**Insulin Resistance and Metabolic Derangements in Obese Mice Are Ameliorated by a Novel Peroxisome Proliferator-activated Receptor γ-sparing Thiazolidinedione**

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**Background:** Thiazolidinediones may have insulin-sensitizing effects independent of the nuclear receptor PPARγ.

**Results:** A novel PPARγ-sparing thiazolidinedione ameliorated insulin resistance and inflammation in obese mice.

**Conclusion:** The insulin-sensitizing effects of thiazolidinediones are separable from the ability to bind PPARγ.

**Significance:** Identification of other molecular targets of thiazolidinediones may generate new therapeutics for treatment of insulin resistance and diabetes.

Currently approved thiazolidinediones (TZDs) are effective insulin-sensitizing drugs that may have efficacy for treatment of a variety of metabolic and inflammatory diseases, but their use is limited by side effects that are mediated through ectopic activation of the peroxisome proliferator-activated receptor γ (PPARγ). Emerging evidence suggests that the potent anti-diabetic efficacy of TZDs can be separated from the ability to serve as ligands for PPARγ. A novel TZD analog (MSDC-0602) with very low affinity for binding and activation of PPARγ was evaluated for its effects on insulin resistance in obese mice. MSDC-0602 treatment markedly improved several measures of multiorgan insulin sensitivity, adipose tissue inflammation, and hepatic metabolic derangements, including suppressing hepatic lipogenesis and gluconeogenesis. These beneficial effects were mediated at least in part via direct actions on hepatocytes and were preserved in hepatocytes from liver-specific PPARγ−/− mice, indicating that PPARγ was not required to suppress these pathways. In conclusion, the beneficial pharmacology exhibited by MSDC-0602 on insulin sensitivity suggests that PPARγ-sparing TZDs are effective for treatment of type 2 diabetes with reduced risk of PPARγ-mediated side effects.

Clinically approved thiazolidinediones (TZDs; pioglitazone and rosiglitazone) are effective insulin-sensitizing agents. However, their use is associated with side effects, including weight gain, edema, and increased risk of fractures (1, 2). Compound-specific effects, including liver toxicity with troglitazone (3) and increased risk of cardiac mortality with rosiglitazone (4, 5), have also arisen. These side effects of TZDs have limited their potential applications for a variety of inflammatory and obesity-related metabolic diseases. The future widespread use of TZDs as insulin sensitizers and for treatment of other metabolic and inflammatory diseases clearly requires a breakthrough that would allow better therapeutic profiles.

Although discovered without respect to mechanism, many TZDs are now known to be ligands for a nuclear receptor transcription factor, the peroxisome proliferator activated receptor γ (PPARγ) (6). Activation of PPARγ in adipose tissue is thought to mediate several of the beneficial effects of TZDs (7, 8). Unfortunately, there are clear data that many of the side effects of TZDs are also mediated through direct and ectopic activation of PPARγ. Various tissue-specific knockouts of PPARγ are protected from increased adiposity (adipocyte-specific (9, 10)), plasma fluid volume expansion (collecting duct-specific (11, 12)), and hepatic steatosis (hepatocyte-specific (13, 14)) in response to the strong PPARγ agonist, rosiglitazone.

Although TZDs are widely regarded as purely PPARγ agonists, evidence also exists for PPARγ-independent, anti-diabetic pharmacology (15, 16). Pioglitazone, although 10-fold less potent at activating PPARγ compared with rosiglitazone (6), is equally efficacious as an insulin-sensitizing agent and has superior effects on lipids, hepatic steatosis, and cardiovascular outcomes (17–19). Studies of isolated perfused liver, which expresses little PPARγ, have shown effects of TZDs on hepatic

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4. The abbreviations used are: TZD, thiazolidinedione; PPAR, peroxisome proliferator activated receptor γ; PsTZD, PPARγ-sparing TZD; DIO, diet-induced obesity; LS, liver-specific; TG, triglyceride.
glucose production in a matter of minutes (20, 21). Supratherapeutic doses of rosiglitazone and pioglitazone also activate AMP-activated protein kinase (AMPK) within minutes in myocytes and adipocytes (22), which is again consistent with a non-genomic effect. Interestingly, TZDs are also known to bind specifically to mitochondrial membrane proteins and may modulate mitochondrial metabolism through this direct binding (23). We have hypothesized that TZDs with potent antidiabetic pharmacology that do not activate PPARγ at physiologic concentrations (PPARγ-sparing TZDs (PsTZDs)) can be identified (24) and used to treat insulin resistance, diabetes, and other metabolic and inflammatory conditions with reduced risk of PPARγ-driven side effects.

