The Novel Hypoglycemic Agent YM440 Normalizes Hyperglycemia Without Changing Body Fat Weight in Diabetic db/db Mice

Akiyoshi Shimaya, Eiji Kurosaki, Ryosuke Nakano, Reiko Hirayama, Masayuki Shibasaki, and Hisataka Shikama

To determine the relationship between hypoglycemic activity and body weight gain induced by insulin sensitizers, we compared the effects of thiazolidinedione analogs (troglitazone and pioglitazone) and the oxadiazolidinedione analog (Z)-1,4-bis[4-[(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)methyl]phenoxy]but-2-ene (YM440) in diabetic db/db mice. Oral treatment with YM440 (100 mg/kg) for 28 days decreased the blood glucose concentration (control *v* YM440, 418 ± 12 *v* 243 ± 44 mg/dL). The hypoglycemic activity of this agent was comparable to that of troglitazone (300 mg/kg) and pioglitazone (100 mg/kg). There were no changes in food intake among the groups. Troglitazone and pioglitazone, but not YM440, significantly increased body weight gain during treatment (control, 7.2 ± 0.5 g; YM440, 7.5 ± 0.8 g; troglitazone, 10.9 ± 0.8 g; and pioglitazone, 14.5 ± 1.1 g). To further assess whether the increase in body weight by troglitazone or pioglitazone was due to adipogenesis, the weight of intraabdominal fat tissue (epididymal, retroperitoneal, and perirenal) was determined. There were no differences in the total weight of visceral fat between the control and YM440 treatment (3.53 ± 0.23 and 3.60 ± 0.16 g). In contrast, troglitazone and pioglitazone significantly increased the fat weight (4.31 ± 0.13 and 4.66 ± 0.19 g). Thiazolidinediones are known as ligands for peroxisome proliferator–activated receptor γ (PPARγ), a nuclear receptor responsible for adipogenesis. Troglitazone and pioglitazone activated PPARγ and increased triglyceride accumulation and mRNA expression of fatty acid–binding protein (FABP) in 3T3-L1 cells. However, YM440 had no effect on these indices for adipocyte differentiation. These results suggest that the mechanism is different for the hypoglycemic action of YM440 versus the thiazolidinediones. YM440 ameliorates hyperglycemia without changing PPARγ activity, adipocyte differentiation, or fat weight. Thus, YM440 could be a useful hypoglycemic agent for the treatment of non–insulin-dependent diabetes mellitus (NIDDM) without affecting body weight.

INSULIN AND SULFONYLUREAS have been used in the management of hyperglycemia in patients with non–insulin-dependent diabetes mellitus (NIDDM), but both agents are associated with side effects including hypoglycemia and weight gain.[1] Recently, a new family of antidiabetic agents, the thiazolidinediones, has attracted considerable attention. One member of this family, troglitazone, has been shown to have potent effects to reduce hyperglycemia and hyperinsulinemia in diabetic patients and diabetic animals with insulin resistance.[2,3] However, this agent has also been reported to increase the mean weight gain in patients with NIDDM.[4] The mean weight gain observed in patients receiving troglitazone at a dose of 200 or 600 mg was 1.9 and 3.6 kg for 26 weeks, respectively, whereas the placebo group had a mean weight gain of 1.5 kg. These findings suggested that the activation of insulin action is normally accompanied by weight gain. However, the relationship between the improvement of insulin resistance and weight gain induced by insulin sensitizers remains to be elucidated, because metformin was shown to improve insulin sensitivity without increasing body weight gain in both animal models and clinical studies.[5,6] Since obesity is one of the recognized risk factors for development of NIDDM, the management of obesity is important in the treatment of diabetes.[7,8] Some thiazolidinediones activate the nuclear receptor peroxisome proliferator–activated receptor (PPARγ), which induces the differentiation of preadipocytes to adipocytes.[9-11] These effects may be attributable to adipogenesis and weight gain in rats and mice treated with thiazolidinedione insulin sensitizers.

In this study, we compared two different types of insulin sensitizers, ie, troglitazone, pioglitazone, and the oxadiazolidinedione analog (Z)-1,4-bis[4-[(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)methyl]phenoxy]but-2-ene (YM440), with respect to their potency for improving hyperglycemia and body weight gain in diabetic db/db mice. We also examined the relationship between body weight gain and adipogenesis. To investigate the potential mechanism of action of YM440, we examined its effects on the ligand activity of PPARγ and adipocyte differentiation in cultured cells.

MATERIALS AND METHODS

Materials

All reagents used in the study were of analytical grade and were obtained commercially. YM440, troglitazone, and pioglitazone hydrochloride (pioglitazone) were synthesized at Yamanouchi Pharmaceutical (Tokyo, Japan) (Fig 1).

