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The PPARα/γ dual agonist chiglitazar improves insulin resistance and dyslipidemia in MSG obese rats

[1]Ping-Ping Li, [2]Song Shan, [1]Yue-Teng Chen, [2]Zhi-Qiang Ning, [1]Su-Juan Sun, [1]Quan Liu, [2]Xian-Ping Lu, [1]Ming-Zhi Xie & *,[1]Zhu-Fang Shen

[1]Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, China and [2]Shenzhen Chipscreen Biosciences Ltd, Shenzhen, 518057, China

1 The aim of this study was to investigate the capacity of chiglitazar to improve insulin resistance and dyslipidemia in monosodium L-glutamate (MSG) obese rats and to determine whether its lipid-lowering effect is mediated through its activation of PPARα.

2 Chiglitazar is a PPARα/γ dual agonist.

3 The compound improved impaired insulin and glucose tolerance; decreased plasma insulin level and increased the insulin sensitivity index and decreased HOMA index. Euglycemic hyperinsulinemic clamp studies showed chiglitazar increased the glucose infusion rate in MSG obese rats.

4 Chiglitazar inhibited alanine gluconeogenesis, lowered the hepatic glycogen level in MSG obese rats. Like rosiglitazone, chiglitazar promoted the differentiation of adipocytes and decreased the maximal diameter of adipocytes. In addition, chiglitazar decreased the fibrosis and lipid accumulation in the islets and increased the size of islets.

5 Chiglitazar reduced plasma triglyceride, total cholesterol (TCHO), nonesterified fatty acids (NEFA) and low density lipoprotein-cholesterol levels; lowered hepatic triglyceride and TCHO contents; decreased muscular NEFA level. Unlike rosiglitazone, chiglitazar showed significant increase of mRNA expression of PPARα, CPT1, BIFEZ, ACO and CYP4A10 in the liver of MSG obese rats.

6 These data suggest that PPARα/γ coagonist, such as chiglitazar, affect lipid homeostasis with different mechanisms from rosiglitazone, chiglitazar may have better effects on lipid homeostasis in diabetic patients than selective PPARγ agonists.
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Abbreviations: ACO, acyl-CoA oxidase; BIFEZ, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; CPT1, carnitine parmitoyl transferase 1; CYP4A10, cytochrome *P*450, family 4, subfamily a, polypeptide 10; PPAR, peroxisome proliferator-activated receptor; HOMA, homeostasis model assessment

Introduction

Type II diabetes mellitus is a chronic disease characterized by glucose intolerance, hyperinsulinemia, and dyslipidemia (Stumvoll *et al*., 2005). The World Health Organization estimated that by the year 2025, up to 300 million people will suffer from type II diabetes (Etgen *et al*., 2002). Blood glucose levels and insulin sensitivity can be improved by treating patients with thiazolidinediones (TZDs) such as pioglitazone (Waugh *et al*., 2006) and rosiglitazone (Campbell, 2005), which act by binding and activating a transcription factor known as peroxisome proliferator-activated receptor gamma (PPARγ). Whereas rosiglitazone can markedly reduce insulin resistance (Aronoff *et al*., 2000), it has modest effects on plasma lipids and increases fat mass and body weight associated with adipocyte proliferation (Brand *et al*., 2003; Berthiaume *et al*., 2004) in type II diabetic patients (Khan *et al*., 2002; Herz *et al*., 2003). On the other hand, the increased triglycerides and decreased high-density lipoprotein cholesterol (HDL-C) levels, the most common lipid abnormalities in patients with insulin resistance, can be effectively altered by treatment with fibrates such as fenofibrate and gemfibrozil, which have been used to treat hypertriglyceridemia and reduce cardiovascular risk (Robins, 2001; Devroey *et al*., 2005). The fibrates act by binding and activating PPARα (Robins, 2001). Both PPARα and PPARγ agonists have actions with distinct benefits for type II diabetes.

PPARs are a class of nuclear transcription factors that have been found to be very important in various aspects of metabolism, including lipids, adipogenesis, and glucose control. PPARs have three subtypes: α, δ (β), and γ, PPARα is predominantly expressed in the liver and regulates the transcription of genes involved in hepatic fatty acid uptake and oxidation (Escher & Wahli, 2000). PPARγ is present mainly in white adipose tissue (WAT), where its activation stimulates the expression of genes involved in fatty acid uptake and storage. In skeletal muscle, significant levels of PPARα and PPARγ proteins are detected although their mRNA expression levels are low (Loviscach *et al*., 2000).

The overwhelming source of morbidity and mortality in type II diabetes is cardiovascular disease (Peter *et al*., 2003). Therefore, there is an urgent medical need for the development

*Author for correspondence; E-mail: shenzhf@imm.ac.cn

of antidiabetic drugs that can treat not only hyperglycemia but also dyslipidemia. In theory, the dual PPARα/γ agonist should be useful for the treatment of the major metabolic disorders of type II diabetes, such as hyperglycemia, insulin resistance, and dyslipidemia, improving both complications resulting from elevated blood glucose and the cardiovascular risk associated with 'syndrome X' (Stumvoll *et al*., 2005). In the present study, we investigated the effects of chiglitazar, a novel PPARα/γ agonist, on reducing insulin resistance and improving lipid profiles in monosodium L-glutamate (MSG) obese rats and revealed the lipid-lowering mechanisms of chiglitazar.

Methods

Chemicals

The chiglitazar, the rosiglitazone and the pioglitazone were provided by Shenzhen Chipscreen Biosciences Ltd, (China), and the WY14643 was purchased from BIOMOL International LP (U.S.A.). The structure of chiglitazar (3-[4-[2-(9H-carbazol-9-yl)ethoxy]phenyl]-2(*S*)-[2-(4-fluorobenzoyl)phenylamino] propionic acid) is shown in Figure 1.