Herein, we demonstrate that a PsTZD (MSDC-0602), which is in Phase 2 clinical trials, markedly improved measures of systemic insulin sensitivity and metabolism and reduced adipose tissue inflammation. We also provide evidence that at least some of the beneficial pharmacology of the TZDs on hepatocytes is direct and independent of PPARγ. These studies suggest that PsTZDs could prove efficacious for treatment of obesity-related derangements in insulin sensitivity and metabolism and provide the framework for further evaluation of PPARγ-sparing mechanisms to treat aspects of metabolic disease.

**EXPERIMENTAL PROCEDURES**

**Materials**—MSDC-0602 was synthesized initially at Kalex-syn (Kalamazoo, MI) as part of a series of TZDs designed to have reduced ability to bind to PPARγ. Production scale synthesis of all TZDs for mouse studies was conducted by USV Ltd. (Mumbai, India), and TZDs were incorporated into the experimental diets by Research Diets Inc.

**Animal Studies**—Male mice were used in all studies. Liver-specific (LS)-PPARγ−/− mice were generated by intercrossing mice harboring floxed PPARγ alleles (9, 25) with mice expressing Cre recombinase in a liver-specific manner by using the albumin promoter (26) and extensively back-crossed into the C57BL/6 background. LS-PPARγ−/− mice were compared with littermate PPARγflox/flox mice not expressing Cre. Ob/ob and ob/+ control mice were purchased from The Jackson Laboratory. To cause diet-induced obesity (DIO), C57BL/6 wild-type or LS-PPARγ−/− mice were fed a diet providing 60% of its calories as fatty acids (Research Diets Inc. (catalog #D12492i)) starting at 6 weeks of age. DIO mice were compared with age-matched C57BL/6 male mice maintained on a matched low fat diet (10% fat; #D12450Bi).

For in vivo drug treatment, 6-week-old ob/ob mice were placed on a diet (Purina #5015) containing rosiglitazone, pioglitazone, or MSDC-0602 (300 ppm) for 4 weeks before sacrifice. This dose was chosen based on pilot studies showing that 300 ppm in diet resulted in a concentration of 2–5 μM MSDC-0602 in the blood of obese mice. DIO mice and that had been maintained on the high fat (60% fat) diet for 8 weeks were randomized to receive 60% fat diet containing MSDC-0602 (300 ppm) for the last 2 or 4 weeks of the trial or to receive 60% fat diet not containing MSDC-0602 for the duration. The dosage selected for these studies provides ~30 mg/kg/day of MSDC-0602.

Mice were sacrificed for tissue collection after a 4-h fast. To examine insulin-stimulated protein phosphorylation, a subset of mice was injected intraperitoneal with human insulin (10 milliunits/g of body weight) 5 min before sacrifice, and tissue was dissected and snap-frozen for protein isolation.

Glucose and insulin tolerance test studies were performed as described (27). Tail blood glucose was determined at 0, 30, 60, and 120 min after challenge using a One-Touch Ultra glucometer (LifeScan, Inc.). Total area under the curve was calculated using the trapezoidal rule. All animal experiments were approved by the Animal Studies Committee of Washington University School of Medicine.

