A Novel Peroxisome Proliferator-Activated Gamma (PPARγ) Agonist, CLX-0921, Has Potent Antihyperglycemic Activity With Low Adipogenic Potential

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Agonists of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) are pharmacologically active antihyperglycemic agents that act by increasing peripheral tissue sensitivity to insulin. Many of these agonists have antihyperglycemic activity that is directly proportional to their ability to bind and activate PPARγ; however, recent data bring this relationship into question. In this report we describe a new PPARγ agonist, CLX-0921, that is derived from a natural product. This thiazolidinedione (TZD) has a spectrum of activity that differs from commercially available TZDs. It is a weak activator of PPARγ (EC_{50} of 0.284 μmol/L) compared to rosiglitazone (EC_{50} 0.009 μmol/L). Despite this difference, the drug maintains potent glucose uptake activity in vitro and glucose-lowering activity in vivo that is equipotent to that of rosiglitazone. Moreover, CLX-0921 showed a 10-fold reduction in in vitro adipogenic potential compared to rosiglitazone. CLX-0921 also increases glycogen synthesis, an activity not typically associated with rosiglitazone or pioglitazone. Thus CLX-0921 appears to have a distinct spectrum of activity relative to other TZDs.

EXTRACTS MADE from the bark of plants of the *Pterocarpus* genus have a long history of safety and clinical efficacy in the treatment of diabetes mellitus in the Indian Ayurvedic system of medicine.[1,2] More recent evidence has suggested that polyphenolic compounds isolated from these plants lower blood glucose concentrations in rodent models of type 2 diabetes,[3,4] and we have been able to reproduce these findings (S.M. and B.N., unpublished observations). In an effort to increase the antihyperglycemic potency of such polyphenols, a structurally related molecule (CLX-0901) has been synthesized that in preliminary experiments appeared to exert its antihyperglycemic effect by an interaction with the insulin receptor.[5] Based on this information, we explored the possibility that combining CLX-0901 with a thiazolidinedione (TZD) moiety might result in a compound that, by interacting with both the insulin receptor and peroxisome proliferator-activated receptor gamma (PPARγ), would ultimately prove to be an effective therapeutic agent to treat type 2 diabetes (P.N., D.D., S.M., M.G., J.C., S.D.S., and B.N., manuscript submitted). In this report we describe a novel TZD (CLX-0921, Fig 1), that is an orally active antihyperglycemic agent in several different rodent models of type 2 diabetes. Importantly, although the antihyperglycemic potency of CLX-0921 is comparable to that of other TZDs currently available for clinical use, the compound is a weaker activator of PPARγ and is much less potent in promoting adipogenesis.

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MATERIALS AND METHODS

Glucose Uptake

Basal glucose uptake was measured in differentiated 3T3-L1 adipocytes following the protocol of Tafuri[6] with modifications. Briefly, 3T3-L1 fibroblasts, obtained from ATCC (Manassas, VA), were differentiated to adipocytes by treating cells with porcine insulin (1 μg/mL for 4 days), dexamethasone (0.25 μmol/L for first 2 days), and isobutyl methyl xanthine (IBMX, 0.5 mmol/L for first 2 days) (all from Sigma Chemicals, St Louis, MO) following the protocol of Frost and Lane.[7] Differentiated adipocytes were incubated in Dulbecco's modified Eagle medium (DMEM) containing 10 % fetal bovine serum (GibcoBRL, Gaithersburg, MD) with various concentrations of CLX-0921 or vehicle (0.1% dimethyl sulfoxide [DMSO]) for 48 hours in 24-well plates, in triplicate. Cells were washed with phosphate-buffered saline ([PBS], 150 mmol/L NaCl, 1 mmol/L KH_2PO_4, 3 mmol/L Na_2HPO_4; pH 7.4) and incubated in glucose-free DMEM for 2 hours at 37°C. The cells were washed 3 times with Krebs Ringer phosphate buffer (KRP). Glucose uptake was initiated by addition of 0.25 μCi 2-^{14}C(U)-deoxy-D-glucose (300 μCi/mmol, American Radiolabeled Chemicals, St Louis, MO) per well and the cells incubated for 10 minutes at room temperature in the presence of 0.1 mmol cold 2-deoxy-d-glucose. Finally, the cells were washed 3 times with ice-cold PBS containing 10 mmol/L cold glucose, lysed with 0.5 % sodium dodecyl sulfate (SDS), and counted in a scintillation counter (Beckman LS6500, Fullerton, CA).

Glycogen Synthesis

Glycogen synthesis was measured as net conversion of ^{14}C-D-glucose to cellular glycogen in HepG2 cells as described by Ciaraldi et al.[8] Briefly, HepG2 cells (ATCC) in 6-well plates were treated with CLX-0921 or other compounds for 48 hours. They were washed with 10 mmol/L HEPES buffer (150 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $CaCl_2$, 2.5 mmol/L $CaCl_2$, 10 mmol/L HEPES; pH 7.4) containing 1% bovine serum albumin (BSA). Cells were incubated in the same buffer for 30 minutes prior to addition of 0.2 μCi/well of ^{14}C-D-glucose (5 mmol/L; final concentration, 10 μCi/mmol; American Radiolabeled Chemicals, St Louis, MO). After incubation for 2 hours at 37°C, the cells were washed with ice-cold PBS and solubilized with 1 mol/L KOH at 55°C. Converted glycogen was precipitated by ethanol after addition of 10 mmol/L carrier glycogen. The pellet was washed and resuspended in water, and an aliquot was counted in a scintillation counter (Beckman LS6500). Total protein was assayed and results were reported as cpm/mg of protein.