Animals and Experimental Design

Male C57BL/KsJ-db/db and db/+ mice were purchased from Charles River Japan (Tokyo, Japan) and maintained under a 12-hour light/dark cycle. The animals were fed a moderately high-calorie laboratory chow (CMF, 373 kcal/100 g; Oriental Yeast Industry, Tokyo, Japan). YM440 (30, 100, and 300 mg/kg), troglitazone (30, 100, and 300 mg/kg), and pioglitazone (10, 30, and 100 mg/kg) were administered orally to db/db mice at 8 weeks of age for 28 days, and blood samples were collected from the tip of the tail vein 16 hours after the last dosing. Glucose concentrations were determined by the glucose oxidase method[12,13] on days 0, 4, 14, 22, 29, 31, and 36. The mice were killed and the weight of intraabdominal fat tissue (epididymal, retroperitoneal, and perirenal) was determined 16 hours after the last dosing.

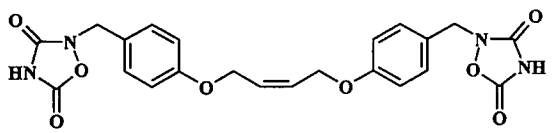
From the Division of Metabolic Diseases Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical, Tsukuba, Ibaraki, Japan.

Submitted June 14, 1999; accepted August 25, 1999.

Address reprint requests to Hisataka Shikama, PhD, Metabolic Diseases Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical, 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan.

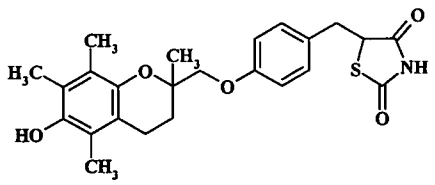
0026-0495/00/4903-0022$10.00/0

Oxadiazolidinedione analogue

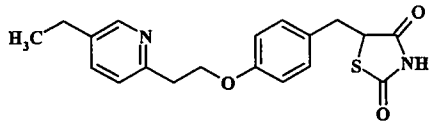


YM440

Thiazolidinedione analogues



Troglitazone



Pioglitazone

Fig 1. Chemical structure of YM440, troglitazone, and pioglitazone.

Analysis of Cell Differentiation and Triglyceride Content in 3T3-L1 Cells

3T3-L1 preadipocytes (American Type Cell Collection, Rockville, MD) were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO^2 in air.[10] To evaluate the effects of test compounds on the differentiation of 3T3-L1 preadipocytes to adipocytes, confluent 3T3-L1 preadipocytes in 6-well culture plates were incubated in the presence of 0.5 mmol/L isobutylmethylxanthine and 0.25 μmol/L dexamethasone for 48 hours and then with various concentrations of the test compound and 0.01 μg/mL insulin for a further 96 hours. The triglyceride concentration in the cells was quantified using colorimetric assay kits (Wako Pure Chemicals, Osaka, Japan) after solubilization in 0.1% sodium dodecyl sulfate.

RNA Extraction and Hybridization

The expression levels of mRNAs encoding fatty acid–binding protein (FABP), a marker of adipose differentiation,[14] and β-actin in 3T3-L1 cells were determined as follows. Briefly, total RNA was extracted from the cells using Isogen, a mixture of acid guanidium isothiocyanate: phenol:chloroform, according to the manufacturer's instructions (Nippon Gene, Toyama, Japan). Extracted RNA (10 μg) was hybridized to [α-^{32}P]uridine triphosphate–labeled antisense riboprobe at 50°C overnight. ^{32}P-labeled antisense riboprobes were prepared as described elsewhere.[15] Samples were digested with RNase A/T1 according to the manufacturer's instructions (Ambion, Austin, TX) at 37°C for 1 hour and analyzed by electrophoresis on a 3.5% polyacrylamide gel including 8 mol/L urea. The bands were detected with a BAS2000 bioimaging analyzer (Fuji Film, Tokyo, Japan). The length of the protected fragments of FABP and β-actin was 150 (bp 192 to 342, Accession No. M23384) and 135 (bp 675 to 809, Accession No. X03672) nucleotides, respectively. The signal for FABP was normalized against that for β-actin.

PPARγ Activation Assay

The following two plasmids were prepared and transfected into CV-1 monkey kidney cells.[16] The GAL4-PPARγ receptor expression vector contained the GAL4 DNA binding domain and murine PPARγ ligand binding domain[17] in the pSG5 expression vector (Stratagene, La Jolla, CA). The reporter plasmid 17m2-G-CAT contained two GAL4 17M binding sites and the β-globin promoter region upstream of the chloramphenicol acetyltransferase (CAT) gene.[18] CV-1 cells were plated at 1.5 × 10^5/well in 12-well plates in DMEM containing 10% FBS. After 24 hours, the cells were transfected using LipofectAMINE reagent (GIBCO-BRL, Gaithersburg, MD). The transfection mixes contained 200 ng reporter plasmid, 20 ng receptor expression vector, 400 ng β-galactosidase expression vector pCH110 (Pharmacia, Uppsala, Sweden) as an internal control, and 400 ng carrier plasmid. Following transfection, the cells were incubated for 48 hours with the test agent. Cell extracts were prepared and the activities of CAT and β-galactosidase were determined.