**Hyperinsulinemic-Euglycemic Clamp Studies**—The right jugular vein and carotid artery of DIO mice that were untreated or treated for 3 weeks with MSDC-0602 were surgically catheterized and mice recovered for 1 week. After a 5–6-h fast, hyperinsulinemic clamp studies were performed on conscious mice using the protocol adopted from the Vanderbilt Mouse Metabolic Phenotyping Center (28) by the University of Michigan Animal Phenotyping Core consisting of a 90-min equilibration period followed by a 120-min experimental period (t = 0–120 min). Insulin was infused at 2.5 milliunits/kg/min. To estimate insulin-stimulated glucose uptake in individual tissues, a bolus injection of 2-[1-14C]deoxyglucose (PerkinElmer Life Sciences) (10 μCi) was given at t = 78 min while continuously maintaining the hyperinsulinemic-euglycemic steady state. At the end of the experiment, animals were anesthetized with an intravenous infusion of sodium pentobarbital, and tissues were collected and immediately frozen in liquid nitrogen for later analysis of tissue 14C radioactivity.

Plasma insulin was measured using the Linco rat/mouse insulin ELISA kits. For determination of plasma radioactivity of [3-3H]-glucose and 2-[1-14C]deoxyglucose, plasma samples were deproteinized and counted using a liquid scintillation counter. For analysis of tissue 2-[1-14C]deoxyglucose 6-phosphate, tissues were homogenized in 0.5% perchloric acid, and the supernatants were neutralized with KOH. Aliquots of the neutralized supernatant with and without deproteinization were counted for determination of the content of 2-[1-14C]deoxyglucose P.

**Cell Culture Studies**—Primary cultures of mouse hepatocytes were prepared as described (29). For isolation of hepatocytes from DIO mice, mice that had been fed a high fat diet for 12 weeks were used. Rates of fatty acid synthesis were assessed 2–3 h after cells were plated and were performed using [14C]acetate (30). TG synthesis rates were quantified by using [3H]glycerol in the presence of 300 μM oleate in the culture media as previously described (31, 32). To measure the rate of glucose output, after a pretreatment period of 18 h with MSDC-0602 or vehicle, the hepatocytes were washed extensively with PBS and then incubated for 3 h in 1 mM Krebs-Ringer-Hepes buffer containing 10 mM lactate and 0.5 μM glucagon with or without insulin (0.5 μM) and MSDC-0602. Glucose contents in the media were determined enzymatically (Sigma).

Hepatocytes from WT or LS-PPARγ−/− mice were isolated, plated, and then treated with vehicle (DMSO), rosiglitazone (1 μM), or MSDC-0602 (1 μM). Protein and RNA was collected 18 h after treatment. Rates of glucose production were determined in hepatocytes after 24 h of drug treatment.

3T3-L1 mouse preadipocytes were purchased from Zen-Bio. Preadipocytes were grown to confluence in complete medium.
containing 10% calf serum. Two days after reaching confluence, adipocyte differentiation was initiated by adding complete medium containing 10% fetal bovine serum and a hormone differentiation mixture (115 μg/ml methylisobutylxanthine, 10 μg/ml insulin, and 390 ng/ml dexamethasone). In addition to the differentiation mixture, cells were treated with vehicle (DMSO), rosiglitazone (0.1 μM), pioglitazone (0.1 μM), or MSDC-0602 (0.1 μM). RNA was collected after 48 h of drug treatment.

Plasma and Tissue Metabolite Quantification—Plasma glucose concentrations were determined using a colorimetric assay (Sigma) using plasma collected at time of sacrifice. Plasma insulin and adiponectin concentrations were determined by commercially available ELISAs from Crystal Chemical Co. and Linco Research Inc., respectively. Hepatic triglyceride content was measured using the lipid assay (Sigma) using plasma collected at time of sacrifice. Plasma cose concentrations were determined using a colorimetric assay (Sigma) using plasma collected at time of sacrifice.

Quantitative RT-PCR—Total RNA was isolated using the RNAzol method (Tel-Test). Real-time PCR was performed using the ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) and the SYBR Green kit. The Quantitative RT-PCR analyses can be provided upon request.

Western Blotting Analyses—Total cell proteins were collected using radioimmune precipitation assay buffer containing protease and phosphatase inhibitors. Western blotting studies were performed using antibodies directed against AKT (Cell Signaling), phospho-Ser-473 or phospho-Thr-308 AKT (Cell Signaling), fatty acid synthase (Abcam Inc.), glucose-6-phosphatase α (Santa Cruz Biotechnology), phosphoenolpyruvate carboxykinase (Cayman Chemical), and actin (Sigma). Densitometries of the specifically labeled bands were conducted using ImageJ software (NIH).

