG2 cell line were used to examine this effect according to the available methodology from publication [22]. Compound A dose-dependently increased palmitic acid β-oxidation as indicated by increasing the amount of tritiated water in the medium. The effect was more pronounced in the primary skeletal muscle cells, with up to 2-fold higher β-oxidation at 10 μmol/L than that in vehicle controls (Fig. 2A). Compound A also produced a slight but significant increase in fatty acid oxidation in HepG2 cells (Fig. 2B).

3.2. In vivo pharmacologic effects

The in vivo efficacy of compound A was assessed in rodent models of dyslipidemia and insulin resistance/type 2 diabetes mellitus and in a higher species, obese beagle dog with mild hypertriglyceridemia.

3.2.1. Models of dyslipidemia

3.2.1.1. Atherogenic rat model

3.2.1.1.1. Compound A improved dyslipidemia in atherogenic rats. When fed a high-cholesterol diet for 14 days, male Sprague-Dawley rats (at 8 weeks old) developed hypercholesterolemia with reductions in serum HDLc, increases in LDLc and total cholesterol, and slight increases in TG and FFA. Oral administration of compound A at 0.01 to 3 mg/(kg d) for 8 days (starting on day 6 of the

Table 2
Effect of (A) compound A and (B) fenofibrate on lipid profile of male rats maintained on a high-cholesterol diet

Diet	Dose (mg/kg)	TG (mg/dL)	Total chol (mg/dL)	HDLc (mg/dL)	HDLc/Total chol	LDLc (mg/dL)	FFA (mEq/L)
A. Compound A (8 d of oral dosing)							
Regular	Vehicle	174 ± 31*	73 ± 5**	34 ± 2	0.47	10 ± 1**	0.69 ± 0.04**
High chol	Vehicle	286 ± 38	574 ± 66	12 ± 5	0.02	120 ± 16	2.00 ± 0.15
High chol	0.01	264 ± 33	423 ± 24*	24 ± 3	0.06	93 ± 7	2.26 ± 0.12
High chol	0.03	182 ± 23*	296 ± 19**	36 ± 2	0.12	74 ± 5**	1.6 ± 0.08
High chol	0.1	103 ± 11**	318 ± 28**	23 ± 3	0.07	76 ± 6**	1.28 ± 0.11**
High chol	0.3	121 ± 13**	393 ± 26**	34 ± 7	0.09	71 ± 8**	1.16 ± 0.15**
High chol	1	70 ± 9**	417 ± 30**	123 ± 1**	0.29	40 ± 5**	0.82 ± 0.06**
High chol	3	67 ± 7**	561 ± 34	199 ± 6**	0.35	44 ± 2**	1.14 ± 0.12**
B. Fenofibrate (8 d of oral dosing)							
High chol	Vehicle	227 ± 43	643 ± 63	18.9 ± 1.9	0.03	158.8 ± 15.3	ND
High chol	30	150 ± 19	324 ± 21**	68.3 ± 10.8**	0.21	46.7 ± 3.4**	ND
High chol	100	136 ± 11*	319 ± 14**	129.8 ± 4.3**	0.41	29.0 ± 2.62**	ND
High chol	300	188 ± 16	394 ± 25**	149.7 ± 8**	0.38	34.2 ± 3.6**	ND

Data are expressed as mean ± standard error of mean (n = 8). High chol indicates high cholesterol; total chol, total cholesterol; ND, not determined.
*$P < .05$ and **$P < .01$, significantly different from vehicle group on high-cholesterol diet.

diet) reduced serum concentrations of total cholesterol, LDLc, TG, and FFA, with approximate EC_{50} of 0.03 mg/kg (Table 2A). At low doses, serum HDLc increased to normal diet levels. At higher doses, the HDLc concentrations increased substantially, which also resulted in higher total cholesterol at these doses. However, the HDLc to total cholesterol ratio also increased in a dose-dependent manner. In a parallel study, fenofibrate at 30, 100, and 300 mg/(kg d) was examined in this model. As shown in Table 2B, fenofibrate decreased serum lipid levels to a similar extent at much higher dose(s) in the atherogenic rats.