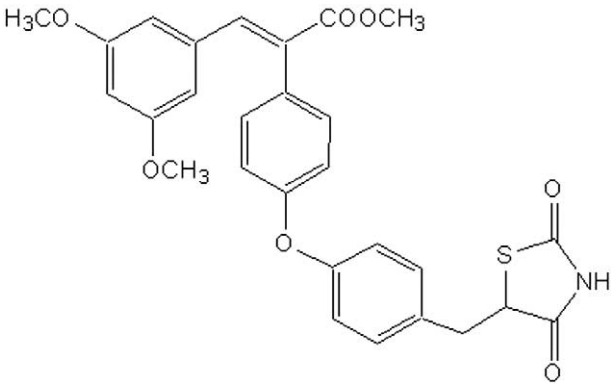

Fig 1. Chemical structure of CLX-0921.

Adipogenesis

Adipogenesis in 3T3-L1 fibroblasts was performed as described by Wu et al.[9] After 2 days of growth in 6-well plates, cells were treated either with vehicle (0.1% DMSO) or with compounds for 14 days. Fresh medium with compounds or vehicle was replenished every 48 hours. Cells were washed with PBS twice and fixed in 10% formalin (Sigma) in PBS. After washing in PBS, cells were stained with freshly diluted Oil Red O in isopropanol for 1 hour at room temperature. The cells were washed 5 times with PBS and visualized under an Olympus BH2 microscope (Melville, NY). Quantitative accumulation of triglyceride was also measured under similar experimental conditions, except in this case cells were plated in 100-mm tissue culture dishes and culture continued for 10 days. Triglyceride was extracted with methanol:chloroform (2:1) mixture. To monitor the efficiency of recovery, ^{3}H-cholesterol oleate (50,000 cpm/well, American Radiolabeled Chemicals) was added in each tube as tracer before extraction following the protocol of Brown et al.[10] Extracted triglyceride was measured by a colorimetric assay (GPO-Trinder, Sigma) according to manufacturer's instructions.

Transfection and Transactivation Assays

Human PPARγ2 expression vector was constructed by inserting the PPARγ2 cDNA coding region into the pcDNA3.1+ vector (Invitrogen, Carlsbad, CA). The PPRE-luciferase reporter gene was the kind gift of Dr Kenneth Feingold. The control vector, pRL-SV40, containing the Renilla luciferase cDNA was purchased from Promega (Madison, WI). About 2.7×10^4 HEK293 human embryonal kidney cells (ATCC) were plated into a 35-mm tissue culture dish and maintained in Eagle modified essential medium (EMEM, ATCC) containing 10% heat-inactivated horse serum for 24 hours. Expression, reporter (100 ng/dish), and control (2.5 ng/dish) vectors were transfected using LIPOFECTAMIN PLUS Reagent (GibcoBRL) according to manufacturer's recommendation. At 24 hours after transfection, cells were treated with vehicle (0.001% DMSO in medium) or compounds at the indicated concentration and incubated for 24 hours. Each treatment was conducted in triplicate. Each culture dish was assayed for firefly luciferase activity normalized by Renillla luciferase activity to account for differences in transfection efficiency. Luciferase activity was measured using the Dual-luciferase Reporter Assay System (Promega, Madison, WI) and a Sirius luminometer (Berthold Detection System, Pforzheim, Germany).

PPARγ Affinity by Radioligand Binding Assay

Ligand binding assays were performed using human recombinant PPARγ as previously described Berger et al.[11] Briefly, a GST-hPPARγ ligand binding domain chimeric cDNA construct was used to generate PPARγ in COS-1 cells. A radiolabled TZD ligand ([^{3}H-AD-5075], K_D ~1 nmol/L) was incubated with transfected COS-1 cell lysates in the presence or absence of test compounds (CLX-0921 or rosiglitazone). Unbound ligand was removed with dextran/charcoal and supernatant fractions were measured in a scintillation counter.

PPARγ Cofactor Protein Association

A homogeneous time-resolved fluorescence assay (HTRF) was used to examine the interaction of liganded PPARγ and the coactivator protein CBP as described elsewhere.[12] GST-PPARγ ligand binding domain (LBD), 2 nmol/L anti–GST-(Eu)K, 10 nmol/L biotin-$CBP_{1\text{-}453}$, 20 nmol/L SA/XL665 were added to each well, followed by addition of test compound or vehicle (Me_2SO) in individual wells. Plates were mixed by hand, covered and incubated overnight at 4°C. Fluorescence was measured on a Discovery instrument (Packard, Boston, MA). This assay measures the ligand dependent interaction of PPARγ and CBP through energy transfer from (Eu)K to SA/XL665.