Reporter gene assays

cDNAs for human retinoid X receptor (RXR), PPARα, and PPARγ were obtained by reverse transcriptase–polymerase chain reaction (RT–PCR) from the human liver or adipose tissues. Amplified cDNAs were cloned into pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA, U.S.A.). The integrity and fidelity of all constructs made were verified by DNA sequencing. Luciferase reporter plasmids, ACOX PPRE (for PPARγ) and pHD(X3)*luc* (for PPARα), were kind gifts of Drs T.M. McIntyre (University of Utah) and R.A. Rachubinski (University of Alberta, Canada), respectively. U-2OS human osteosarcoma cells (passage 20, American Type Culture Collection, U.S.A.) were cultured in McCoy's 5A with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C. Cells were seeded in 96-well plates the day before transfection to give a confluence of 50–80% at transfection. A total of 60 ng of DNA containing 10 ng of hRXR, 10 ng of pCMV Gal, 10 ng of nuclear receptor expression vectors, and 30 ng of the corresponding reporters were cotransfected per well using FuGene6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN, U.S.A.) according to the manufacturer's instructions. Following 24 h after transfection, cells were incubated with 10% charcoal-stripped fetal bovine serum Dulbecco's modified Eagle's medium and were treated with the individual compounds dissolved in dimethylsulfoxide (DMSO) for 24 h. The final concentration of DMSO in culture medium was 0.1%. Cells were lysed and prepared for measurement of luciferase activity using a luciferase assay kit from the Promega (U.S.A.). Luciferase enzyme activity was detected by the Ascent Fluoroskan FL reader (Thermo Labsystems, Finland). To measure α-galactosidase activity, 50 μl of supernatant from each transfection lysate was transferred to a new microplate, and the enzyme activity was detected by a reagent kit from Promega and read in a microplate reader (Bio-tek Instruments Inc. U.S.A.). The α-galactosidase data were used to normalize the luciferase data.

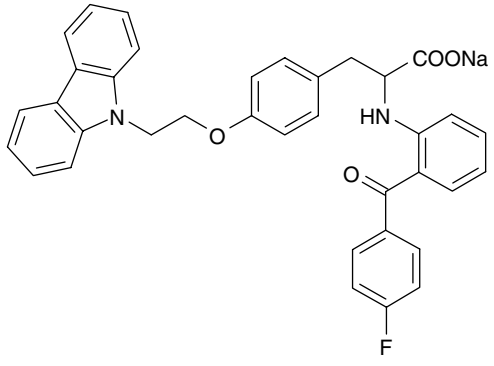

Figure 1 The structure of the dual PPARα/γ agonist chiglitazar ($C_{36}H_{28}N_2O_4FNa$).

Animals and treatment

MSG obese rats were rendered by subcutaneous injection of MSG at 4 g kg^{-1} day^{-1} to newborn wistar rats for 7 days. In contrast to normal wistar rats, the MSG rats developed increased plasma triglyceride, cholesterol, and free fatty acid contents as well as impaired insulin and glucose tolerance at 6 months of age (Hirata *et al*., 1997; Ding *et al*., 2001). Normal

Table 1 Sequences of the primers used in the PCR measurements

Gene	*Sense*	*Sequence*	*GenBank no./ref.*
GAPDH	Forward	ATG CCA TCA CTG CCA CCC	X02231
	Reverse	GCC TGC TTC ACC ACC TTC TT	
PPARα	Forward	GCA AAA CTG AAA GCA GAA ATT CT	NM_013196
	Reverse	AGC TCC GTG ACG GTC TCC A	
BIFEZ	Forward	GGT GCA AGA GGA ACC CAG CT	NM_133606
	Reverse	GAG GGA GTT GAC CAC TTA TTT GC	
CYP4A10	Forward	GCT TGC AGT GTT CAG GTG GAT GGA A	NM_153307
	Reverse	GCA TCT TTG TTG GTG CTT AAAATC AAG CGG G	
ACO	Forward	TGA AAT CAA GCA AAG CGA ACC	NM_017340
	Reverse	TGA CAC CAT ACC ACC CAC CAA C	
CPT1	Forward	CCC ATG TTG TAC AGC TTC CAG	NM_031559
	Reverse	TGG ATG GTG TCT GTC TCC TC	

wistar rats (weighing 200 ± 5 g each) were purchased from Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences & Peking Union Medical College (Beijing, China). All animals were maintained at controlled temperature ($22 \pm 1^{\circ}$C), under 12 h light/dark cycles and given standard laboratory chow and water *ad libitum*.

MSG obese rats (6 months old) were sorted into five treatment groups ($n = 10$ each, male and female in half) based on decreased blood glucose in the insulin tolerance test, glucose levels, blood total triglyceride (TG), total cholesterol (TCHO), and initial body weight. From the next day, MSG obese rats received single daily oral treatment with chiglitazar (5, 10, and 20 mg kg^{-1} day^{-1}, respectively), rosiglitazone (5 mg kg^{-1} day^{-1}) or vehicle (water, 0.05% Tween 80) for 40 days. Normal wistar rats ($n = 10$) served as a normal group were treated with vehicle. Experiments were performed during treatment, and all protocols for animal use and killing were in accordance with National Institutes of Health guidelines.

Intraperitoneal glucose tolerance test, Insulin tolerance test, and alanine gluconeogenesis test

For intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) animals were fasted for 4 h and a basal blood sample was taken, followed by intraperitoneal injection of glucose (2 g kg^{-1}), subcutaneous injection of insulin (Humulin R, Lilly, France, 0.4 U kg^{-1}), respectively. Blood samples were taken at 30, 60, and 120 min after the glucose load, at 40 and 90 min after the injection of insulin. For alanine gluconeogenesis test, using a modified method (Chiasson *et al*., 1977), animals were fasted for 4 h, and then intraperitoneally injected by L-alanine (1.5 g kg^{-1}), blood samples were taken before and at 60 min after the alanine load. These samples were analyzed for blood glucose using glucose-oxidase method, and the area under the curves (AUC) generated from the data collected during the IPGTT were calculated.