The antidyslipidemic activity of compound A was also tested in a high-fat– and high-fructose–fed hamster model, where compound A not only effectively normalized LDLc, TG, and HDLc levels, but also reduced total cholesterol and glucose levels (data not shown).

3.2.1.2. Obese and hyperlipidemic dog model

3.2.1.2.1. Compound A decreased serum lipids in obese dogs. The in vivo antihyperlipidemic activity of compound A was further confirmed in obese and mild hypertriglyceridemic female beagle dogs. Obese dogs were administered compound A (0.03-3 mg/[kg d]) by oral gavage for 17 consecutive days. There was a dose-dependent reduction in serum TG (Table 3). Total cholesterol and FFA were also dose-dependently reduced. Although HDLc levels were slightly decreased (primarily because of the decreases in total cholesterol levels), the ratios of HDLc to total cholesterol tended to increase. Low-density lipoprotein cholesterol levels were not detectable in these animals. Serum glucose levels and body weight did not change (data not shown).

3.2.1.3. Apo A-I transgenic mouse model. Apolipoprotein A-I is a key lipoprotein of HDLc. High-density lipoprotein cholesterol plays a very important role in reverse cholesterol transport [26]. Peroxisome proliferator–activated receptor α agonists have been shown to increase apo A-I and HDLc levels. Using apo A-I transgenic mouse model as a tool, the PPARα-mediated regulation on apo A-I and its correlation to HDLc were studied. In apo A-I transgenic mice, compound A dose-dependently increased serum levels of human apo A-I and HDLc, and reduced serum TG after 7 days of oral dosing (Table 4A). The effect of compound A was also compared with that of fenofibrate, where fenofibrate significantly increased serum levels of human apo A-I and HDLc (Table 4B). Compound A at 0.3 mg/kg elevated HDLc levels more than 2-folds over vehicle, whereas fenofibrate doubled HDLc levels at doses greater than 30 mg/kg.

Table 4
Effect of (A) compound A and (B) fenofibrate on serum apo A-I, HDLc, and TG concentrations in human apo A-I transgenic male mice

Dose (mg/kg)	Apo A-I (mg/dL)	HDLc (mg/dL)	TG (mg/dL)
A. Compound A (7 d of oral dosing)			
Vehicle	991 ± 106	168 ± 6	109 ± 7
0.3	1657 ± 19	441 ± 35**	70 ± 4**
1	1582 ± 111	470 ± 29**	56 ± 2**
3	2061 ± 432*	487 ± 38**	71 ± 6**
10	1962 ± 516*	NA	77 ± 9*
B. Fenofibrate (7 d of oral dosing)			
Vehicle	732 ± 28	127.4 ± 30.8	139.6 ± 11.6
10	893 ± 47	191.5 ± 7*	107.4 ± 9.8*
30	1135 ± 58*	238.5 ± 8.7**	85.9 ± 9.3**
100	1562 ± 86**	246.6 ± 5.5**	61.1 ± 4.4**
300	1928 ± 232**	253.6 ± 6.5**	46.6 ± 3.2**

Data are expressed as mean ± standard error (n = 8). NA indicates not available.
*$P < .05$ and **$P < .01$, significantly different from vehicle.

3.2.2. Diabetic and obese db/db mouse model

The *db/db* mouse strain carries inactive mutation in the leptin receptor gene and develops obesity and severe insulin resistance [27]. The antidiabetic activity of compound A was evaluated in *db/db* mice.