Statistical Analysis

Comparisons between experimental groups were made using 1-way ANOVA, followed by Dunnett's multiple-range test. Differences were considered significant at a P level less than .05.

RESULTS

Effects of YM440, Troglitazone, and Pioglitazone on Blood Glucose, Body Weight, and Intraabdominal Fat Weight in Diabetic db/db Mice

YM440, troglitazone, and pioglitazone decreased blood glucose (Fig 2). The hypoglycemic activity of YM440 (100 mg/kg) was comparable to that of troglitazone (300 mg/kg) and pioglitazone (100 mg/kg). The hypoglycemic effect of YM440 was still observed 7 days after cessation of treatment, but the other two agents had no effect on glucose levels at this time point. Although food intake was not significantly different among the groups (Table 1), pioglitazone significantly increased the body weight and body weight gain (Fig 3). Troglitazone (300 mg/kg) also increased the body weight gain, but YM440 (up to 300 mg/kg) had no significant effect on these parameters. Troglitazone and pioglitazone, but not YM440, increased the weight of intraabdominal fat (Table 1).

Effects of YM440, Troglitazone, and Pioglitazone on PPARγ Reporter Activity

Troglitazone and pioglitazone can serve as ligands of PPARγ, one of the important nuclear receptors for adipogenesis. These two agents dose-dependently increased the transcriptional activity mediated through PPARγ in CV-1 cells expressing the PPARγ reporter gene (Fig 4). However, YM440 did not increase the transcriptional activity, suggesting that YM440 is not a ligand of PPARγ.

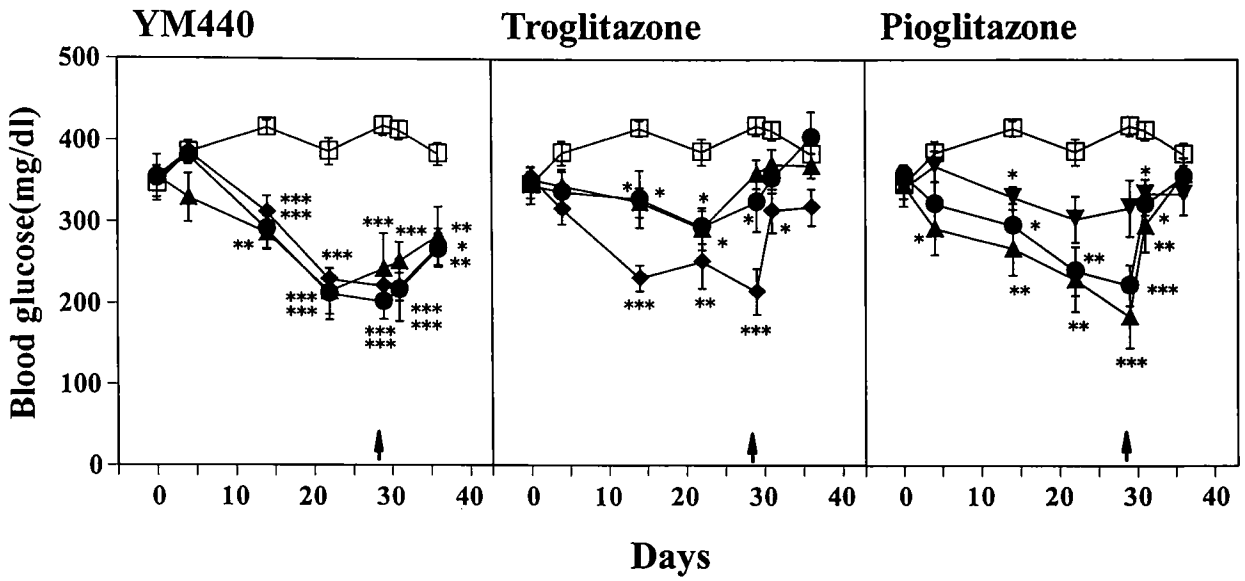


Fig 2. Effects of YM440, troglitazone, and pioglitazone on blood glucose in diabetic db/db mice. Male db/db mice were treated with YM440, troglitazone (30, 100, and 300 mg/kg), or pioglitazone (10, 30, and 100 mg/kg) orally once per day for 28 days: □, 0 mg/kg; ▼, 10; ●, 30; ▲, 100; ◆, 300. Hypoglycemic activity was measured 16 hours after the last dosing. Arrows indicate the end of treatment. Data are the mean ± SEM (n = 6). *$P < .05$, **$P < .01$, ***$P < .001$ *v* untreated control at the same time point.

Effects of YM440, Troglitazone, and Pioglitazone on FABP mRNA Expression and Triglyceride Accumulation in 3T3-L1 Cells

Troglitazone and pioglitazone increased FABP mRNA expression (Fig 5) and cellular triglyceride accumulation (Fig 6) in a dose-dependent manner, indicating that these agents stimulated adipocyte differentiation. In contrast, YM440 had no significant effect on these parameters related to adipocyte differentiation.