In Vivo Studies

All procedures performed were in compliance with the Animal Welfare Act and US Department of Agriculture regulations and were

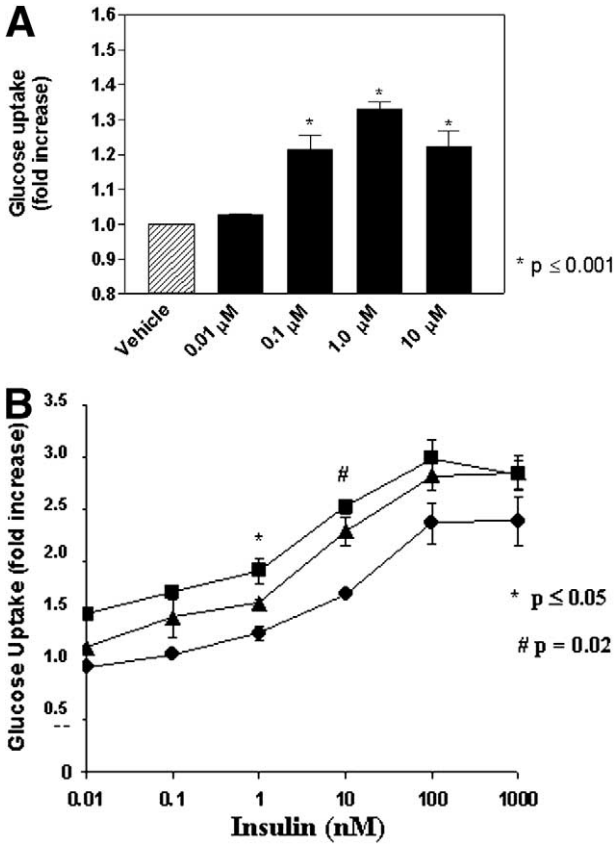

Fig 2. Glucose uptake in 3T3-L1 adipocytes. (A) In vitro glucose uptake was measured in differentiated 3T3-L1 adipocytes after 48-hour treatment with increasing concentrations of CLX-0921 (black bars) or 0.1% DMSO as vehicle (hatched bars). *$P \leq .001$. (B) Glucose uptake in differentiated 3T3-L1 adipocytes was measured in the presence of increasing concentrations of insulin pretreated with vehicle, rosiglitazone (5 μmol/L) or CLX-0921 (5 μmol/L). *$P \leq .05$, #$P = .02$ *v* vehicle. Vehicle (●), CLX-0921 (■), rosiglitazone (▲).

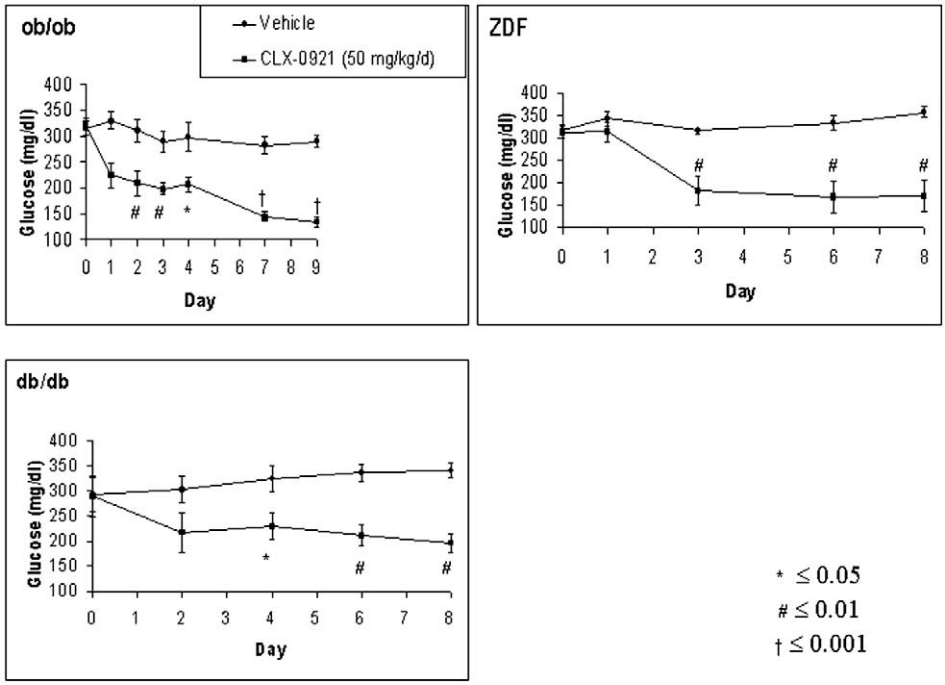

Fig 3. In vivo antihyperglycemic activity of CLX-0921 in diabetic animals. Diabetic *ob/ob* and *db/db* mice and diabetic ZDF rats were treated with single daily doses of CLX-0921 (50 mg/kg = 96.2 μmol/kg) or vehicle (0.5% carboxymethyl cellulose) by oral gavage for 8 or 9 days. Blood glucose measurements were made in the fed state. *$P \leq .05$, #$P \leq .01$, †$P \leq .001$. Vehicle (●), CLX-0921 (▲).