RESULTS

MSDC-0602 Is a Ps-TZD—MSDC-0602 is a modified TZD with alterations in the carbon backbone (Fig. 1A) that limit the ability to bind PPARγ (Fig. 1B). The IC₅₀ for PPARγ binding was 0.08 μM for rosiglitazone, 1.147 μM for pioglitazone, and 18.25 μM for MSDC-0602. Using a Gal4-PPARγ construct containing the ligand binding domain of mouse PPARγ, we confirmed that MSDC-0602 only minimally activated Gal4-PPARγ in HepG2 cells even at a concentration of 50 μM, whereas rosiglitazone and pioglitazone activated the Gal4-PPARγ construct at much lower concentrations (Fig. 1C). MSDC-0602 also did not activate Gal4-PPARα (Fig. 1C) or PPARδ (not shown) at physiologic concentrations. The EC₅₀ for activation of PPARα or PPARδ is 100 μM for MSDC-0602, rosiglitazone, and pioglitazone. Rosiglitazone and pioglitazone (0.1 μM) induced the expression of the known PPARγ target genes Fabp4, Cidec, Cd36, and Plin4 in differentiating 3T3-L1 adipocytes (Fig. 1D).

In contrast, MSDC-0602 (0.1 μM) did not lead to a significant induction in the expression of these genes. Finally, TZDs are also known to bind competitively to mitochondrial membranes (23), and rosiglitazone, pioglitazone, and MSDC-0602 bound to mitochondrial membranes with an equivalent affinity (Fig. 1E). These data demonstrate that, although these TZDs differ greatly in their ability to bind and activate PPARγ, they each have similar affinity for mitochondrial binding, which we

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hypothesize mediates at least some of the anti-diabetic pharma-
cology of TZDs.

Comparison of the Anti-diabetic Effects of MSDC-0602 with
Other TZDs—We compared the efficacy of MSDC-0602
with pioglitazone and rosiglitazone in ob/ob mice. Treatment
with any of the three TZDs corrected plasma glucose, non-
esterified fatty acids, triglycerides, cholesterol, and insulin con-
centrations, which were elevated in ob/ob mice compared with
lean controls (Table 1). Treatment with MSDC-0602, piogli-
tazone, and rosiglitazone also significantly improved glucose and

![Diagram A](image1.png)

![Diagram B](image2.png)

![Diagram C](image3.png)

![Diagram D](image4.png)

![Diagram E](image5.png)
insulin tolerance in ob/ob mice (Fig. 2A). Consistent with this, insulin-mediated phosphorylation of Ser-473 and Thr-308 of Akt in gastrocnemius muscle was significantly improved by TZD treatment (Fig. 2B). Thiazolidinediones are known to increase insulin sensitivity by acting in adipose tissue to increase expression of beneficial adipokines and to reduce inflammation and macrophage infiltration. All three TZDs corrected the expression of Fabp4, adiponectin (Adipoq), and TNFα (Tnfa), which were dysregulated in ob/ob adipose tissue compared with lean controls (Fig. 2C). Plasma adiponectin was also increased by all three TZDs (Table 1). MSDC-0602, rosiglitazone, and pioglitazone also reduced the expression of macrophage cell surface markers (Cd68 and F4/80) (Fig. 2C). Collectively, these data indicate that these TZDs are equally efficacious with similar pharmacology despite marked differences in the ability to bind and activate PPARγ.