Clamp experiments in MSG obese rats

The effect of chiglitazar on insulin sensitivity was analyzed using euglycemic hyperinsulinemic clamp technique (Kraegen *et al*., 1983) in anesthetized MSG obese rats ($n = 6$) treated with chiglitazar for 40 days. On the day of the clamp experiment, the rats fasted for 6 h were anesthetized intraperitoneally with 45 mg kg^{-1} sodium pentobarbital. After tracheotomy, the rats were fitted with a carotid artery catheter for blood glucose measurement (Accu-Chek Glucotrend 2, Roche Diagnostics GmbH, Germany) and a Y-type catheter in a jugular vein for infusion of insulin and glucose. Insulin (8 mU kg^{-1} min^{-1}) was infused using a syringe pump (KD Scientific 100, KD Scientific Inc., U.S.A.) to elevate circulating insulin levels, and glucose (20%) was infused using another pump (Master Flex-L/S digital Economy Drive, Cole-Parmer Instrument Company, U.S.A.). The blood glucose was sampled once every 5 min. The steady state can be reached about 2 h later with constant glucose infusion. During the steady state, the blood glucose was maintained with 5% of the euglycemia level for at least 30 min. Glucose infusion rate (GIR) (mg kg^{-1} min^{-1}) was defined as this steady-state glucose infusion rate.

Biochemical analysis

TG, TCHO, low-density lipoproteins cholesterol (LDL-C) and nonesterified fatty acids (NEFA) in the blood, TG, TCHO in the liver and NEFA in the muscle were determined using enzymatic colorimetric methods by commercial kits (Biosino Bio-Technology and Science Inc., Beijing, China) and plasma insulin (PI) was measured using radioimmunoassay kit (Chinese Institute of Atomic Energy, Beijing, China). Tissue content of glycogen was estimated by anthrone method in a liver homogenate. The homeostasis model assessment (HOMA) index and insulin sensitivity index (ISI) were calculated by the values of fasting plasma glucose (FPG) and PI. $ISI = 1/(FPG \times PI)1000$, where FPG is expressed as mg dl^{-1} and PI as mU l^{-1}. $HOMA = FPG \times PI/22.5$, where FPG is expressed as mM and PI as mU l^{-1}.

Preparation of RNA and cDNA from the liver

Tissues were homogenized in Trizol and total RNA was isolated according to the manufacture's protocol (Invitrogen, U.S.A.). RNA concentration was quantified by its absorbance at 260 nm. For the PCR analysis of RNA, cDNA was prepared by RT–PCR. Briefly, 5 μg of each RNA sample was added to reaction mixture of 50 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 0.002 mM ethylene diamine tetra-acetic acid, 0.2 mM dithiothreitol, 0.8 mM deoxynucleotide triphosphates, 1 μg Oligo (dT), 2 U of RNase Block Ribonuclease Inhibitor, and 50 U of moloney murine leukemia virus reverse transcriptase (Stratagene, U.S.A.). The reaction mixture was incubated at 42°C for 1 h and at 95°C for 5 min. The cDNAs were used for the following PCR amplifications.

The PCR amplifications were performed in 10 μl of reaction mixture volume containing 0.8 μl of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM deoxynucleotide triphosphates, 0.4 mM primers and 0.25 U of *Taq* polymerase (TaKaRa Biotechnology Co., Ltd, China). Primers for PCR are shown in Table 1. *Taq* DNA polymerase was added to each tube after a treatment of 2 min at 94°C. This was followed by 22–30 cycles of denaturation (23 s at 94°C), annealing (20 s at 55°C), extension (30 s at

Table 2 Chiglitazar activated both PPARα and PPARγ

Compound	*PPARα*		*PPARγ*	
	EC_{50} (μM)	*% WY* (*5* μM)	EC_{50} (μM)	*% ROS* (*10* μM)
Chiglitazar	1.2	147	0.08	117
Rosiglitazone	7.3	16	0.04	100
Pioglitazone	3.2	152	0.18	90.8
WY14643	18.0	100	IA[a]	IA[a]

Transactivation by human PPARα and PPARγ receptors was determined in U-2OS cells cotransfected with PPARα expression plasmid and pHD(X3)*luc* or PPARγ expression plasmid and ACOX PPRE, and both hRXR and pCMV Gal. Following 24 h after transfection, these cells were than incubated with a series of concentrations of compound for 24 h before the luciferase activities were determined. The results are shown as the mean value obtained from at least three independent experiments performed in triplicate.
[a]Inactive, WY: WY14643, ROS: rosiglitazone.

72°C), and a final extension step of 2 min. The number of cycles used (22 for CYP4A10, 24 for BIFEZ, 25 for CPT1 and ACO, 26 for PPARα) was predetermined to be the greatest number of cycles within the linear range. PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium-bromide, and the intensity of the fluorescent signal emitted by the PCR products was quantified by densitometry. Each cDNA was amplified in triplicate.