approved by the Calyx Therapeutics Institutional Animal Care and Use Committee. Animals were housed at 22°C and 50% relative humidity, with a 12-hour light and dark cycle, and received a regular rodent diet (Harlan Teklad, Madison, WI) ad libitum with free access to water. Male C57BL/KsJ-*db/db* and C57BL/6J-*ob/ob* mice were obtained from Jackson Laboratories (Bar Harbor, ME) at age 5 weeks. Seven-week-old animals (6 animals per group) were dosed with CLX-0921, rosiglitazone (purified from commercially available tablets), or vehicle (0.5% carboxymethyl cellulose [Sigma] in water) orally once daily by gavage. Blood glucose measurements were made with a One Touch Glucose Meter (Life Scan, Milpitas, CA) and/or a glucose oxidase assay (Glucose Trinder, Sigma) prior to administering the next dose and in the fed state. Body weights were monitored throughout the study. Eight-week-old male Zucker diabetic fatty (ZDF-*fa/fa*) rats (Genetic Models, Indianapolis, IN) were kept on a 6.5% fat Formulab Diet 5008 (PMI Feeds, Richmond, IN) for 2 weeks prior to dosing as described above.

Statistical Analysis

Data are presented as the mean ± SE and statistical comparisons were made by *t* test or analysis of variance (ANOVA) with Tukey/Kramer post hoc testing where appropriate using StatView 5 software (SAS Institute, Cary, NC)

RESULTS

CLX-0921 Stimulates Glucose Uptake In Vitro

Differentiating 3T3-L1 adipocytes represent an insulin-sensitive cell-culture model for studying glucose uptake and is often used to characterize potential antidiabetic compounds. Although TZDs increase glucose uptake in these cells, both in the absence and presence of insulin,[6,13] the majority of this effect appears to be the result of non–insulin-mediated glucose disposal. As shown in Fig 2A, glucose uptake was increased to a maximum of 1.33 ± 0.02 (mean ± SE) fold over basal levels in response to increasing concentrations of CLX-0921 (0.01, 0.1, 1.0, and 10 μmol/L). We also examined the effect of CLX-0921 and rosiglitazone on insulin-stimulated glucose uptake in 3T3-L1 adipocytes (Fig 2B). There was no difference in dose-response curves of glucose uptake in response to insulin in the presence (5 μmol/L) or absence of the TZDs. Differences in the maximal responses can be accounted for by the increased amount of basal glucose uptake in the absence of insulin and either CLX-0921 or rosiglitazone, indicating an additive, not synergistic, effect on glucose uptake. These results suggest this enhancement of glucose uptake is mediated through a non–insulin-dependent mechanism, such as an increase in GLUT-1 transporters.[6,14]

In Vivo Antihyperglycemic Effect of CLX-0921

The antihyperglycemic activity of CLX-0921 was examined in several models of type 2 diabetes mellitus. Figure 3 summarizes the effect of CLX-0921 given as single daily oral doses of 50 mg/kg (96.2 μmol/kg) over 8 to 9 days. At the end of each study the drug led to marked decreases in blood glucose levels in *ob/ob* mice (59% *v* baseline), *db/db* mice (32% *v* baseline), and ZDF rats (50% *v* baseline). In *ob/ob* mice, CLX-0921 led to a 58% decrease in insulin levels after the 9-day dosing period compared to control (7.3 ± 1.1 *v* 17.6 ± 0.6 ng/mL, respectively, $P < .001$). Serum triglycerides and free fatty acids (FFA) were also lower in CLX-0921–treated *ob/ob* mice compared to controls by 64% (CLX-0921, 51.2 ± 6.7; control, 143 ± 7.4 mg/dL, $P < .001$) and 34% (CLX-0921, 1.75 ± 0.29; control, 2.64 ± 0.12 mg/dL, $P < .05$), respectively. Weight gain in treated and control animals was similar except for ZDF rats, where CLX-0921–treated animals gained 13% more weight than control animals.

To examine the dose-response relationship between blood

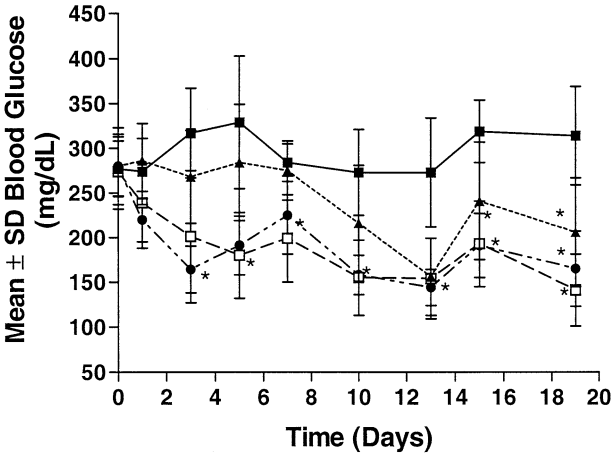

Fig 4. In vivo dose-dependent antihyperglycemic activity of CLX-0921 in *ob/ob* mice. Diabetic *ob/ob* mice were treated once daily, by oral gavage, with either vehicle (0.5 % carboxymethyl cellulose) or different doses of CLX-0921 (3.1, 6.3, and 12.5 mg/kg body weight) for 19 days and blood glucose was monitored in alternate days. Vehicle (■), CLX-0921 3.1 mg/kg (▲), 6.3 mg/kg (□), and 12.5 mg/kg (●).