3.2.2.1. Compound A ameliorated hyperglycemia and hyperinsulinemia in db/db mice. In *db/db* mice (6-7 weeks old), oral administration of compound A for 11 days normalized serum glucose and TG, and reduced insulin concentrations (Fig. 3). Compound A also significantly reduced weight gain at higher doses (1 mg/kg and above) compared with vehicle control. However, the effect of compound A on TG and glucose/insulin occurred at 0.03 and 0.3 mg/(kg d), respectively, without changing body weight, suggesting that the effects of compound A on lipid and glucose metabolism are independent of weight lost. Food intake was not measured in this study. Treatment of *db/db* mice with fenofibrate at 30, 100, and 300 mg/(kg d) for

Table 3
Effect of compound A (17 days of oral dosing) on serum concentrations of TG, total cholesterol, HDLc, and FFA in female beagle dogs

Dose (mg/kg)	TG (mg/dL)	Total chol (mg/dL)	HDLc (mg/dL)	HDLc/Total chol	FFA (mEq/L)
Vehicle	364.4 ± 65.4	397.2 ± 34.9	294 ± 9	0.74	0.89 ± 0.13
0.03	270.3 ± 51.3	388.1 ± 24.4	319 ± 19	0.82	0.92 ± 0.16
0.1	291.6 ± 32.4	355.3 ± 14.2	282 ± 5	0.79	1.09 ± 0.11
0.3	129.8 ± 27.4**	318.2 ± 28.9	272 ± 15	0.86	0.67 ± 0.14
1	150.7 ± 13.2**	300.1 ± 24.6*	267 ± 11	0.89	0.79 ± 0.08
3	104.7 ± 8.4**	274.6 ± 20.8**	254 ± 17	0.93	0.46 ± 0.04*

Data are expressed as mean ± standard error (n = 8).
*$P < .05$ and **$P < .01$, significantly different from vehicle.