DISCUSSION

The severity of hyperglycemia in db/db mice increases with age.[19] At an early stage, the mice exhibit hyperphagia, marked obesity, hyperglycemia, and hyperinsulinemia, which are characteristic of insulin resistance. However, plasma insulin in db/db mice eventually decreases because of islet degeneration of pancreatic β cells, and then severe hyperglycemia develops.[20] Several laboratories have reported that insulin sensitizers such as ciglitazone and troglitazone ameliorate hyperglycemia in db/db mice.[21,22] In this study using diabetic db/db mice, we compared two types of insulin sensitizers, thiazolidinedione analogs (troglitazone and pioglitazone) and an oxadiazolidinedione analog (YM440), with respect to their potency in improving hyperglycemia and body weight gain. We also examined the relationship between body weight gain and adipogenesis. YM440 significantly decreased blood glucose concentrations in db/db mice. Its hypoglycemic activity was comparable to that of troglitazone and pioglitazone reported previously as insulin sensitizers.[10,23,24] However, the reduction in blood glucose by YM440 was not dose-dependent, and especially the effect at the maximal dose (300 mg/kg) was saturated. The dose range used in this study might be too high, since this agent caused dose-related hypoglycemic effects in other diabetic mouse

Table 1. Effects of YM440, Troglitazone, and Pioglitazone on Food Intake and Intraabdominal Fat Weight in db/db Mice

Parameter	Control	YM440 (100 mg/kg)	Troglitazone (300 mg/kg)	Pioglitazone (100 mg/kg)
Food intake (g/d)	6.43 ± 0.55	5.87 ± 0.24	6.02 ± 0.14	6.66 ± 0.25
Intraabdominal fat weight (g)				
Epididymal	2.46 ± 0.17	2.45 ± 0.10	2.82 ± 0.06	2.99 ± 0.11*
Retroperitoneal	0.65 ± 0.05	0.71 ± 0.05	0.90 ± 0.03†	0.96 ± 0.06‡
Perirenal	0.41 ± 0.04	0.45 ± 0.02	0.60 ± 0.06	0.71 ± 0.04‡
Total	3.53 ± 0.23	3.60 ± 0.16	4.31 ± 0.13	4.66 ± 0.19‡
Per body weight (%)				
Epididymal	5.59 ± 0.14	5.43 ± 0.12	5.50 ± 0.10	5.51 ± 0.11
Retroperitoneal	1.48 ± 0.07	1.56 ± 0.07	1.75 ± 0.06*	1.75 ± 0.08*
Perirenal	0.93 ± 0.05	0.99 ± 0.03	1.16 ± 0.08*	1.30 ± 0.05‡
Total	8.00 ± 0.17	7.97 ± 0.18	8.41 ± 0.08	8.56 ± 0.14*

NOTE. Each value represents the mean ± SEM of 6 mice.
*$P < .05$, †$P < .01$, ‡$P < .001$ *v* control.

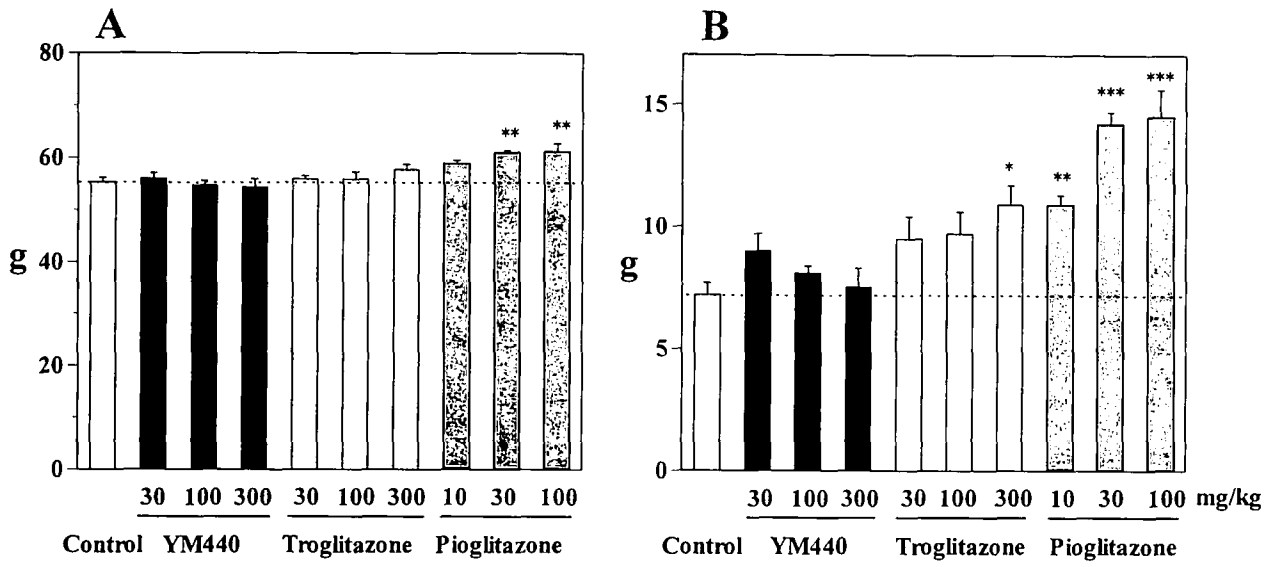


Fig 3. Effects of YM440, troglitazone, and pioglitazone on (A) body weight and (B) body weight gain in diabetic db/db mice. Male db/db mice were treated with the test compound orally once per day for 28 days. Each bar shows the mean ± SEM of 3 mice. *$P < .05$, **$P < .01$, ***$P < .001$ *v* untreated control.