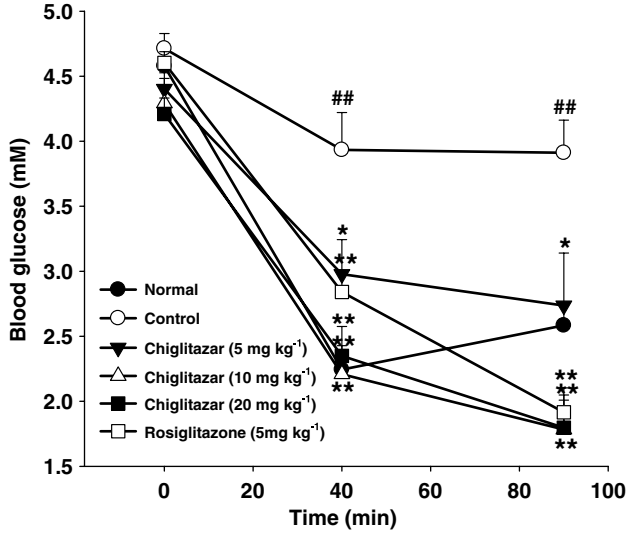

Figure 2 Insulin tolerance test in MSG obese rats. Wistar rats were treated with vehicle (Normal), MSG obese rats were treated with vehicle (Control), chiglitazar at doses of 5, 10, and 20 mg kg^{-1}, or rosiglitazone at dose of 5 mg kg^{-1} for 9 days, and then subjected to subcutaneous injection of insulin (0.4 U kg^{-1}) after 4 h fasting. Plasma glucose concentrations (0, 40, 90 min after insulin injection) were plotted on the graph (mean ± s.e.m., $n=9$–10); $^{\#\#}P<0.01$ for Con *versus* Nor; $^{*}P<0.05$, $^{**}P<0.01$ compared with Con (ANOVA).

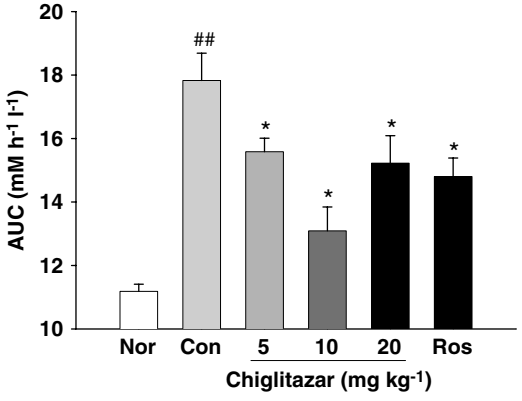

Figure 3 Glucose tolerance test in MSG obese rats. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and 20 mg kg^{-1} dose or rosiglitazone at 5 mg kg^{-1} dose (Ros) for 23 days, and then subjected to intraperitoneal injection of glucose (2 mg kg^{-1}) after 4 h fast. The area under the curve (AUC) was calculated from the glucose concentrations on 0, 30, 60, 120 min after glucose load and were plotted on the graph. The values are expressed as mean ± s.e.m. ($n=9$–10); $^{\#\#}P<0.01$ for Con *versus* Nor; $^{*}P<0.05$, compared with Con (ANOVA).

Histological analysis of adipose tissue and pancreatic morphology

At the end of experiment, MSG rats' infrarenal fat pads were excised and fixed with 2.5% formalin in ethanol saline. Fixed specimens were dehydrated and embedded in paraffin. Sections (10 μm) were sliced and stained with hematoxylin and eosin (HE) and adipocyte sizes were measured. The pancreas was fixed in bourin for paraffin embedding and stained with HE and gomori for analysis of islet morphology and pancreatic beta cell mass.

Statistical analyses

All values are presented as mean ± standard error of the mean (s.e.m.). Statistical analysis was performed by one-way ANOVA (two-tailed test). All analyses were performed using SPSS version 11.

Results

In vitro *characterization of chiglitazar*

Comparative dose–response study of chiglitazar was performed with rosiglitazone and pioglitazone for PPARγ, and WY14643 for PPARα. As shown in Table 2, chiglitazar showed significant activation of both the isoforms. Chiglitazar showed weaker PPARγ activating activity than rosiglitazone, but stronger than pioglitazone. In terms of PPARα activation, chiglitazar showed more potent activity than rosiglitazone, pioglitazone, or WY14643 which is a selective PPARα agonist. We then chose rosiglitazone as a control compound for the *in vivo* study of chiglitazar, as shown follow.

Insulin resistance in MSG obese rats

The results of ITT are shown in Figure 2. After insulin injection, plasma glucose levels in the MSG rats treated with

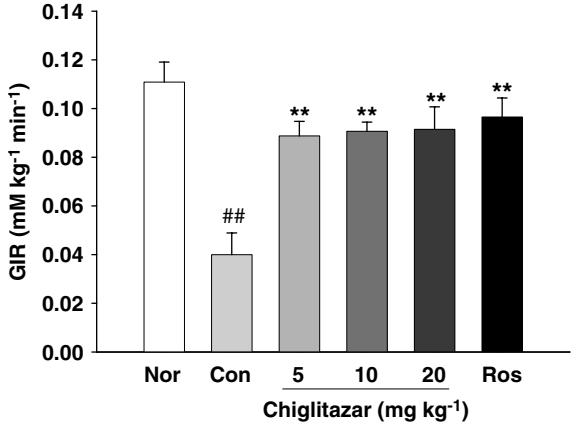

Figure 4 The glucose infusion rate (GIR) in euglycemic hyperinsulinemic clamp studies. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and 20 mg kg^{-1} dose or rosiglitazone at 5 mg kg^{-1} dose (Ros) for 40 days. The values are expressed as mean ± s.e.m. ($n=5$–6); $^{\#\#}P<0.01$ for Con *versus* Nor; $^{**}P<0.01$ compared with Con (ANOVA).

chiglitazar or rosiglitazone were significantly reduced compared with the control group treated with vehicle at all time points.