Administration of MSDC-0602 Improves Insulin Sensitivity of DIO Mice—We also sought to evaluate the efficacy of MSDC-0602 endpoints germaine to insulin sensitivity in DIO mice, which are more relevant to human obesity. At the time of sacrifice, plasma glucose and insulin levels were significantly lower, and adiponectin was increased in obese mice treated with MSDC-0602 for 2 or 4 weeks compared with DIO mice not receiving the drug (Table 2). Hyperinsulenic-euglycemic clamp studies demonstrated that treatment of DIO mice with MSDC-0602 reduced basal plasma insulin concentration (Fig. 3A) and, under hyperinsulenic conditions, MSDC-0602 increased the glucose rate of infusion (Fig. 3B). MSDC-0602 also increased glucose uptake into gastrocnemius, adipose tissue, and heart (Fig. 3C). MSDC-0602 treatment enhanced the suppression of hepatic glucose production by insulin (Fig. 3D). These data indicate that MSDC-0602 treatment improves insulin sensitivity in multiple tissues including striated muscle, adipose tissue, and liver. The phosphorylation status of Ser-473 and Thr-308 residues of Akt/PKB in liver (Fig. 3E) and skeletal muscle (data not shown) of DIO mice after a bolus insulin injection was again markedly improved by either 2 or 4 weeks of MSDC-0602 administration. Consistent with this, the expression of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (Pck1) and glucose-6-phosphatase (G6pc), which is suppressed by insulin action and elevated in DIO liver, was reduced by treatment with MSDC-0602 (Fig. 3E).

MSDC-0602 Treatment Increases Mitochondrial Respiratory Rate and Leads to Reduced Expression of Genes Encoding Lipogenic Enzymes—Oxygen consumption rates by liver mitochondria supplied with succinate as a metabolic substrate were increased by 4 weeks of treatment with MSDC-0602 (Fig. 4A). This was observed under basal conditions as well as when respiration was maximally stimulated by ADP or uncoupled with a chemical uncoupler. These data are consistent with a mitochondrial site of action of MSDC-0602 and suggest that treatment with this novel TZD enhances the mitochondrial oxidative capacity in hepatocytes. Obesity is known to increase hepatic de novo lipogenesis, and quantitative RT-PCR demonstrated that several genes involved in glycolysis (Gk and Lpk) and fatty acid synthesis (Acly, Fasn, Me1, and Thrsp) were robustly induced in liver of untreated DIO mice compared with lean controls (Fig. 4B). Treatment with MSDC-0602 for either 2 or 4 weeks led to a significant reduction in the expression of each of these genes in DIO mice, and this was confirmed at the protein level for FAS (Fig. 4B). The expression of many of these genes is known to be regulated by SREBP-1 (Sreb1f) (35), the expression of which was up-regulated in liver of DIO mice compared with lean controls but down-regulated by administration of MSDC-0602 (Fig. 4B). As predicted by these changes in gene expression, rates of de novo fatty acid synthesis from [14C]acetate in isolated DIO hepatocytes were significantly suppressed by in vivo MSDC-0602 treatment (Fig. 4C). MSDC-0602 also significantly suppressed expression of enzymes involved in triglyceride synthesis (Lpin1 and Scd1) (Fig. 4D). Consistent with this, rates of oleate-stimulated TG synthesis in isolated hepatocytes were suppressed by in vivo MSDC-0602 (Fig. 4E). These data suggest that a molecular signature of MSDC-0602-mediated insulin sensitization is a down-regulation of lipogenic pathways regulated by SREBP-1.

Administration of rosiglitazone is known to activate the hepatic expression of PPARγ target genes in obese mice and...
can actually exacerbate hepatic steatosis (13, 14). MSDC-0602 did not affect the hepatic expression of various PPARγ genes that promote lipid storage including Fabp4, Cidec, Cd36, and Plin4 in DIO (Fig. 5A) or ob/ob (Fig. 5B) mice. In contrast, rosiglitazone increased the expression of Fabp4, Cidec, Cd36, and Plin4 in ob/ob liver, whereas pioglitazone also induced the expression of Cidec (Fig. 5B). Collectively, these data indicate that the PsTZD markedly improves measures of hepatic and systemic insulin sensitivity and does not lead to PPARγ activation in liver.

**MSDC-0602 Suppresses Gluconeogenesis and Lipogenesis in Primary Hepatocytes**—To determine whether MSDC-0602 might affect lipogenesis and gluconeogenesis directly, hepatocytes from drug-naïve DIO mice were isolated and treated for 18 h with MSDC-0602 or rosiglitazone. The expression of mRNA and protein of the lipogenic and gluconeogenic
enzymes that are induced in DIO hepatocytes was diminished by MSDC-0602 or rosiglitazone treatment (Fig. 6A). Consistent with these signaling and protein expression results, MSDC-0602 treatment of isolated hepatocytes reduced rates of glucose production (Fig. 6B) and de novo lipogenesis (data not shown). These data are consistent with a direct hepatic effect of MSDC-0602 on metabolic pathways that are dysregulated in insulin-resistant hepatocytes.