models (KK mice and ob/ob mice, data not shown). An improvement of hyperglycemia by these agents was not due to a reduction of food intake, since food consumption was not significantly different among the groups. Furthermore, pioglitazone (≥30 mg/kg) and troglitazone (300 mg/kg) significantly increased body weight gain, leading to a significant increase in body weight in pioglitazone-treated mice. In contrast, YM440 (≤300 mg/kg) had no significant effect on body weight or body weight gain. The body weight gain in each group was compared under conditions in which the three agents were equipotent in decreasing blood glucose (YM440 and pioglitazone 100 mg/kg and troglitazone 300 mg/kg; Fig 2), and YM440, troglitazone, and pioglitazone increased the body weight gain by 113%, 151%, and 201%, respectively, compared with the control (100%). Fujiwara et al[21] reported similarly that troglitazone (200-mg/kg dosing for 3 weeks) did not affect food intake but caused a small but significant increase in body weight in db/db mice. These findings clearly indicate that YM440 had no effect on body weight or body weight gain under conditions in which YM440 and the other two thiazolidinedione insulin sensitizers ameliorated the hyperglycemia.

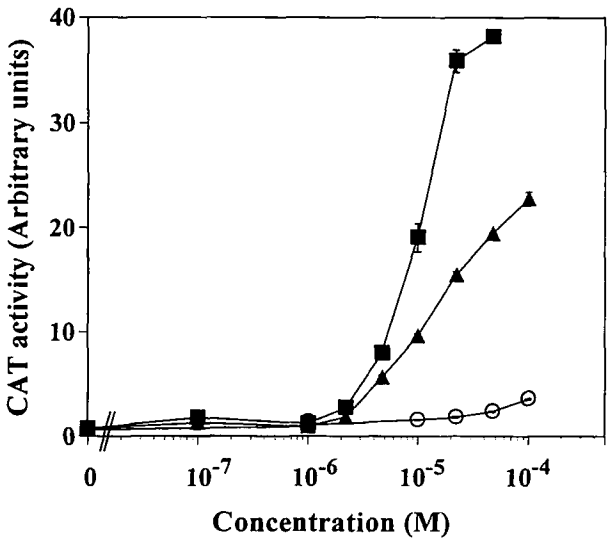


Fig 4. Effects of YM440, troglitazone, and pioglitazone on PPARγ activation in CV-1 cells. (○) YM440, (■) troglitazone, or (▲) pioglitazone were incubated with CV-1 cells expressing the plasmid containing the DNA binding domain of GAL4, ligand binding domain of PPARγ, and reporter plasmid containing GAL4 binding element, β-globin promoter region, and CAT gene. CAT activity was determined after a 48-hour incubation with the test compound. Data are the mean ± SEM (n = 3).

Troglitazone and pioglitazone significantly increased the body weight gain in this study, but it remains to be determined which organ is mainly responsible for body weight gain. We measured the weight of some intraabdominal fat tissues in this study (Table 1). Pioglitazone (100 mg/kg) significantly increased the weight of intraabdominal fat from a control value of 3.53 ± 0.23 g to 4.66 ± 0.19 g (132% of control), and also increased the ratio of intraabdominal fat weight to body weight from 8.00 ± 0.17 to 8.56 ± 0.14 (107% of control). Troglitazone also tended to increase these parameters, but YM440 had no effect at all. These findings suggest that increases in intraabdominal fat weight are correlated with body weight gain. However, these results could partially explain the body weight gain in pioglitazone- or troglitazone-treated mice, since if we calculate the net increase in body weight gain in each group (total body weight gain in each treated group minus that in the control group), about 8%, 21%, and 16% of the net increase in total body weight gain was due to increases in intraabdominal fat in YM440-, troglitazone-, and pioglitazone-treated groups, respectively. Rebuffé-Scrive et al[25] determined the effect of a high-fat, high-carbohydrate diet on the distribution of visceral and subcutaneous fat in C57/BL/6J mice, one of the best-