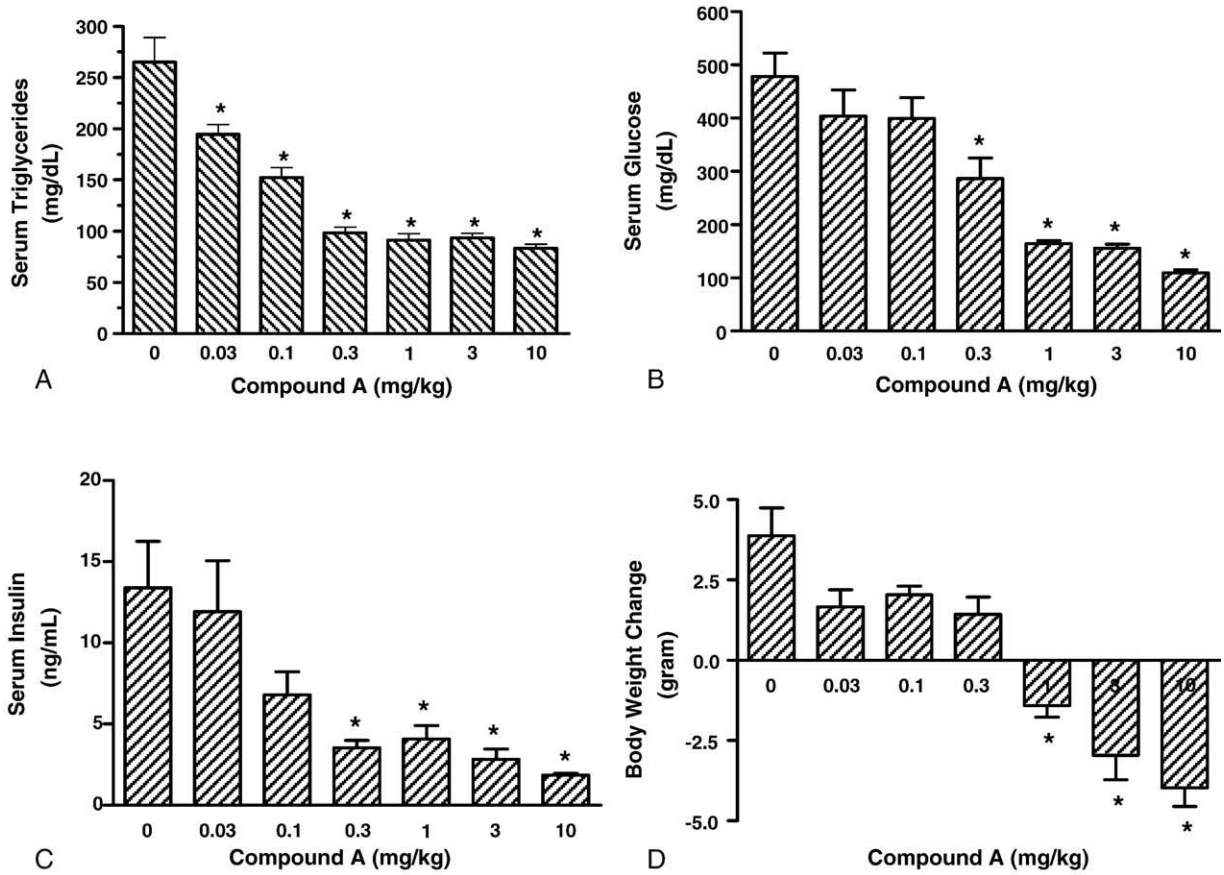

Fig. 3. Effect of compound A on serum TG, glucose, insulin, and body weight change in *db/db* mice after 11 days of oral administration. Data are expressed as mean ± SEM (n = 7-8). *$P < .001$, compound A vs vehicle.

11 days dose-dependently decreased serum TG, but had no effect on serum glucose levels (Fig. 4). The effect of fenofibrate on TG was also much less potent than that of compound A in *db/db* mice.

3.2.2.2. Compound A up-regulated hepatic target gene expression in db/db mice. To further elucidate the mechanism of action of compound A, the expression levels of selected PPARα target genes in lipid metabolism and fatty acid oxidation were quantified. As shown in Fig. 5A, there was a dose-dependent induction of genes for proteins/enzymes involved in lipid metabolism in the liver of *db/db* mice after 11 days of treatment with compound A. These genes included HD (7×, maximal increase), FATP (21×), MCAD (2×), and UCP2 (5×). For comparison, fenofibrate was also tested. Fenofibrate at 100 mg/(kg d) up-regulated hepatic gene expression of HD, FATP, MCAD, and UCP2 in *db/db* mice, but with less potency than compound A (Fig. 5B). Fenofibrate at 30 mg/kg also increased HD gene expression (10×) (data not shown).

3.2.2.3. Compound A improved glucose tolerance in db/db mice. To further characterize the antidiabetic activity of compound A in *db/db* mice, an OGTT was performed. As shown in Fig. 6, fasting plasma glucose and insulin levels decreased after 11 days of treatment of *db/db* mice with compound A at 0.3 and 3 mg/(kg d). Compared with vehicle controls, compound A suppressed the glucose challenge–induced elevations in both plasma glucose and insulin. There were corresponding decreases in the area under the curve (AUC) for both glucose and insulin. Insulin resistance indexes (calculated by multiplying fasting glucose levels by fasting insulin levels) were significantly reduced compared with those in the vehicle control group (Fig. 6, inset). These results indicate that compound A improved glucose tolerance and insulin sensitivity in diabetic *db/db* mice.

A long-term effect of compound A was evaluated in *db/db* mice after 56 days of treatment. Compound A at 0.3 mg/(kg d) was shown to reduce hemoglobin A_{1c} by 29% at the end of the study (data not shown), suggesting that compound A had a sustained glycemic control.

3.2.3. DIO mouse model of insulin resistance

3.2.3.1. Compound A decreased hyperinsulinemia and body weight in DIO mice. The DIO mouse is a model of obesity and insulin resistance without a defect in the leptin pathway. Diet-induced obesity mice were fed a high-fat diet for

