Thiazolidinediones Downregulate Stearoyl-CoA Desaturase 1 Gene Expression in 3T3-L1 Adipocytes

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Thiazolidinediones (TZDs) are known to have potent increases of insulin sensitivity. Because peroxisome proliferator-activated receptor-γ (PPAR-γ), a receptor for TZDs, is mainly expressed in adipocytes, we tried to search the TZD-targeted genes in mouse 3T3-L1 adipocytes. By the mRNA differential display method, one band repressed by troglitazone was obtained, which corresponded to the partial sequences of the stearoyl-CoA desaturase 1 (SCD1) gene. Troglitazone dramatically decreased SCD1 mRNA levels in 3T3-L1 adipocytes in a dose-dependent manner. Pioglitazone also repressed the SCD1 mRNA expression, whereas WY-14,643 had no apparent effect. Both troglitazone and pioglitazone raised the composition (weight percentage) of myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), but lowered the composition of the Δ^9-*cis* desaturated fatty acids such as myristoleic acid (C14:1, Δ^9), palmitoleic acid (C16:1, Δ^9), oleic acid (C18:1, Δ^9), and linoleic acid (C18:2, $\Delta^{9,12}$). These results indicate that TZDs repress SCD1 activity in 3T3-L1 adipocytes via downregulating SCD1 enzyme gene expression. *Diabetes* 46:2115–2118, 1997

Thiazolidinediones (TZDs), which are known to have potent increases of insulin sensitivity, have been developed for the treatment of NIDDM (1). The mechanism of the insulin-sensitizing effect of TZDs is not precisely determined, although several reports showed their stimulatory effect on insulin receptor and insulin receptor substrate (IRS)-1 tyrosine phosphorylation, phosphatidylinositol-3-kinase activity, and GLUT4 expression (1–3). Recently, it has been found that TZDs are high-affinity ligands for the peroxisome proliferator–activated receptor-γ (PPAR-γ), which belongs to a nuclear receptor superfamily (4). The expression of PPAR-γ has been shown to be induced very early in adipose cell differentiation, which provided potency of adipocyte differentiation cooperatively with CCAAT/enhancer binding protein α (C/EBPα) (5). After the expression of PPAR-γ and C/EBPα, adipocyte-specific genes are induced to be expressed after the treatment of adipogenic agents, such as insulin, glucocorticoid, and isobutylmethylxanthine (IBMX). The addition of TZDs to preadipocytes is known to induce differentiation into mature adipocytes and enhance expression of the adipocyte-specific genes (6). Thus, TZDs are indicated to elicit some potential effects on preadipocytes via activation of PPAR-γ. By contrast, the effects of these molecules on mature adipocytes have not been fully investigated. It is also necessary to identify their target genes on the mature adipocytes to analyze the in vivo effects of TZDs.

In this study, we tried to search the genes regulated by TZDs in fully differentiated 3T3-L1 adipocytes. For this purpose, we used the mRNA differential display method (7). We obtained one clone whose expression was downregulated by TZDs. This clone corresponded to the partial sequences of stearoyl-CoA desaturase 1 (SCD1) gene (8). We demonstrate that TZDs decreased SCD1 mRNA levels in 3T3-L1 adipocytes. We also show that TZDs changed fatty acid composition in these cells.

RESEARCH DESIGN AND METHODS

Chemicals. Troglitazone was from Sankyo Pharmaceuticals (Tokyo, Japan), pioglitazone was from Takeda Pharmaceuticals (Osaka, Japan), and WY-14,643 was from Sigma (St. Louis, MO). All other reagents were of analytical grade.

Cell culture. Mouse preadipocyte cell line 3T3-L1 (9) was obtained from American Type Culture Collection (Rockville, MD). The cells were grown in basal medium (Dulbecco's modified Eagle's medium [DMEM]) containing 4.5 g/l glucose, 50 μg/ml streptomycin sulfate, 50,000 U/l penicillin G), supplemented with 10% fetal bovine serum (FBS). After confluence, the cells were treated with basal medium supplemented with 100 ng/ml insulin, 0.15 mmol/l IBMX, and 1 μmol/l dexamethasone for 2 days. The medium was replaced with fresh basal medium every 2 days. After the cells were fully differentiated, the indicated drugs were added to the culture medium and incubated for the indicated time. Then cells were harvested to prepare total RNA for differential display, polymerase chain reaction (PCR), and Northern blot analysis, and for estimation of fatty acid composition.