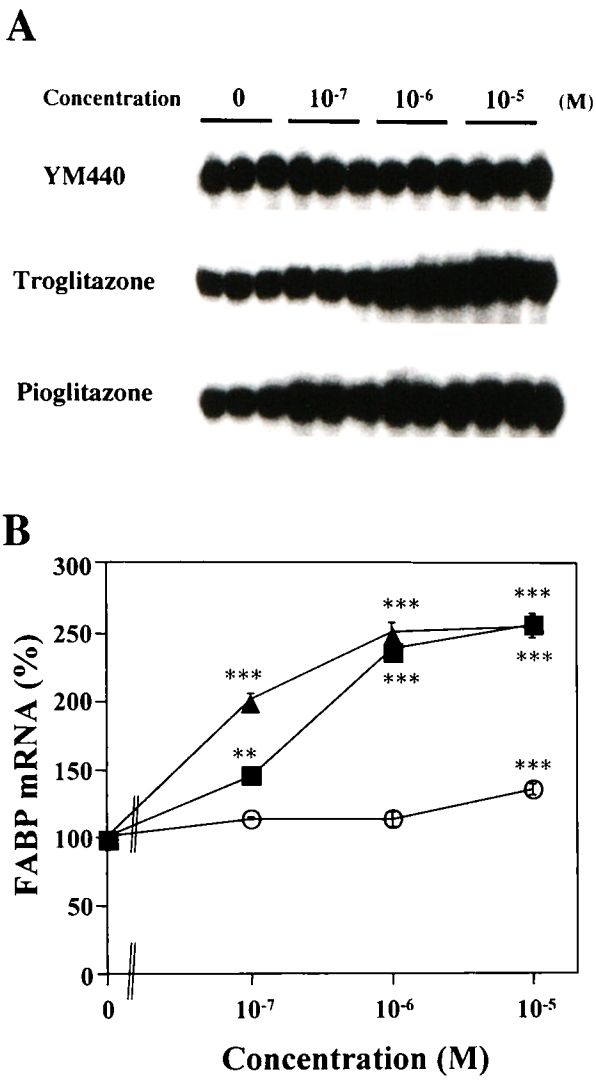


Fig 5. Effects of YM440, troglitazone, and pioglitazone on FABP mRNA levels in 3T3-L1 preadipocytes. 3T3-L1 cells were incubated for 48 hours with 0.5 mmol/L IBMX and 0.25 μmol/L dexamethasone followed by a further 72-hour incubation with various concentrations of (○) YM440, (■) troglitazone, or (▲) pioglitazone in the presence of 0.01 μg/mL insulin. (A) Representative autoradiographs of FABP mRNA. (B) Relative abundance of FABP mRNA. The signal for FABP was normalized against that for β-actin. Data are the mean ± SEM (n = 3). **$P < .01$, ***$P < .001$ *v* control.

characterized models of NIDDM.[26] The subcutaneous fat was 1.9- to 2.1-fold heavier than the visceral fat in mice fed either a standard diet or a high-fat, high-carbohydrate diet. Based on these values (about 2-fold), about 24%, 63%, and 48% of the net increase in total body weight gain may be attributable to the increases in total visceral and subcutaneous fat in YM440-, troglitazone-, and pioglitazone-treated groups, respectively. Yamashita et al[27] demonstrated that the volume and distribution of body fat has an important role in the incidence of metabolic complications such as diabetes and hyperlipidemia. Further studies are needed to determine the changes in the volume and distribution of intraabdominal and subcutaneous fat and the relationship between fat accumulation and insulin resistance.

Troglitazone and pioglitazone can serve as ligands of PPARγ, one of the most important nuclear receptors for adiposity.[28-30] These agents dose-dependently increased the transcriptional activity through PPARγ in CV-1 cells expressing the human PPARγ reporter gene. In addition, these agents increased FABP mRNA expression and cellular triglyceride accumulation, indicating that they stimulated adipocyte differentiation through PPARγ. On the other hand, YM440 did not increase the expression of PPARγ reporter gene and had no significant effect on adipocyte differentiation, supporting the idea that YM440 is not a ligand for PPARγ. The lack of effect of this agent on PPARγ was also observed in other cell types, such as NIH3T3 cells expressing mouse PPARγ reporter gene (data not shown). Thus, the properties of this agent were totally distinct from those of troglitazone or pioglitazone. Recently, Reginato et al[31] reported that MCC-555 is a novel thiazolidinedione ligand for PPARγ. Although this agent showed higher potency than BRL49653 in improving hyperglycemia in the mouse KK-A^y model of obesity and diabetes, its binding affinity for PPARγ was less than one fiftieth that of BRL49653. This agent induced adipogenesis but showed lower potency than BRL49653. We believe it is unlikely that YM440 is a partial agonist for PPARγ, as this agent had no effect at all with respect to PPARγ activation and adipocyte differentiation in vitro. In contrast, MCC-555 activated PPARγ but with lower potency than BRL49653, suggesting that this agent functioned as a partial agonist of PPARγ activation and adipocyte differentiation. There have been no previous reports showing the effect of MCC-555 on body weight gain in vivo. When such information becomes available, more detailed comparisons between the two compounds will be possible.