glucose and CLX-0921, *ob/ob* mice were treated once daily with vehichle or 3 different doses of CLX-0921 (3.1, 6.3, and 12.5 mg/kg body weight by oral gavage; n = 8 per group). As shown in Fig 4, there was a dose dependent decrease in blood glucose levels that was most apparent during the first week of treatment. Compared to the vehicle group, mean blood glucose levels decreased by 35%, 55%, and 47%, for the 3.1-, 6.3-, and 12.5-mg/kg dose groups, respectively, at day 19 ($P \leq .01$, .001, and .001, respectively). In a subsequent study, the in vivo potency of CLX-0921 was compared to rosiglitazone in *ob/ob* mice (Fig 5). CLX-0921 and rosiglitazone treatment (both at 10 mg/kg/d; 19.2 and 28.0 μmol/kg/d for CLX-0921 and rosiglitazone, respectively) demonstrated similar antidiabetic potency over the 8-day treatment period. Weight gain was similar in vehicle and drug-treated groups.

CLX-0921 Is Less Adipogenic Than Rosiglitazone

Because TZDs are ligands for PPARγ and induce adipocyte differentiation,[15-17] we sought to determine the adipogenic potential of CLX-0921 using the 3T3-L1 preadipocyte model. In these studies, 3T3-L1 fibroblasts were incubated with various concentrations (0.1, 1, 10 μmol/L) of CLX-0921, rosiglitazone, or vehicle in the absence of dexamethasone, insulin, and IBMX and on day ten cells were lysed and assayed for triglyceride accumulation. As shown in Fig 6A there was a dose-dependent increase in triglyceride accumulation in response to CLX-0921 and rosiglitazone. The dose response of triglyceride accumulation in response to CLX-0921 is right-shifted in comparison to rosiglitazone. In 6-well plates, after 14 days of treatment, cells were stained with Oil Red O, and counterstained with methylene blue for visual assessment (Fig 6B).

Moreover, the maximal amount of triglyceride accumulated in response to CLX-0921 was significantly less than that seen in response to rosiglitazone (3.96- *v* 9.22-fold increase over control, respectively; $P < .0001$, ANOVA). CLX-0921 at concentrations of 100 μmol/L and higher was cytotoxic in this system (data not shown).

CLX-0921 Is a Weak Agonist of PPARγ

We examined the ability of rosiglitazone and CLX-0921 to transactivate a PPRE-Luc reporter gene in HEK293 cells co-transfected with a human PPARγ2 expression vector. CLX-0921 was a substantially less potent activator of PPARγ than rosiglitazone (Fig 7). The EC_{50} for transactivation in this system was 0.009 ± 0.0007 μmol/L for rosiglitazone, and 0.284 ± 0.036 μmol/L for CLX-0921 (n = 5). Similar dose-response curves were obtained using a reporter-gene assay in cells transfected with heterologous cDNA constructs of GAL4-DNA binding domain/PPARγ−ligand binding domain and 5X upstream activator sequence (UAS)-luciferase constructs (data not shown). The comparative data of rosiglitazone and CLX-0921 on PPARγ- binding, CBP cofactor association, and transactivation are shown in Table 1. CLX-0921 was 9.6 fold less potent in CBP recruitment relative to rosiglitazone, which is