mRNA differential display. mRNA differential display was performed using RNA image kit (GenHunter, Nashville, TN), according to the manufacturer's protocol. Briefly, RNA was isolated from mature 3T3-L1 adipocytes treated with 10 μmol/l troglitazone for 8 or 48 h, using the guanidine isothiocyanate extraction method (10). Subsequently, each RNA was processed to a reverse transcription reaction using the three 3′ poly(A) tail-anchored primers and MMLV reverse transcriptase. Each cDNA was used for subsequent PCR amplification. PCR was performed with cDNA, the same 3′-anchored primer, 5′ arbitrary primer, dNTPs, [α-^{35}S]dATP 1,200 Ci/mmol (Amersham, Buckinghamshire, U.K.), and *Taq* DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT). Parameters for PCR were 40 cycles of denaturing at 94°C for 30 s, annealing at 40°C for 2 min, and extension at 72°C for 30 s. After the electrophoresis of the PCR products, candidate bands were excised

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C/EBPα, CCAAT/enhancer binding protein α; HPLC, high-performance liquid chromatography; PCR, polymerase chain reaction; PPAR-γ, peroxisome proliferator-activated receptor-γ; PUFA, polyunsaturated fatty acids; SCD1, stearoyl-CoA desaturase 1; TZD, thiazolidinedione.

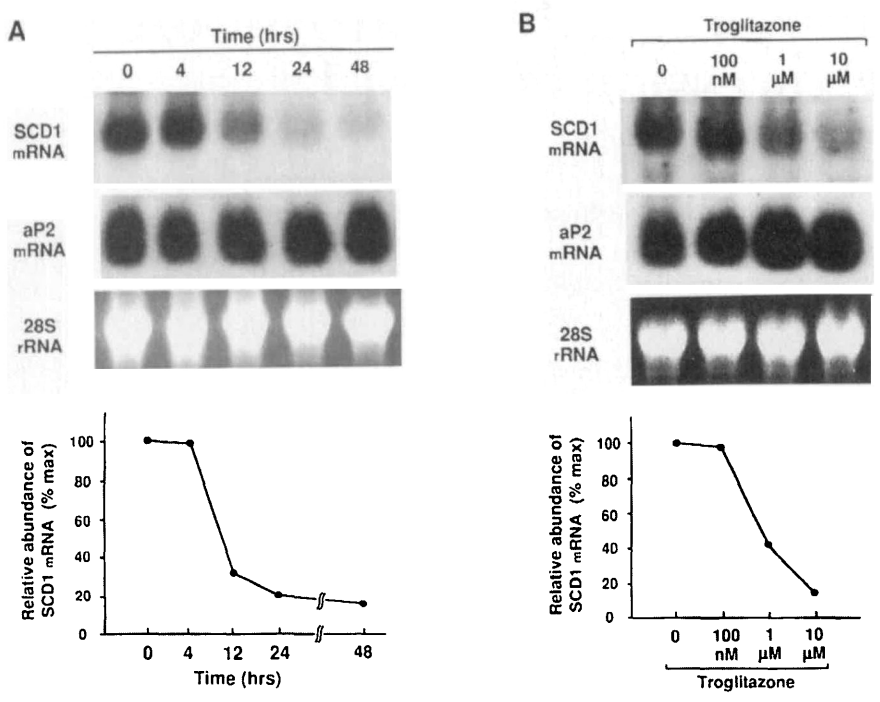

FIG. 1. Troglitazone decreases SCD1 mRNA levels in 3T3-L1 adipocytes. *A*: total cellular RNA was isolated from fully differentiated 3T3-adipocytes treated with 10 μmol/l troglitazone for various periods of time. *B*: total cellular RNA was isolated from these cells treated for 2 days with the indicated concentrations of troglitazone. In the both experiments, 10 μg total RNA was subjected to Northern blot analysis. The blot was hybridized sequentially with DNA probes for SCD1 and aP2 mRNA. Densitometrical data were plotted as relative abundance of SCD1 mRNA normalized to 28S rRNA. Data are typical of two separate experiments.

from the sequencing gel. The eluted DNA was reamplified by using the same primer pair, which was cloned into the TA cloning vector (Invitrogen, Carlsbad, CA).

Northern blot analysis. Of the total RNA prepared from the 3T3-L1 cells, 10 μg was separated on 1% denaturing formaldehyde agarose gel. Northern blot analysis was performed using ^{32}P-labeled cDNA probes for SCD1 mRNA and aP2 mRNA (11), as previously described (12). cDNA used as a probe for SCD1 mRNA is a clone amplified in differential display PCR.

Estimation of composition of fatty acid. The 3T3-L1 cells were washed with phosphate-buffered saline and scraped from the dish. The pelleted cells were homogenized in 1 ml chloroform and vortexed vigorously. After centrifugation at 1,500*g* for 5 min, the chloroform phase was taken for the determination of fatty acids. High-performance liquid chromatography (HPLC) was performed for determining the composition of total fatty acids, as previously described (13).