Paulik et al,[32] using infrared thermography, have reported that troglitazone and the other related PPARγ agonists suppress thermogenesis in adipocytes. They also observed that the mitochondrial uncouplers, such as carbonyl cyanide *p*-

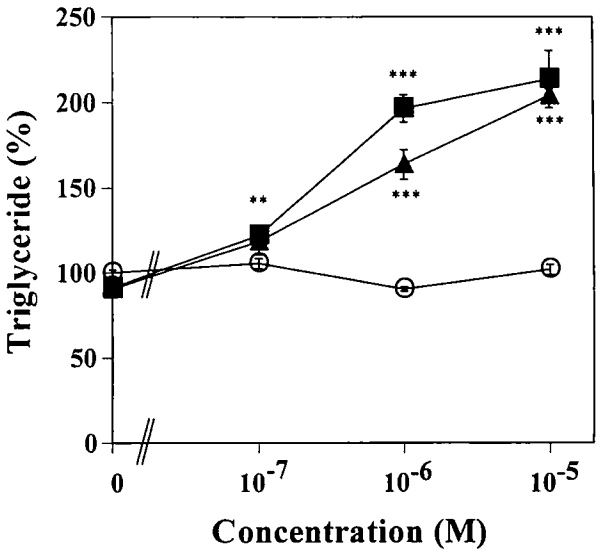


Fig 6. Effects of YM440, troglitazone, and pioglitazone on triglyceride accumulation in 3T3-L1 preadipocytes. Experimental conditions are the same as in Fig 5. ○, YM440; ■, troglitazone; ▲, pioglitazone. Data are the mean ± SEM (n = 3). **$P < .01$, ***$P < .001$ *v* control.

(trifluoromethoxy)phenylhydrazone and rotenone, and β3-adrenoceptor agonists increase thermogenesis. These findings may indicate a possibility that PPARγ agonists increase fat accumulation by reducing thermogenesis, presumably by inhibiting uncoupling protein (UCP) expression in the mitochondria of adipocytes. In contrast, there are several reports showing that PPARγ agonists increase UCP mRNA in vitro and in vivo.[33-35] Thiazolidinedione BRL49653 increased UCP1 in human preadipocytes.[33] Furthermore, our previous report demonstrated that pioglitazone increased UCP2 mRNA but decreased UCP3 mRNA levels in skeletal muscle of diabetic KK mice.[34] The role of thermogenesis or UCP expression in the hypoglycemic effects of PPARγ agonists remains to be elucidated, and further study will be required to examine the relationship between UCP expression and the hypoglycemic effect of YM440.

In conclusion, we have examined the relationship between hypoglycemic activity and body weight gain induced by insulin sensitizers in diabetic db/db mice. Thiazolidinedione analogs decreased blood glucose but increased the body weight gain. In contrast, YM440, an oxadiazolidinedione analog, showed hypoglycemic activity without affecting body weight gain. The increase in body weight gain induced by troglitazone or pioglitazone may be accounted for, in part, by the adipogenesis in intraabdominal fat tissue. Troglitazone or pioglitazone, but not YM440, activated PPARγ and promoted adipocyte differentiation in cultured cells. Therefore, it is highly likely that the mechanism of the hypoglycemic action of YM440 is different from that of thiazolidinediones. Although the precise mechanism by which YM440 ameliorates hyperglycemia in diabetic animals remains unknown, our recent study using a euglycemic-hyperinsulinemic clamp technique indicates that this agent ameliorates the impaired regulation of hepatic glucose output in obese Zucker rats by improving insulin sensitivity (R. Nakano, E. Kurosaki, unpublished observations, June 1998). YM440 improves hyperglycemia without inducing adiposity and may be a useful hypoglycemic agent in the treatment of NIDDM.