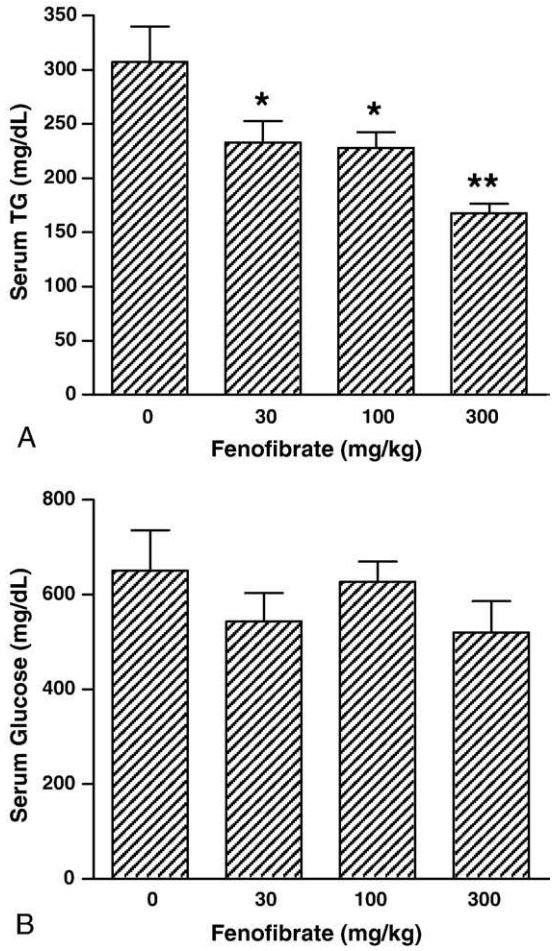

Fig. 4. Effect of fenofibrate on serum TG and glucose in *db/db* mice after 11 days of oral administration. Data are expressed as mean ± SEM (n = 7-8). *$P < .05$ and **$P < .01$, fenofibrate vs vehicle.

4 months and developed hyperinsulinemia and mild hyperglycemia reminiscent of an insulin-resistant state. Oral administration of compound A for 11 days induced dose-dependent reductions in serum insulin, TG, glucose, and FFA (Table 5). There was also additional weight loss over the treatment period compared with vehicle group, as with the *db/db* mice.

3.2.3.2. Compound A increased energy expenditure and decreased fat deposition in DIO mice. To further understand the whole-body metabolic effect of compound A, the effect of compound A on energy metabolism was evaluated in a longer-term (28 days) study in DIO mice. The control animals lost weight early during the study but later stabilized and did not gain weight; the average weight of these animals was around 50 g. As summarized in Table 6, compound A treatment (at 0.1 mg/[kg d]) resulted in substantial sustained weight loss (throughout the period measured, with significant effects observed starting from day 9; data not shown). At the end, the weights of epididymal fat pads and brown adipose tissues were reduced. Compound A decreased total food consumption; however, feeding efficiency (or calorie efficiency) calculated from the ratio of weight gain to calorie intake was decreased, suggesting increased energy expenditure. Furthermore, indirect calorimetry measurements indicated that compound A significantly increased oxygen consumption (VO_2), suggesting increases in metabolic rate and energy expenditure. In addition, there was no effect on spontaneous movements, suggesting that the increase in VO_2 was not due to an increase in locomotor activity. There was no change in the respiratory quotient, which may be due to the high-fat diet feeding.

4. Discussion

In the present study, compound A was demonstrated to activate both rat and human PPARα receptors. The compound induced expression of the hepatic genes involved in fatty acid metabolism and lipid transport in *db/db* mice. Compound A was active to increase the