To determine the requirement for PPARγ in this response to TZDs, LS PPARγ/H9253/H11002/H11002 mice were generated and induced to become obese with high fat diet. The expression of PPARγ, which was increased in WT mice by DIO, was reduced by more than 99% in isolated LS-PPARγ/H9253/H11002/H11002 hepatocytes (Fig. 6A). Consistent with PPARγ-independent effects on insulin sensitivity, MSDC-0602 and rosiglitazone were equally effective at suppressing lipogenic and gluconeogenic enzyme expression (Fig.

| Parameter                  | Lean   | DIO control | DIO 2 wk MSDC | DIO 4 wk MSDC |
|----------------------------|--------|-------------|---------------|---------------|
| Weight (g)                 | 31.1 ± 0.45 | 43.9 ± 1.63* | 45.1 ± 1.83* | 42.7 ± 1.91* |
| Glucose (mg/dl)            | 149.6 ± 3.10 | 180.5 ± 5.32* | 145.2 ± 5.71 | 152.8 ± 6.17 |
| Insulin (ng/ml)            | 0.79 ± 0.09 | 4.34 ± 0.93* | 1.56 ± 0.36* | 1.61 ± 0.24* |
| TG (mg/dl)                 | 99.2 ± 19.6 | 146.5 ± 26.4 | 103.9 ± 21.9 | 122.2 ± 20.9 |
| Adiponectin (mg/dl)        | 7.3 ± 0.72 | 15.6 ± 1.54* | 22.5 ± 2.20* | 27.05 ± 1.60* |
| Liver TG (mg/g)            | 13.3 ± 1.2 | 121.8 ± 10.8* | 95.4 ± 21.6* | 109.7 ± 15.1* |

*p < 0.05 versus lean controls.

*p < 0.05 versus DIO control and lean controls.

**FIGURE 3.** MSDC-0602 treatment improves whole body and tissue insulin sensitivity in DIO mice. Hyperinsulinemic clamp studies performed after 3 weeks of treatment with MSDC-0602 in DIO mice demonstrate a significant improvement in plasma insulin concentration (A), glucose infusion rate (B), tissue glucose uptake (C), and hepatic glucose production (D). n = 11 per group, *, p < 0.05 versus DIO control diet mice. **, p < 0.05 versus clamped DIO control mice and basal values for MSDC-0602-treated mice. AUC, area under the curve. E, representative Western blots using liver lysates from mice injected with insulin (I) before sacrifice and the antibodies listed at the left are shown. The table inset represents the normalized and corrected (to total Akt) band intensity for insulin-stimulated Akt phosphorylation for n = 3 per group. Expression of the indicated genes in liver is depicted in the graphs. n = 8 per group. *, p < 0.05 versus lean and MSDC-0602-treated mice. **, p < 0.05 versus DIO control and lean control mice. AU, absorbance units.
A mitochondrial respiration was performed on isolated mitochondria and using succinate-rotenone as a substrate. *, p < 0.05 versus other groups mice. FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone. **, p < 0.05 versus other groups mice. *p < 0.05 versus lean mice. **, p < 0.05 versus lean mice. Inset, representative Western blots using liver lysates and the antibodies listed at the left are shown. The table represents the normalized and corrected (to actin) band intensity for FAS (n = 6 per group). AU, absorbance units. C, the graph depicts rates of fatty acid synthesis in hepatocytes. *, p < 0.05 versus lean mice. **, p < 0.05 versus DIO and lean mice. D, the graph depicts the hepatic expression of genes encoding enzymes involved in TG esterification. *, p < 0.05 versus lean mice. **, p < 0.05 versus DIO and lean mice. E, the graph depicts rates of triglyceride synthesis in hepatocytes. *, p < 0.05 versus lean mice. **, p < 0.05 versus DIO and lean mice.