REFERENCES

1. Edelman SV: Importance of glucose control. Med Clin North Am 82:665-687, 1998
2. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045. Diabetes 31:1549-1558, 1988
3. Suter SL, Nolan JJ, Wallace P, et al: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15:193-203, 1992
4. Schwartz S, Raskin P, Fonseca V, et al: Effect of troglitazone in insulin-treated patients with type II diabetes mellitus. N Engl J Med 338:861-866, 1998
5. Matthaei S, Reibold JP, Hamann A, et al: In vivo metformin treatment ameliorates insulin resistance: Evidence for potentiation of insulin-induced translocation and increased functional activity of glucose transporters in obese (fa/fa) Zucker rat adipocytes. Endocrinology 133:304-311, 1993
6. Stumvoll M, Nurjahan N, Perriello G, et al: Metabolic effects of metformin in non–insulin-dependent diabetes mellitus. N Engl J Med 333:550-554, 1995
7. Serrano Rios M: Relationship between obesity and the increased risk of major complications in non–insulin-dependent diabetes mellitus. Eur J Clin Invest 28:14-18, 1998 (suppl 2)
8. Berger M: Risk of obesity in type II diabetes mellitus. Int J Obes Relat Metab Disord 4:S29-S33, 1992 (suppl)
9. Speigelman BM: PPAR-γ: Adipogenic regulator and thiazolidinedione receptor. Diabetes 47:507-514, 1998
10. Lambe KG, Tugwood JD: A human peroxisome-proliferator–activated receptor-γ is activated by inducers of adipogenesis, including thiazolidinedione drugs. Eur J Biochem 239:1-7, 1996
11. Shimaya A, Kurosaki E, Shioduka K, et al: YM268 increases the glucose uptake, cell differentiation, and mRNA expression of glucose transporter in 3T3-L1 adipocytes. Horm Metab Res 30:543-548, 1998
12. Shimaya A, Noshiro O, Hirayama R, et al: Insulin sensitizer YM268 ameliorates insulin resistance by normalizing the decreased content of GLUT4 in adipose tissue of obese Zucker rats. Eur J Endocrinol 137:693-700, 1997
13. Noshiro O, Hirayama R, Shimaya A, et al: Role of plasma insulin concentration in regulating glucose and lipid metabolism in lean and obese Zucker rats. Int J Obes Relat Metab Disord 21:115-121, 1997
14. Bernlohr DA, Angus CW, Lane MD, et al: Expression of specific mRNA during adipose differentiation: Identification of an mRNA encoding a homologue of myelin P2 protein. Proc Natl Acad Sci USA 81:5468-5472, 1984
15. Ezaki O, Flores-Riveros JR, Kaestner KH, et al: Regulated expression of an insulin-responsive glucose transporter (GLUT4) minigene in 3T3-L1 adipocytes and transgenic mice. Proc Natl Acad Sci USA 90:3348-3352, 1993
16. Robb JA, Huebner K: Effect of cell chromosome number on simian virus 40 replication. Exp Cell Res 81:120-126, 1973
17. Kliewer SA, Forman BM, Blumberg B, et al: Differential expression and activation of a family of murine peroxisome proliferator–activated receptors. Proc Natl Acad Sci USA 91:7355-7359, 1994
18. Kato S, Endoh H, Masuhiro Y, et al: Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270:1491-1494, 1995
19. Prochazka M, Premdas FH, Leiter EH, et al: Estrone treatment dissociates primary versus secondary consequences of "diabetes" (db) gene expression in mice. Diabetes 35:725-728, 1986
20. Like AA, Chick WL: Studies in the diabetic mutant mouse. I. Light microscopy and radioautography of pancreatic islets. Diabetologia 6:207-215, 1970
21. Fujiwara T, Wada M, Fukuda K, et al: Characterization of CS-045, a new oral antidiabetic agent. II. Effects on glycemic control and pancreatic islet structure at a late stage of the diabetic syndrome in C57BL/sJ-db/db mice. Metabolism 40:1213-1218, 1991
22. Diani AR, Peterson T, Sawada GA, et al: Ciglitazone, a new hypoglycemic agent. IV. Effect on pancreatic islets of C57BL/6J-ob/ob and C57BL/KsJ-db/db mice. Diabetologia 27:225-234, 1984
23. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator–activated receptor γ (PPAR γ). J Biol Chem 270:12953-12956, 1995
24. Young PW, Buckle DR, Cantello BC, et al: Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator–activated receptor gamma. J Pharmacol Exp Ther 284:751-759, 1998
25. Rebuffé-Scrive M, Surwit R, Feinglos M, et al: Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non–insulin-dependent diabetes mellitus. Metabolism 42:1405-1409, 1993
26. Onodera T, Yoon JW, Brown KS, et al: Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. Nature 274:693-696, 1978
27. Yamashita S, Nakamura T, Shimomura I, et al: Insulin resistance and body fat distribution. Diabetes Care 19:287-291, 1996
28. Okuno A, Tamemoto H, Tobe K, et al: Troglitazone increases the

number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 101:1354-1361, 1998

29. Hallakou S, Doaré L, Foufelle F, et al: Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rats. Diabetes 46:1393-1399, 1997

30. Kletzien RF, Foellmi LA, Harris PKW, et al: Adipocyte fatty acid–binding protein: Regulation of gene expression in vivo and in vitro by an insulin-sensitizing agent. Mol Pharmacol 42:558-562, 1992

31. Reginato MJ, Bailey ST, Krakow SI, et al: A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor γ-activating properties. J Biol Chem 273:32679-32684, 1998

32. Paulik MA, Buckholz RG, Lancaster ME, et al: Development of infrared imaging to measure thermogenesis in cell culture: Thermogenic effects of uncoupling protein-2, troglitazone, and β-adrenoceptor agents. Pharm Res 6:944-949, 1998

33. Digby JE, Montague CT, Sewter CP, et al: Thiazolidinedione exposure increases the expression of uncoupling protein 1 in cultured human preadipocytes. Diabetes 47:138-141, 1998

34. Shimokawa T, Kato M, Watanabe Y, et al: In vivo effects of pioglitazone on uncoupling protein-2 and -3 mRNA levels in skeletal muscle of hyperglycemic KK mice. Biochem Biophys Res Commun 251:374-378, 1998

35. Foellmi-Adams LA, Wyse BM, Herron D, et al: Induction of uncoupling protein in brown adipose tissue: Synergy between norepinephrine and pioglitazone, an insulin-sensitizing agent. Biochem Pharmacol 52:693-701, 1996