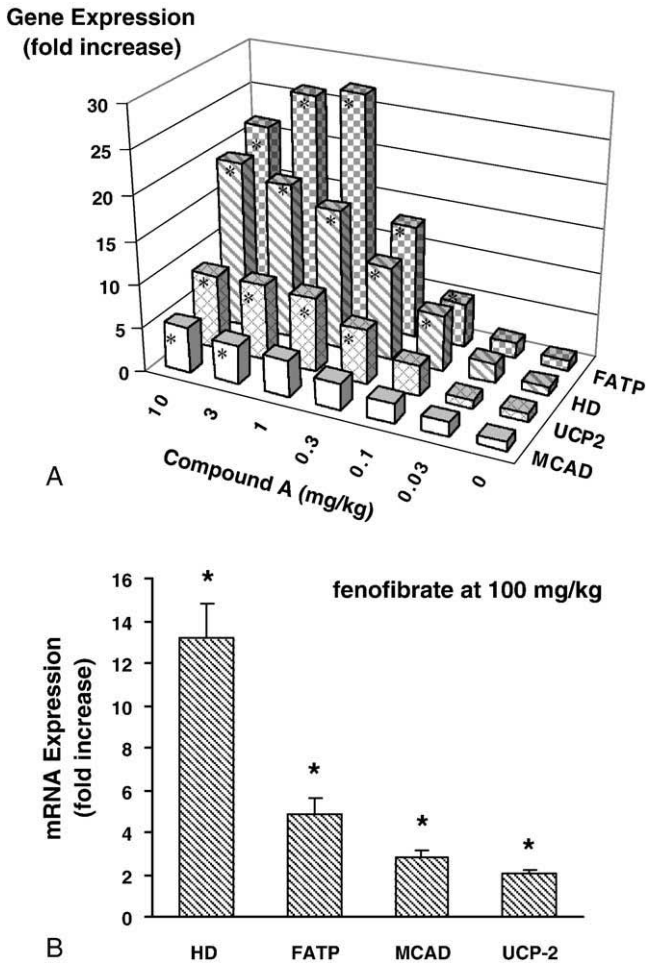

Fig. 5. Effect of compound A and fenofibrate on hepatic gene expression in *db/db* mice after 11 days of oral administration. Data are expressed as fold increase over vehicle (n = 4). A, Effect of compound A. *$P < .01$, compound A vs vehicle. B, Effect of fenofibrate at 100 mg/kg. *$P < .01$, fenofibrate vs vehicle.