Fasting PI levels were lower in animals treated with chiglitazar and rosiglitazone (35.0 ± 3.3, 23.8 ± 1.6, 36.2 ± 4.4, and $38.8\pm3.2\,mU\,l^{-1}$ for 5, 10, $20\,mg\,kg^{-1}$ chiglitazar-treated and $5\,mg\,kg^{-1}$ rosiglitazone-treated MSG obese rats, respectively, $P<0.01$) than control ($63.4\pm9.7\,mU\,l^{-1}$). The ISIs of MSG obese rats treated with chiglitazar (3.7 ± 0.5, 5.2 ± 0.4, and 3.7 ± 0.4 for 5, 10, and $20\,mg\,kg^{-1}$, respectively, $P<0.01$) and rosiglitazone (3.2 ± 0.3, $P<0.01$) were significantly higher than control (2.0 ± 0.2). Furthermore, chiglitazar ameliorated the HOMA indices (7.6 ± 0.8, 5.0 ± 0.3, 7.4 ± 1.0, 8.3 ± 0.7, and

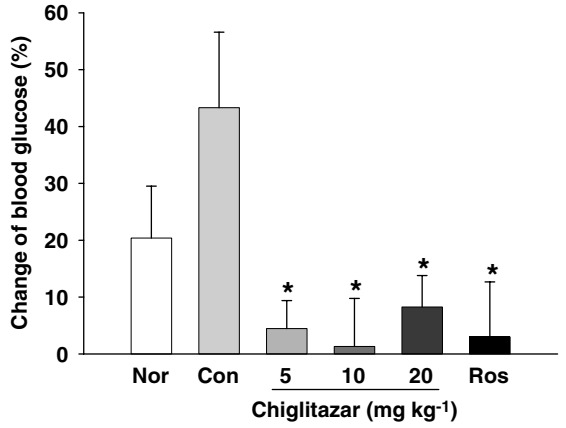

Figure 5 The gluconeogenesis test from alanine in MSG obese rats. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and $20\,mg\,kg^{-1}$ dose or rosiglitazone at $5\,mg\,kg^{-1}$ dose (Ros) for 29 days, and then subjected to intraperitoneal injection of L-alanine ($1.5\,g\,kg^{-1}$) after 4 h fasting. Changes of plasma glucose concentrations were plotted on the graph (mean ± s.e.m., $n=7$–8); $^{*}P<0.05$ compared with Con (ANOVA).

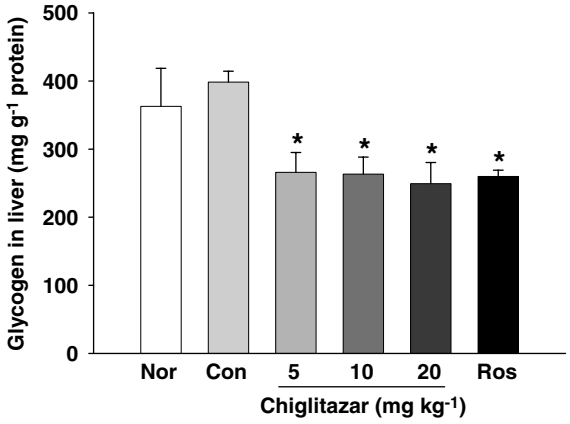

Figure 6 Hepatic glycogen content in MSG obese rats. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and $20\,mg\,kg^{-1}$ dose or rosiglitazone at $5\,mg\,kg^{-1}$ dose (Ros) for 40 days. The values are expressed as mean ± s.e.m., ($n=5$–6); $^{*}P<0.05$ compared with Con (ANOVA).

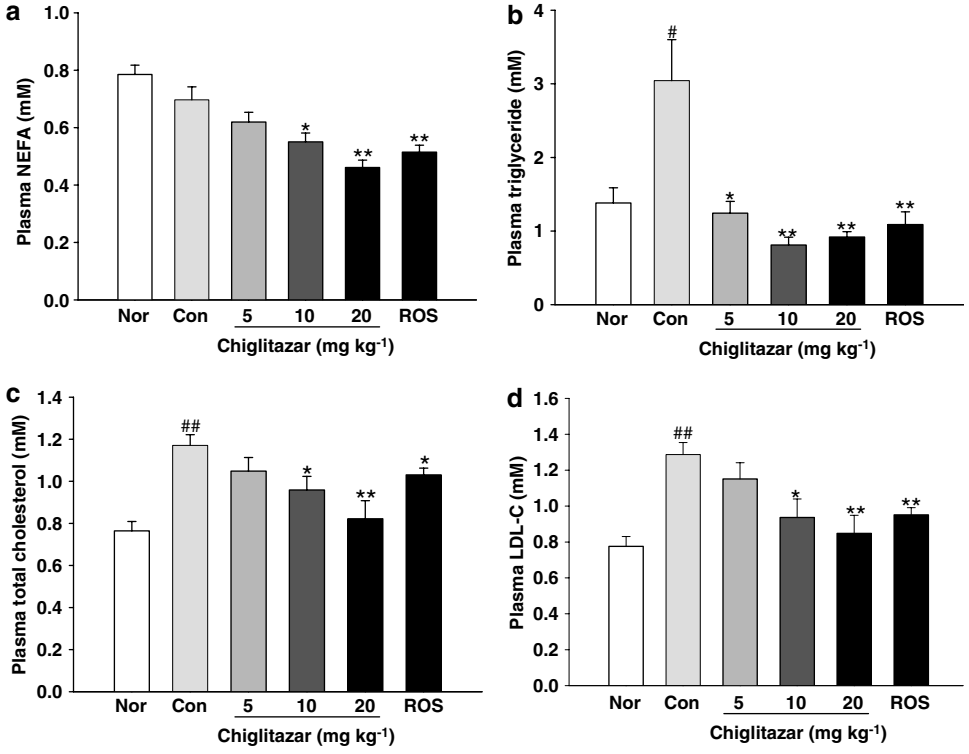

Figure 7 Effect of chiglitazar on plasma lipid profile in MSG obese rats. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and $20\,mg\,kg^{-1}$ dose or rosiglitazone at $5\,mg\,kg^{-1}$ dose (Ros). (a) NEFA, (b) triglyceride, (c) TCHO, and (d) LDL-C. The values are expressed as mean ± s.e.m. ($n=9\sim10$); $^{\#}P<0.05$, $^{\#\#}P<0.01$ for Con *versus* Nor; $^{*}P<0.05$, $^{**}P<0.01$ compared with Con (ANOVA).