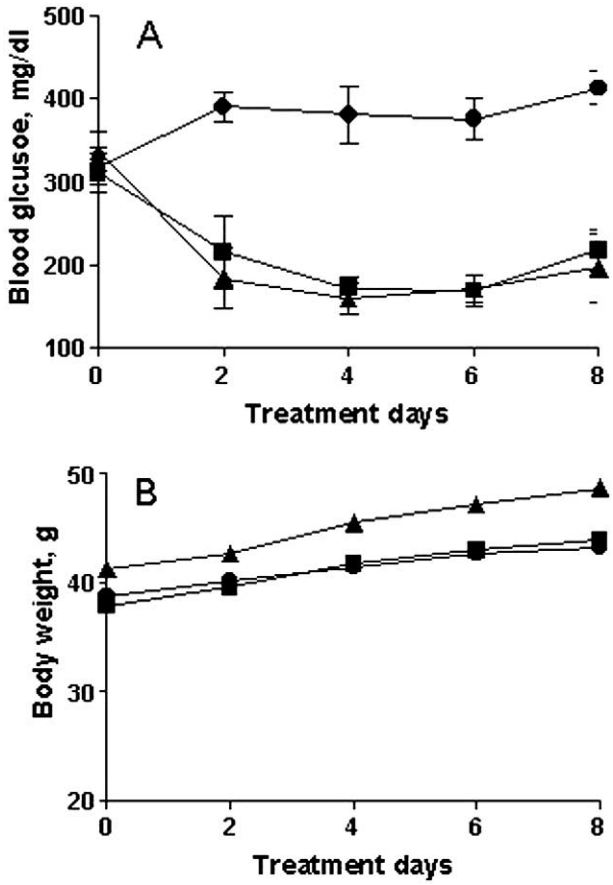

Fig 5. In vivo antihyperglycemic activity of CLX-0921 *v* rosiglitazone in *ob/ob* mice. Diabetic *ob/ob* mice were treated with single daily doses of CLX-0921 and rosiglitazone at 10 mg/kg (19.2 and 28.0 μmol/kg, respectively) or vehicle (0.5% carboxymethyl cellulose) by oral gavage. Blood glucose (A) and body weight (B) were measured during the 8-day treatment period. Vehicle (●), CLX-0921 (■), rosiglitazone (▲).

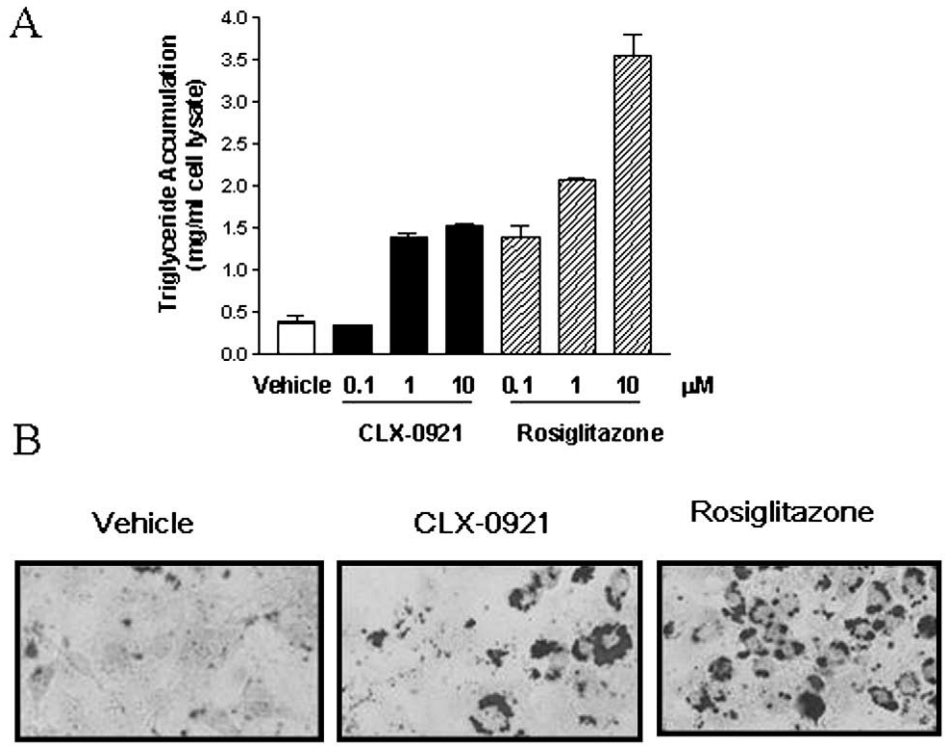

Fig 6. In vitro adipogenic activity of CLX-0921. 3T3-L1 cells were cultured with vehicle, CLX-0921 or rosiglitazone for 10 days. Total accumulated triglyceride was measured. (A) Quantitative measurement of accumulated triglyceride after 10 days of treatment with increasing concentrations of CLX-0921 (black bars), rosiglitazone (hatched bars), or vehicle (white bar). (B) Qualitative assessment of triglyceride accumulation by Oil Red O after 14-day treatment with 1 μmol/L CLX-0921, rosiglitazone or vehicle.

similar to the differences in PPARγ affinity for these 2 compounds (Table 1).

CLX-0921 Increases Glycogen Synthesis in HepG2 Hepatocytes

Previous reports suggest that hepatic glycogen levels are not affected by TZDs,[18,19] although glycogen levels in muscle may be decreased by these agents despite increased glycogen synthesis.[18] Based on the preliminary studies that indicate the parent compound CLX-0901 interacts with the insulin receptor,[5,20] and the observation that the potency of CLX-0921 at PPARγ activation is less than that of rosiglitazone despite their having equipotent in vivo glucose-lowering activity, we examined the ability of CLX-0921 to increase glycogen synthesis in HepG2 hepatocytes. Figure 8A shows that there is a dose-dependent increase in ^{14}C-glucose incorporated into glycogen in response to CLX-0921 in the absence of insulin; this response was maximal (3.1-fold increase over baseline) at 48 to 72 hours (Fig 8B). In contrast, rosiglitazone did not increase glycogen synthesis (0.9-fold decrease at 10 μmol/L, Fig 8A).