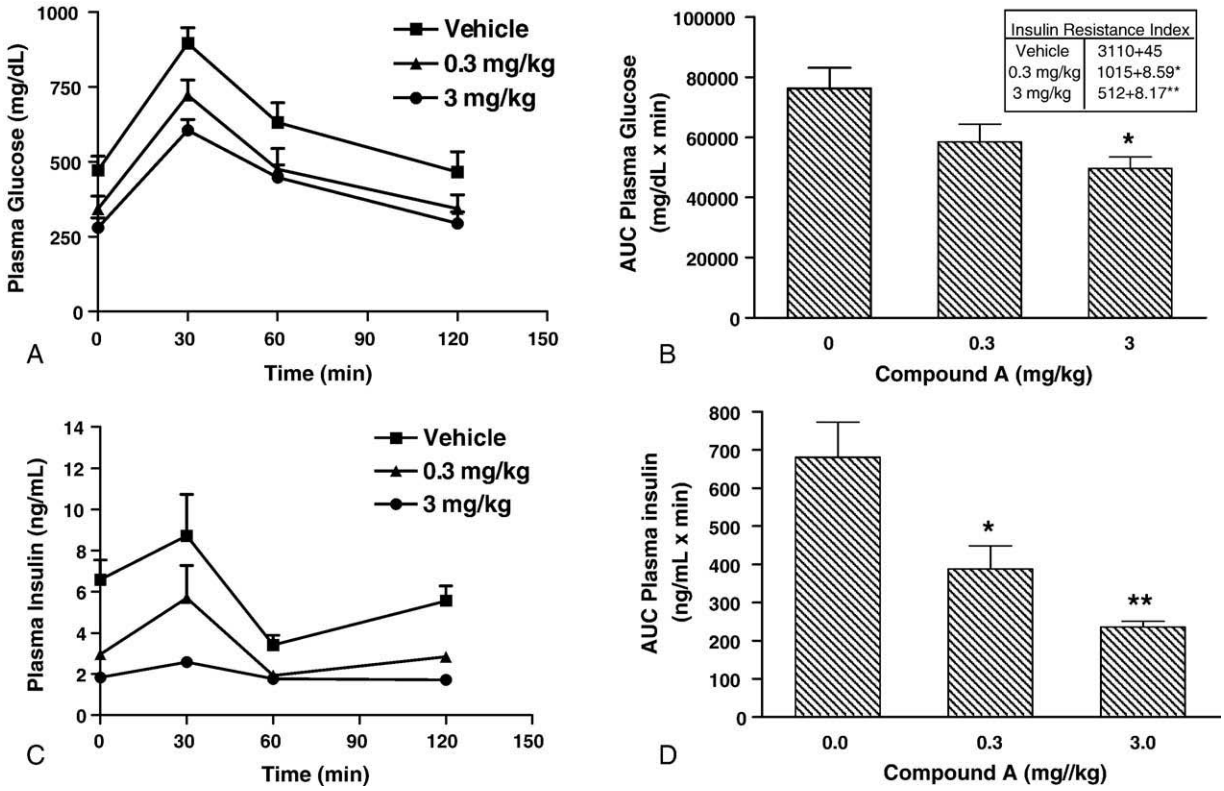

Fig. 6. Effect of compound A on plasma glucose (A), total AUC plasma glucose (B), plasma insulin (C), AUC plasma insulin (D), and insulin resistance index (inset in B) during OGTT in *db/db* mice. Data are expressed as mean ± SEM (n = 8). After oral administration of compound A for 11 days, OGTT was performed 1.5 hours after the last dose in the animals fasted for 6 hours. *$P < .01$, compound A vs vehicle; **$P < .001$, compound A vs vehicle and compound A at 3 mg/kg vs 0.3 mg/kg.

amount of fatty acid β-oxidation in primary HSKMCs and in HepG2 cell line, confirming the functional activation of PPARα.

The pharmacokinetic properties of compound A have been evaluated in healthy male Sprague-Dawley rats, showing a 59% oral bioavailability [18]. The antidyslipidemic effect of compound A was first demonstrated in an atherogenic rat model and compared with fenofibrate. The TG-lowering effect of compound A was much more potent (with an ED_{50} of ~0.03 mg/kg) than that of fenofibrate. Compound A normalized serum HDLc at a dose of 0.3 mg/kg; and at 1 mg/kg, it dramatically increased HDLc above control levels seen in rats on a regular diet. In addition, the TG-lowering effect of compound A was confirmed in a higher species, obese female beagle dog. After a long-term treatment with compound A, serum TG, FFA, and total cholesterol concentrations were significantly reduced in obese dog.

Currently, the first line of defense in reducing the risk of atherosclerosis is targeted at lowering LDLc and increasing

Table 5
Effect of compound A (11 days of oral dosing) on serum chemistry and body weight changes in male C57BL/6 DIO mice

Dose (mg/kg)	TG (mg/dL)	Glucose (mg/dL)	Insulin (ng/mL)	FFA (mEq/L)	BW change (g)
Vehicle	123 ± 12	226 ± 10	10.6 ± 1.3	1.1 ± 0.05	−2.6 ± 0.7
0.03	92± 16	209 ± 12	9.6 ± 1.3	1.08 ± 0.1	−2.6 ± 0.5
0.1	58 ± 5.6*	178 ± 4.0*	2.8 ± 0.7*	0.8 ± 0.08*	−5.7 ± 0.9*
0.3	51 ± 1.5*	110 ± 5.9*	0.8 ± 0.2*	0.78 ± 0.05*	−7.1 ± 0.5*
Normal mice	114 ± 4.98	155 ± 4.5	1.45 ± 0.12	0.05 ± 0.01	–

Data are expressed as mean ± standard error of mean (n = 7). BW indicates body weight.
* $P < .01$, significantly different from vehicle.

Table 6
Effect of compound A on energy metabolism after 28 days of treatment in DIO mice

Test	Vehicle	Compound A 0.1 mg/kg
Body weight (g)	50 ± 3.5	41 ± 2.0*
Total food intake (% of control)	100	80 ± 9*
Feed efficiency (mg wt change/kcal)	−1.17	−25.4*
Weight of epididymal fat pads (g)	1.3 ± 0.1	0.8 ± 0.03*
Weight of brown fat mass (g)	0.5 ± 0.05	0.3 ± 0.03*
Oxygen consumption VO_2 (mL/[kg h])	2668 ± 54	3265 ± 120*
Respiratory quotient VCO_2/VO_2	0.77 ± 0.01	0.77 ± 0.01
Spontaneous movement (counts in X, Y, and Z axes)	936 ±105	959 ± 47

Data are expressed as mean ± standard error of mean (n = 8).
* $P < .05$, significantly different from vehicle.