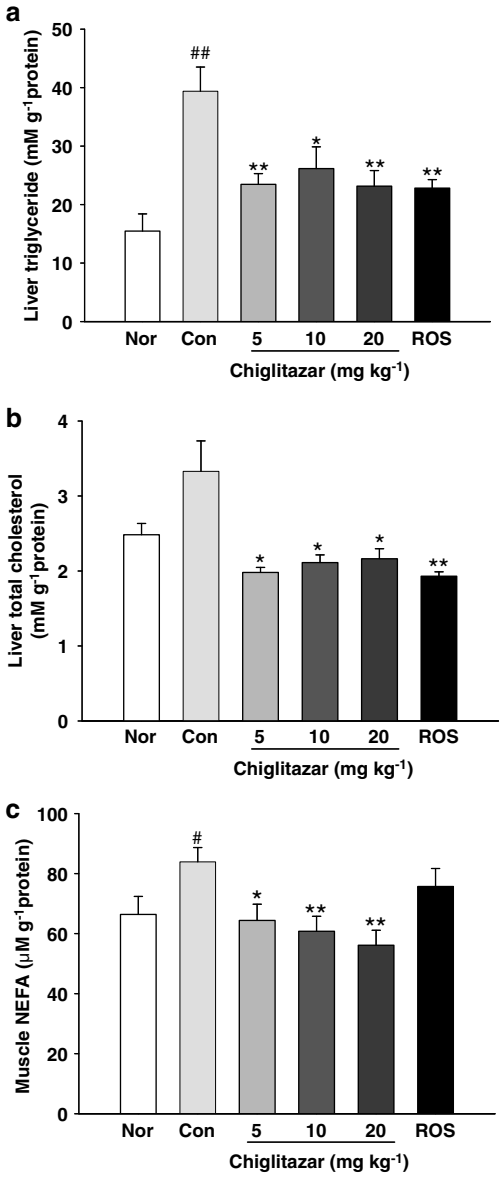

Figure 8 Effect of chiglitazar on hepatic and muscular lipid profile in MSG obese rats. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and 20 mg kg^{-1} dose or rosiglitazone at 5 mg kg^{-1} dose (Ros). (a) hepatic triglyceride, (b) hepatic TCHO, and (c) muscular NEFA. The values are expressed as mean ± s.e.m. ($n = 9$–10); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for Con *versus* Nor; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Con (ANOVA).

14.3 ± 2.2 for 5, 10, and 20 mg kg^{-1} chiglitazar, rosiglitazone, and vehicle-treated rats, respectively, $P < 0.01$).

For IPGTT, at the 30 min after glucose loading, the glucose values in the 5 and 10 mg kg^{-1} chiglitazar- and rosiglitazone-treatment groups were significantly lower than those in the vehicle treatment group. The integrated (AUC, 0–120 min) for the glucose response during the IPGTT in the treatment groups were significantly less than those in the control groups (Figure 3).

MSG Obese rats showed significant insulin resistance, demonstrated by decreased GIR in hyperinsulinemic euglycemic clamp technique. Compared with vehicle-treated MSG rats, both chiglitazar and rosiglitazone increased GIR (Figure 4).

Gluconeogenesis and hepatic glycogen content

Endogenous glucose production is the net result of breakdown of glucose stored as glycogen (glycogenolysis) and the synthesis of new glucose molecules from lactate, amino acids, and glycerol (gluconeogenesis). The rate of gluconeogenesis from alanine increased in MSG obese rats. The increase in the rate of alanine converting to glucose was suppressed by chiglitazar (Figure 5). In addition, both chiglitazar and rosiglitazone decreased liver glycogen content (Figure 6), and the effect of chiglitazar was greater than that of rosiglitazone.

Lipid profile

Figures 7 and 8 showed that chiglitazar had beneficial effects on lipids contents in the serum, liver, and muscle. Both chiglitazar and rosiglitazone significantly decreased TG, TCHO, LDL-C, and NEFA contents in the plasma, lowered TG and TCHO in the liver and only chiglitazar reduced NEFA in the muscle of MSG obese rats.

Genes expression

To study the mechanism of the lipid-lowering effects in treatment groups, we measured genes expression involved in NFFA oxidation in the liver of MSG obese rats. Chiglitazar had greater effect on induction of PPARα, ACO, BIFEZ, CYP4A10, and CPT1 mRNA than rosiglitazone (Figure 9).

Histological analysis of adipose tissue and pancreatic morphology

Like rosiglitazone, chiglitazar promoted the differentiation of adipocytes and decreased the maximal diameter of adipocyte. The maximal diameters of adipocyte in the treatment groups (72.07 ± 10.00, 57.03 ± 7.00, 63.50 ± 7.61, 62.4 ± 6.45 μm for 5, 10, 20 mg kg^{-1} chiglitazar-treated and rosiglitazone-treated group, $P < 0.01$) were significantly lower than those in control group (83.00 ± 13.34 μm). The overall morphology of the islets in the treatment group was also more regulated and rounded. In the vehicle-treated MSG rats, the islets showed substantial fibrosis, whereas there was little fibrosis in the chiglitazar-treated MSG rats. In addition, chiglitazar decreased the lipid accumulation in the islets and increased the size of islets (Figure 10).