FIGURE 4. Mitochondrial oxygen consumption is increased and dysregulated lipogenesis in DIO mice is corrected by MSDC-0602 treatment. A, mitochondrial respiration was performed on isolated mitochondria and using succinate-rotenone as a substrate. *, p < 0.05 versus other groups mice. FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone. B, the graph depicts the hepatic expression of genes encoding glycolytic and lipogenic enzymes. **, p < 0.05 versus lean mice. **, p < 0.05 versus DIO and lean mice. C, the graph depicts rates of fatty acid synthesis in hepatocytes. *, p < 0.05 versus lean mice. **, p < 0.05 versus DIO and lean mice.
Traditional drug discovery programs have been focused on the identification of direct, high affinity PPARγ agonists. However, of the two approved TZDs, pioglitazone is 10-fold less potent at activating PPARγ yet has equivalent or even superior therapeutic effects on hepatic steatosis, circulating lipid profile, and glycemia with fewer reported side effects (18, 36, 37). Mechanistic studies have demonstrated that many of the side effects of TZDs are mediated through ectopic activation of PPARγ. For example, mice lacking PPARγ in the collecting ducts of the kidney are protected from the plasma volume expansion and weight gain caused by rosiglitazone (11, 12). Activation of PPARγ is also the molecular trigger driving white adipose tissue expansion (9, 10). Finally, administration of high doses of rosiglitazone to obese mice is associated with an activation of PPARγ target gene expression profile in hepatocytes, which is abrogated by liver-specific deletion of PPARγ (this study and Refs. 13 and 14).

With these data in mind, we have designed TZD analogs with modifications to the carbon backbone to impair binding to PPARγ with the hypothesis that the anti-diabetic efficacy of TZDs can be separated from the PPARγ-activating functions to minimize the likelihood of side effects. The current studies reveal that the PsTZD, MSDC-0602, lowers blood glucose and insulin concentration, enhances hepatic and systemic measures of insulin sensitivity, and increases the circulating concentration of beneficial adipokines in obese rodents. These beneficial effects are likely translatable, as a Phase 2a clinical trial with a first generation PsTZD, MSDC-0160, demonstrated beneficial effects on glucose, insulin, circulating lipids, and blood pressure without causing weight gain and or blood volume expansion (38). These data indicate that a TZD with a PPARγ-sparing profile promotes systemic insulin sensitivity and has positive effects on adipocyte inflammation and hepatic lipid metabolism while potentially reducing other PPARγ-driven and dose-limiting side effects.

Recently, Choi et al. (39) have demonstrated that PPARγ activity can be modulated by TZDs that have reduced ability to interact with the ligand binding domain of PPARγ. These compounds instead bind PPARγ through other motifs to promote a conformational change that prevents phosphorylation by CDK5, a kinase that is activated by inflammatory signaling cascades in obesity and insulin resistance. Abrogating CDK5-mediated phosphorylation of PPARγ selectively enhanced its activity toward a subset of PPARγ target genes encoding important metabolic regulators, including adiponectin, that are believed to mediate anti-diabetic effects of TZDs. On the other hand, we have found that MSDC-0602 spares PPARγ binding altogether and thus likely does not affect CDK5-mediated phosphorylation of PPARγ. In support of this, the data obtained using LS-PPARγ knock-out mice indicate that PPARγ is dispensable altogether for the effects of MSDC-0602 on lipogenesis and gluconeogenesis.

If MSDC-0602 improves insulin sensitivity and hepatic metabolism independent of PPARγ phosphorylation or activation, what is the molecular mechanism of action of this compound? Our current hypothesis is that this pharmacology...
occurs after the direct interaction of the compound with a binding site in the mitochondrion. During the development of pioglitazone, an unbiased approach identified a mitochondrial binding site for [3H]pioglitazone (23). Initial work suggested that the mitochondrial target of pioglitazone was a protein termed mitoNEET (23), a protein of some interest as it contains a redox active iron-sulfur cluster and may be involved in regulating oxidative metabolism (40, 41). In unpublished studies, however, we have shown the binding of pioglitazone to mitochondria continues unabated in mitoNEET knock-out tissue. Experimentation to identify the protein(s) mediating the mitochondrial binding and to characterize its importance to the pharmacology of these novel agents is being conducted.