In separate experiments, concentrations of rosiglitazone higher than 30 μmol/L produced only minimal increases in glycogen synthesis (1.4 ± 0.06 SD, fold increase over baseline, data not shown). The increase in glycogen synthesis induced by CLX-0921 was dependent on new protein synthesis as it was blocked by cotreatment with cycloheximide (Fig 8C).

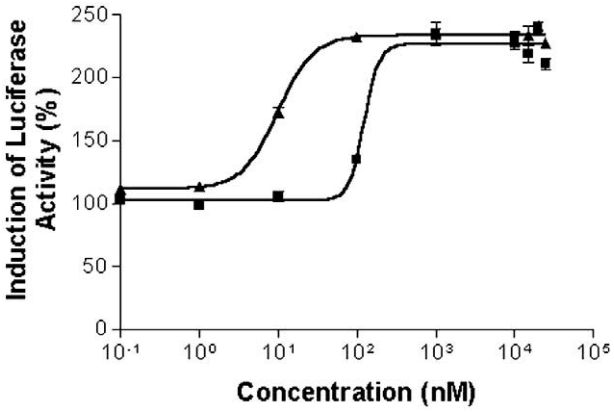

Fig 7. Induction of PPARγ mediated transactivation of PPRE-Luc reporter by CLX-0921 and rosiglitazone. HEK293 cells were transiently cotransfected with a PPARγ expression vector and a PPRE-Luc reporter construct. Cells were also transfected with a cDNA construct containing Renilla luciferase, which was used to control for transfection efficiency. Transfected cells were treated with increasing concentrations of CLX-0921 (■) or rosiglitazone (▲). Luciferase activity is expressed as enhancement over basal levels (no drug) and is corrected for transfection efficiency.

DISCUSSION

Our results indicate that CLX-0921 is effective in lowering blood glucose in several animal models of type 2 diabetes. It

Table 1. Comparative Data on PPARγ Affinity of Rosiglitazone and CLX-0921

Compound	Binding IC_{50} (μmol/L)	Cofactor Association EC_{50} (μmol/L)	Transactivation (μmol/L)
Rosiglitazone	0.237	0.059	0.009
CLX-0921	1.54	0.564	0.284

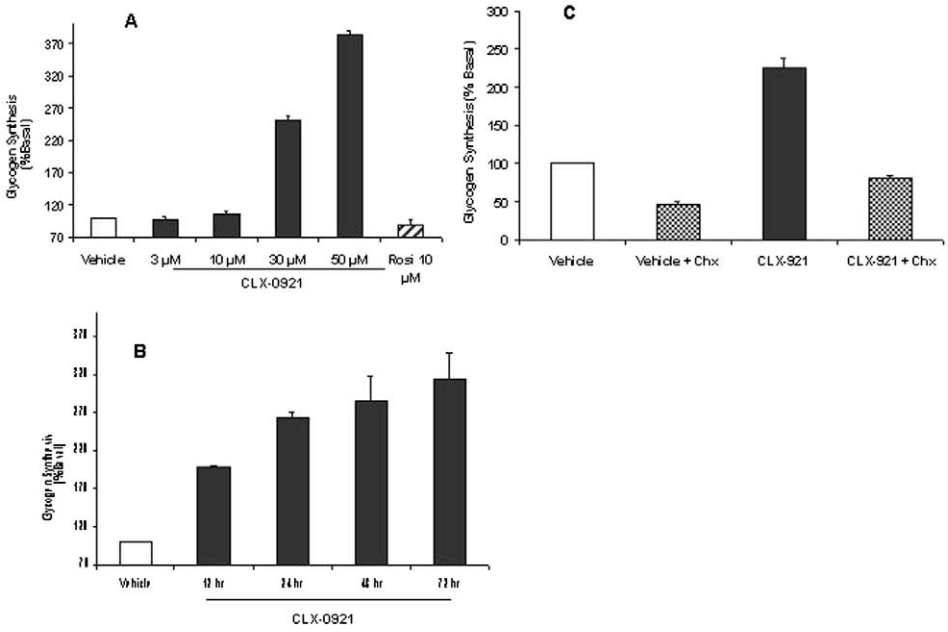

Fig 8. Effect of CLX-0921 on in vitro glycogen synthesis in HepG2 cells. (A) Dose-dependent stimulation of glycogen synthesis from glucose by CLX-0921 in the absence of insulin at 48 h. Stimulation of glycogen synthesis is expressed as a percentage of basal (vehicle), which is defined as 100%. (B) Time-dependent increase in CLX-0921–stimulated glycogen synthesis. The maximal effect occurs at 48 to 72 h of treatment. (C) Cycloheximide blocks glycogen synthesis induced by CLX-0921 (30 μmol/L, 48 h). Vehicle (white bars), CLX-0921 (black bars), rosiglitazone (hatched bars), CHX (cycloheximide, checked bars); rosi, rosiglitazone.

has a robust antihyperglycemic effect in ZDF rats, where it normalizes glucose levels. CLX-0921 demonstrated a steep dose-response effect on lowering blood glucose in diabetic rodents with a maximal effect at 6.3 mg/kg/d. In a side-by-side comparative study with rosiglitazone in *ob/ob* mice with a single daily oral doses, CLX-0921 appears to be equally efficacious to rosiglitazone on a mass basis. In actuality, on a mole-per-mole basis; the molecular weight of rosiglitazone is substantially less than CLX-0921 (357 *v* 520 g/mol), yet the 2 drugs produced the same degree of glucose-lowering in *ob/ob* mice. Because we did not measure drug levels, we cannot rule out differences in metabolic profiles between CLX-0921 and other TZDs.