Discussion

To the best of our knowledge, it is reported for the first time that chiglitazar, a novel agonist of both PPARα and PPARγ, is an effective insulin sensitivity agent. MSG has been shown to induce obesity, insulin resistance, and dyslipidemia associated with chemical ablation of the arcuate nucleus (Hirata *et al*., 1997). In our study, chiglitazar improved the impaired insulin sensitivity and glucose tolerances, decreased hyperinsulinemia and HOMA index. These studies in MSG obese rats clearly indicated that chiglitazar is an efficacious insulin sensitizer. Because most *in vivo* insulin-stimulated glucose disposal occurs in the skeletal muscle (Hevener *et al*., 2003), results from the clamp experiment suggested that chiglitazar and rosiglitazone might reduce the insulin resistance in the muscle of MSG obese rats. It has been demonstrated that the small adipocyte is more

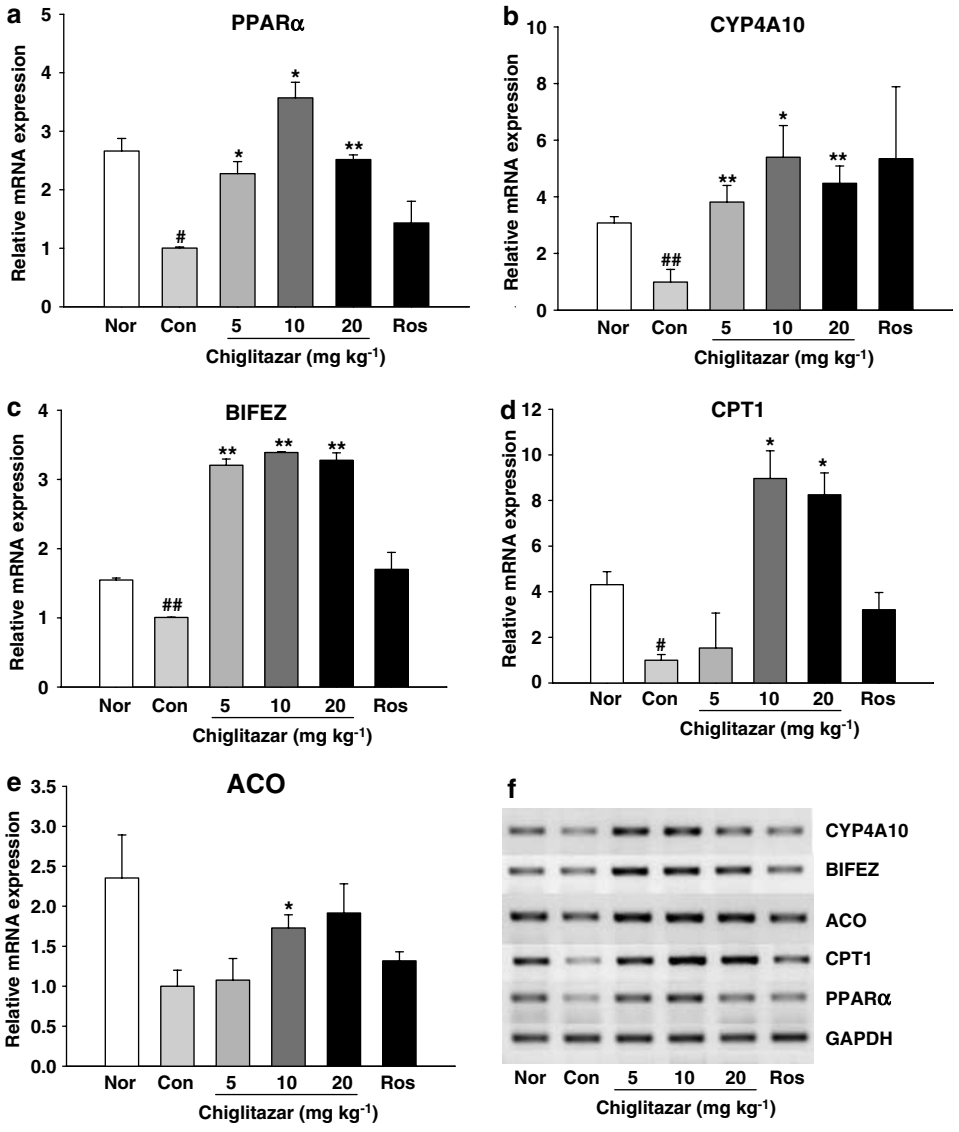

Figure 9 Effects of chiglitazar on genes expression involved in NEFA oxidation. Wistar rats were treated with vehicle (Nor), MSG obese rats were treated with vehicle (Con), chiglitazar at 5, 10, and 20 mg kg^{-1} dose or rosiglitazone at 5 mg kg^{-1} dose (Ros) for 40 days. (a) PPARα, (b) CYP4A10, (c) BIFEZ, (d) CPT1, (e) ACO, and (f) representative gel figure. GAPDH served as internal standard. The values are expressed as mean ± s.e.m.; $^{\#}P<0.05$, $^{\#\#}P<0.01$ for Con *versus* Nor; $^{*}P<0.05$, $^{**}P<0.01$ compared with Con (ANOVA).

sensitive to insulin than the large adipocyte (Havel, 2004), suggesting that chiglitazar improved insulin resistance in adipocytes of MSG obese rats.

The underlying cause of insulin resistance was a combination of decreased insulin-mediated glucose disposal rate in peripheral tissues and impaired suppression of hepatic glucose output (HGO). HGO is the result of gluconeogenesis and glycogen breakdown. In patients with type II diabetes, the rate of hepatic gluconeogenesis is considerably increased compared with healthy subjects, therefore contributing 50–60% of the related glucose (Hundal *et al*., 2000). Insulin is the most important hormone that inhibits gluconeogenesis. It acts predominantly by suppressing the expression of genes for the key gluconeogenic enzymes PEPCK and G-6-Pase. Compared with vehicle-treated MSG obese rats, hepatic alanine gluconeogenesis and glycogen stores were reduced significantly in drug-treated groups. The properties of chiglitazar reducing alanine gluconeogenesis and hepatic glycogen suggested that the compound might inhibit HGO, thereby suppressing insulin resistance in the liver of MSG obese rats.