HDLc. High-density lipoprotein cholesterol level can be regulated by apo A-I, ABC-A1, lipoprotein lipase, SR-BI, etc [23]. Apolipoprotein A-I is the primary protein component of HDLc. The blood levels of apo A-I protein and HDLc have been shown to be inversely correlated with development of atherosclerosis [28,29]. Apolipoprotein A-I transcription in the liver is regulated by PPARα [24]. Human apo A-I transgenic mouse [30-32] provides another model for examining the effect of PPARα agonists on apo A-I. Treatment of human apo A-I transgenic mice with compound A and fenofibrate increased the serum concentrations of human apo A-I protein and HDLc and decreased TG, suggesting that compound A increased HDLc levels at least partially through elevating apo A-I, which was mediated by PPARα activation.

In subchronic studies, compound A was demonstrated to potently normalize serum concentrations of TG, glucose, and insulin in *db/db* mice, where fenofibrate (at 30-300 mg/kg) was not able to improve hyperglycemia after 11 days of treatment. In a longer-term (56 days) *db/db* mouse study, compound A decreased serum concentrations of hemoglobin A_{1c}, suggesting a sustained glycemic control by the compound. In addition, compound A decreased insulin resistance index and improved glucose tolerance. Improvement of insulin sensitivity by PPARα agonists likely involves their ability to enhance hepatic expression of genes implicated in the transport and oxidation of fatty acids [15,22]. In our study, compound A effectively up-regulated the expression of hepatic PPARα target genes in *db/db* mice. These genes play important roles in fatty acid metabolism, fatty acid transport, or energy expenditure [23-25], indicating the possible underlying mechanisms of action of the PPARα agonist in lipid metabolism and energy balance. Numerous studies have demonstrated the association between obesity/dyslipidemia and insulin resistance [33-37]. In patients with visceral obesity or dyslipidemia, large amounts of fatty acids are delivered into the circulation. Elevated FFA levels in skeletal muscle contribute to insulin resistance indirectly by impairing glucose oxidation and directly by affecting glucose transport and glycogen synthesis [34,35,37]. Increased FFA flux in liver can induce hepatic insulin resistance by stimulating TG synthesis and gluconeogenesis [38] as well as contribute to systemic insulin resistance through other mechanisms [39]. Therefore, efficiently increasing fatty acid *β*-oxidation and decreasing TG and FFA by more potent and selective PPARα agonists should subsequently have a beneficial effect on glucose control. This hypothesis was further demonstrated in the present study.

In *db/db* mice, compound A also significantly reduced weight gain at higher doses (1 mg/kg and above) after 11 days of treatment. However, the effect of compound A on TG, glucose, and insulin occurred at the doses without changing body weight. The result suggests that the effects of this compound on lipid and glucose metabolism could be independent of the weight lost. Peroxisome proliferator–activated receptor α has been implicated in the regulation of obesity as shown in a previous finding that the lack of PPARα in rodents leads to weight gain [40]. Our experimental data also indicated that PPARα plays a role in energy homeostasis. In a long-term study, compound A at 0.1 mg/kg produced a reduction in body weight with a decrease in fat deposition and an increase in oxygen consumption in DIO mice. Although the compound decreased total food consumption, the effect did not fully contribute to the weight loss. In fact, the feeding efficiency was reduced by compound A, suggesting increased energy expenditure in these animals. The improvement in energy expenditure could also contribute to the amelioration in insulin resistance.

In conclusion, our studies strongly demonstrate that a novel and potent PPARα agonist improved dyslipidemia, insulin sensitivity, and energy expenditure in animal models. The beneficial effect of the compound on glucose metabolism occurred without weight gain, which is contrary to the currently marketed PPARγ agonists rosiglitazone and pioglitazone. The study suggests a potential therapeutic utility of PPARα agonists in the treatment of dyslipidemia associated with insulin resistance.

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