Regardless of the molecular target, the present data demonstrate that the effects MSDC-0602 on systemic insulin sensitivity and adipose tissue inflammation are comparable to equal doses of the two clinically effective TZDs. Hyperinsulinemic clamp studies detected strong effects of MSDC-0602 on insulin sensitivity in striated muscle, adipose tissue, and liver of DIO mice. The beneficial effects of MSDC-0602 treatment on skeletal muscle or liver could be indirect through the effects on systemic metabolism, as MSDC-0602 reduced circulating hyperinsulinemia and hyperglycemia, which are known to contribute to insulin resistance and activate hepatic lipogenesis (42). The increase in adiponectin concentration elicited by MSDC-0602 administration may also explain some of the observed suppression of hepatic lipogenesis and gluconeogenesis and increased skeletal muscle insulin sensitivity, as this adipokine is well established to play an important role in the insulin-sensitizing effects of TZDs (7, 43). Although generally

**FIGURE 6.** MSDC-0602 suppresses gluconeogenesis and lipogenesis in primary hepatocytes independent of PPARγ. A, the graphs depict the expression of the indicated genes in mouse hepatocytes from WT or LS-PPARγ−/− DIO mice treated in vitro with vehicle (DMSO), rosiglitazone (1 μM), or MSDC-0602 (1 μM) for 18 h. *, p < 0.05 versus DMSO control hepatocytes. Representative Western blots using lysates from mouse hepatocytes from WT or LS-PPARγ−/− DIO mice treated in vitro with vehicle (DMSO), rosiglitazone (1 μM), or MSDC-0602 (1 μM) for 18 h and the antibodies listed at the left are shown. The values below each image represent the normalized and corrected (to actin) band intensity for the blot and group above (n = 4 per group). B, the graph depicts rates of glucose release by hepatocytes isolated from DIO mice treated for 24 h with vehicle (DMSO) or MSDC-0602 (5 μM). *, p < 0.05 versus DMSO control hepatocytes. C, the graphs depict the expression of PPARγ target genes in hepatocytes from WT or LS-PPARγ−/− DIO mice treated in vitro with vehicle (DMSO), rosiglitazone (1 μM), or MSDC-0602 (1 μM) for 18 h. *, p < 0.05 versus DMSO control hepatocytes. AU, absorbance units.
described as a “PPARγ target gene,” several studies have shown that PPARγ does not mediate the induction of adiponectin expression during adipocyte differentiation and that other transcription factors control its expression (for review, see Ref. 44). Moreover, the regulation of adiponectin expression is not related to PPARγ affinity of the TZDs (45). There are a number of insulin sensitizing approaches unrelated to PPARγ biology that can normalize adiponectin expression in insulin-resistant rodents. Last, non-genomic control of adiponectin production by TZDs by regulation of its secretion may not require activation of PPARγ.

Our data also suggest a direct hepatic effect of MSDC-0602, as treatment of isolated hepatocytes suppressed the rates of gluconeogenesis and lipogenesis. This is consistent with previous work demonstrating a direct effect of TZDs on hepatic glucose production in isolated perfused livers, effects that were evident within 20 min of TZD infusion (20, 21). Importantly, the effects observed in this study were preserved in hepatocytes from LS-PPARγ−/− mice demonstrating that MSDC-0602 does not require PPARγ to elicit its beneficial pharmacology on hepatocytes. Interestingly, the strong PPARγ agonist rosiglitazone also suppressed hepatic gluconeogenic and lipogenic gene expression in PPARγ−/− hepatocytes, indicating that rosiglitazone, in addition to being a potent PPARγ agonist, also exhibits PPARγ-independent pharmacology. Whether the beneficial effects of TZDs can also be maintained in adipocytes completely lacking PPARγ cannot be determined due to the requirement for PPARγ in adipocyte differentiation. However, the present data show that MSDC-0602 is less adipogenic in differentiating 3T3-L1 adipocytes (Fig. 1D). These data demonstrate the potential utility of MSDC-0602 for treatment of insulin resistance and diabetes but more importantly provide the framework for the discovery and development of new insulin sensitizers that can operate independent of the activation of PPARγ.

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