Type II diabetes is a serious and growing health threat in the industrialized world. Because diabetic patients are also at high risk for atherosclerosis and myocardial infarction, and this risk can probably be attributed to diabetic dyslipidemia, which is characterized by high TG and low HDL-C levels. Although TZD lower glucose concentrations and increase insulin sensitivity, their nonglycemic effects on body weight, lipid, and blood pressure have been disappointment, implying that this class of medication will not reduce the need to treat dyslipidemia and hypertension with separate therapies. Moreover, PPARγ agonists lower TG much more effectively in db/db mice than they do in type II diabetic humans (Guo *et al*., 2004; Yki-Jarvinen, 2004), the reason for this discrepancy is not fully known. It is clear that PPARγ are highly expressed in

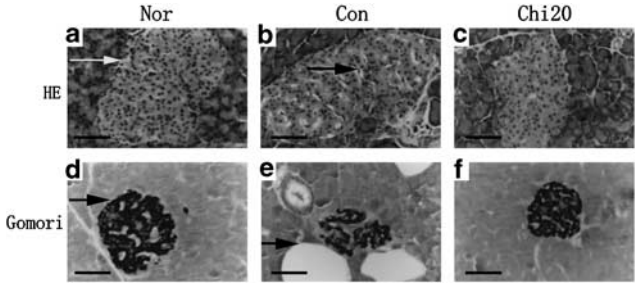

Figure 10 Histopathological examination of the pancreas. Wistar rats treated with vehicle (Nor, left), and MSG obese rats treated with vehicle (Con, middle) or chiglitazar (20 mg kg^{-1}, Chi 20, right). HE, staining; Gomori, gomori staining. (a) Arrow, islet, (b) arrow, fibroblast, (d) arrow, pancreatic beta cell, (e) arrow, fat drop. In (b) (Con) the islets showed substantial fibrosis, whereas there was little fibrosis in the (a) (Nor) and (c) (Chi 20). In both (d) (Nor) and (f) (Chi 20), the staining of the beta cells was much higher than that in (e) (Con). Black bars = 200 μm.

adipocytes and mediate their differentiation. A major mechanism of the insulin-sensitizing action of PPARγ agonists results from the lowering of lipid supply to muscle and liver through a 'lipid-stealing' by PPARγ-mediated effects in adipose tissue (Kersten *et al*., 2000), thus TZD have the nonglycemic effects. Unlike PPARγ, PPARα mediates expression of genes regulating lipid oxidation (Kersten *et al*., 2000). PPARα agonists, such as fibrates, have been used to treat hypertriglyceridemia and reduce cardiovascular risk (Robins, 2001). A number of studies on insulin-resistant animal models have shown marked decreases in the liver triglyceride content and adiposity by PPARα agonists (Guerre-Millo *et al*., 2000). Administration of PPARα agonists increases the expression of peroxisomal and mitochondrial β-oxidation enzymes, in particular ACO, CAT, CPT1, and several other related enzymes in hepatocellular compartments in rodents (Chou *et al*., 2002). It appears that chiglitazar decreased plasma and liver lipids content at least by activating PPARα, as indicated by increased expression levels of liver BIFEZ, CYP4A10, CPT1, and ACO mRNA, in a similar way to a PPARα agonist WY14643. These results are consistent with the finding in obese Zuker rats that the PPARα/γ coligand KRP-297 reduces liver triglyceride content because it stimulates NEFA oxidation (Murakami *et al*., 1998). Interestingly, chiglitazar upregulated the expression of PPARα mRNA. These results strongly suggested that the activation of PPARα is involved in the lipid-lowering effect of chiglitazar and the compound could have better effects on lipid homeostasis in diabetic patients than rosiglitazone.

It is clear that a lipid accumulation in the muscle and liver can cause the development of insulin resistance (Boden, 2002), which has led to various strategies to improve insulin sensitivity by lowering excess lipid accumulation in the liver and muscle (Moller, 2001). Like rosiglitazone, chiglitazar significantly decreased lipids accumulation and enhanced insulin action in the muscle and liver. This result is similar to the previous reports studied dual PPARα/γ agonists such as JTT-501 (Maegawa *et al*., 1999) and LY-465608 (Etgen *et al*., 2002) in other insulin-resistant models.

Insulin resistance in peripheral target tissues and impaired insulin secretory capacity of pancreatic β-cells contribute to the pathogenesis of type II diabetes (Ahren, 2005; Stumvoll *et al*., 2005). It has been demonstrated that PPARα and PPARγ are expressed in pancreatic beta cells (Lupi *et al*., 2004), and lipotoxicity may cause beta cell abnormalities, loss of glucose-stimulated insulin secretion and GLUT2, and triglyceride accumulation (Unger *et al*., 1999; Prentki *et al*., 2002). Chiglitazar reduced the islet lipid content and prevented the loss of beta cells mass in obese MSG rats. Therefore, it is possible that chiglitazar can retard the progression of diabetes under conditions of reduced insulin resistance. These results are consistent with the recent finding that long-term administration of rosiglitazone (Finegood *et al*., 2001) prevented disruption of pancreatic islet architecture in ZDF rats, and pioglitazone has the similar effect in db/db mice (Kawasaki *et al*., 2005).

In summary, effects of dual PPARα/γ agonist (chiglitazar) and PPARγ agonist (rosiglitazone) on insulin sensitivity and lipids homeostasis in MSG obese rats were studied. Both chiglitazar and rosiglitazone improved the impaired insulin and glucose tolerance in MSG obese rats. Although both compounds normalized dyslipidemia, chiglitazar upregulated mRNA expression of genes involved in NEFA oxidation, suggesting that chiglitazar may have better effects on lipid homeostasis in diabetic patients than selective PPARγ agonist. Chiglitazar, *via* its dual PPARα and PPARγ activating properties, may decrease multiple risk factors for morbidity and mortality in diabetic patients and improve the quality and length of their lives.

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