Our results also indicate that CLX-0921 is substantially less adipogenic than rosiglitazone. This effect likely reflects the lower affinity of CLX-0921 for PPARγ in comparison to rosiglitazone. We did detect a small increase in weight gain in ZDF-rats treated with CLX-0921, and we were unable to detect differences in weight gain between rosiglitazone and CLX-0921 treated animals in this short-term study. These data are difficult to interpret because the studies were conducted in genetically obese animals, which may have obscured a differential effect of these drugs on weight gain. Several studies in normal animals dosed with CLX-0921, up to 1 month in duration, have failed to demonstrate statistically significant changes in weight gain compared to vehicle (data not shown).

It is widely held that the weight gain associated with TZDs is partly due to their adipogenic potential, and there has been much effort directed at finding compounds which are potent PPARγ activators but do not cause weight gain. Because CLX-0921 has less adipogenic activity, but maintains antihyperglycemic activity, it appears that it may be possible to develop pharmacologic PPARγ activators that produce less weight gain than current commercially available PPARγ activators. In addition to the contribution of adipogenesis, edema is an important factor in the weight gain associated with PPARγ agonists. This toxicity is believed to be directly related to the PPARγ activation potency of the molecule. Because CLX-0921 is a weak agonist of PPARγ, it may be associated with less edema. Clinical studies underway will help define the effect of CLX-0921 on body weight in humans.

The affinity (*Ki*) of PPARγ for CLX-0921 was 6.5-fold less than its affinity for rosiglitazone (Table1). The transactivation potency was as much as 30-fold less than that of rosiglitazone. In general, there is a relatively strong correlation between PPARγ affinity and glucose-lowering activity[11]; however, recent data indicate that this relationship may not be true for all ligands of this receptor.[13,21,22] Recently a non-TZD PPARγ activator, FMOC-l-Leucine, has been shown to have a similar profile to CLX-0921. FMOC-l-Leucine has approximately 400-fold less affinity for PPARγ, is only weakly adipogenic, but has potent in vivo antihyperglycemic activity.[22] Differences in the in vivo metabolism may explain part of the apparent discrepancy between PPARγ affinity and in vivo antidiabetic potency for CLX-0921 and other ligands. Studies by Reginato et al,[21] Mukherjee et al,[13] and Rocchi et al[22] indicate that ligand-mediated recruitment of the coactivator SRC-1 to PPARγ is an important determinant for differential activities of ligands. At present, we can only speculate on the way in which CLX-0921 influences coactivator recruitment to the PPARγ-RXR complex. It may be that CLX-0921 is less conducive for SRC-1 or PGC-1[23] recruitment and, as a result, transcriptional activation. A recent study[14] reported that in vitro glucose uptake into adipocytes is partially independent of PPARγ. Whether non–PPARγ-mediated activities or coactivator recruitment explains the unique properties of CLX-0921 will require further investigation.

Presently, the mechanism by which PPARγ ligands, including TZDs, produce their antihyperglycemic effects is not known. The prevailing wisdom suggests that the glucose-low-

ering effect of these drugs is mediated through the PPARγ receptor, which enhances insulin sensitivity. Recent data suggest that the relationship between PPARγ, its ligands, and insulin sensitivity is more complex. For example, heterozygous PPARγ null mice actually demonstrate increased insulin sensitivity, and the insulin-sensitizing effect of synthetic ligands may result from a balance between transcriptional activation and repression.[24] Abrogating endogenous PPARγ does not result in the elimination of TZD activity,[14,25] which further highlights the complexity of the mechanism of action of these drugs. At present, a non–receptor-mediated mechanism of action for CLX-0921 and other PPARγ agonists cannot be excluded. However, we have been unable to demonstrate any insulin receptor-mediated activity of CLX-0921, which was expected based on the compound's structural derivation from CLX-0901. Our data indicate that CLX-0921, in contrast to rosiglitazone, increases glycogen synthesis in liver cells, possibly providing an added mechanism for lowering glucose levels in diabetic animals. Recent data suggest that some non-TZD PPARγ agonists may upregulate genes involved in glycogen synthesis.[26] It is becoming apparent that ligands for this receptor will have a spectrum of affinities, transcriptional activities, and in vivo pharmacodynamic profiles.[13,21,27] Therefore, there is substantial clinical value in generating compounds with selective PPARγ modulating activities. The acronym SPPRM, as coined by John Auwerx's group for "selective PPAR modulator,"[13,22] may best describe these molecules. SPPRMs may ultimately prove to have both specific and tailored activities, including the potential avoidance of weight gain associated with currently marketed TZDs. Such agents would have the potential to be of great benefit in treating patients with type 2 diabetes.

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