Statistics. Values are means ± SD. Statistical analyses were performed according to Student's *t* test. A P value <0.05 was considered to be statistically significant.

RESULTS

In searching the genes regulated by TZDs in 3T3-L1 adipocytes, we used the mRNA differential display technique. In PCR using H-T11A (AAGCTTTTTTTTTTTA) and H-AP21 (AAGCTTTCTCTGG) primers, a candidate band in sequence gel was found to be repressed by the treatment with 10 μmol/l troglitazone for 8 and 48 h. By searching the homology in GenBank, this band was found to correspond to the partial sequences of exon 6 in the mouse SCD1 gene (8). Thus, we performed Northern blot analyses using the amplified clone as a probe. As shown in Fig. 1A, 10 μmol/l troglitazone decreased the accumulation of 4.9-kb SCD1 mRNA in 3T3-L1 adipocytes. Repetitive experiments showed that this downregulation was observed within 12 h after the treatment of troglitazone. The greatest repression occurred at 24 h and continued until 48 h. The repression of SCD1 mRNA by troglitazone was also dose-dependent (Fig. 1B). Densitometric scanning analysis revealed that the treatment with 1 μmol/l troglitazone for 48 h repressed SCD1 mRNA levels to 40% of the control levels. The maximal repression to ~20% of the control was observed at 10 μmol/l. By contrast, troglitazone appeared not to alter fatty acid binding protein aP2 mRNA levels. It had no effect on cell viability, as determined by cell number, and cell morphology (data not shown). As demonstrated in Fig. 2, 10 μmol/l pioglitazone also decreased SCD1 mRNA levels in 3T3-L1 adipocytes, whereas 10 μmol/l of the peroxisome proliferator WY-14,643 showed no apparent effect on the mRNA levels.

SCD1 enzyme catalyzes the Δ^9-*cis* desaturation of a spectrum of methylene-interrupted fatty acyl-CoA substrates (14). Therefore, we determined fatty acid composition of 3T3-L1

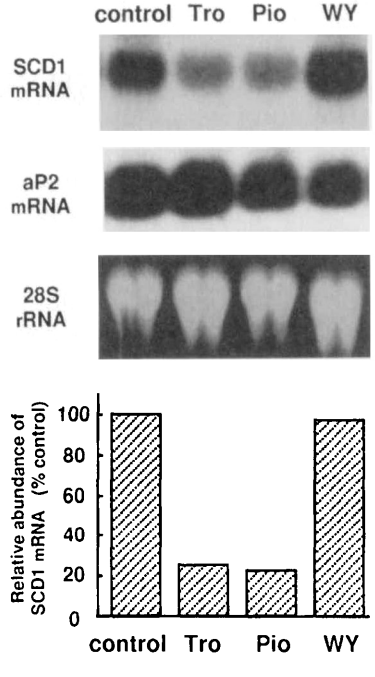

FIG. 2. Downregulation of SCD1 mRNA is specific to thiazolidinedione derivatives. Total cellular RNA was isolated from fully differentiated 3T3-L1 adipocytes treated with 10 μmol/l of troglitazone (Tro), pioglitazone (Pio), or WY-14,643 (WY) for 2 days. Of the total RNA, 10 μg was subjected to Northern blot analysis as described in Fig. 1. Densitometrical data were plotted as relative abundance of SCD1 mRNA normalized to 28S rRNA. Data are typical of two separate experiments.

TABLE 1
Fatty acid composition (weight percentage) of 3T3-L1 adipocytes treated with or without troglitazone, pioglitazone, or WY-14,643

Treatment	Fatty acid (%)									Total saturated fatty acids (%)	Total unsaturated fatty acids (%)
	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:4	Other		
Control	2.9 ± 0.05	1.5 ± 0.02	26.5 ± 0.3	31.5 ± 0.7	6.0 ± 0.5	7.3 ± 0.2	22.4 ± 0.2	1.4 ± 0.06	0.5	35.7 ± 0.8	64.3 ± 0.8
Troglitazone	3.5 ± 0.05§	1.2 ± 0.1†	34.7 ± 0.7§	22.2 ± 0.2§	7.8 ± 0.4†	6.8 ± 0.09*	22.0 ± 0.09*	1.4 ± 0.09	0.3	46.3 ± 0.3§	53.7 ± 0.3§
Pioglitazone	3.4 ± 0.1‡	1.2 ± 0.1†	36.9 ± 0.8§	23.0 ± 0.7§	6.4 ± 1.1	6.4 ± 0.3*	21.3 ± 0.4*	1.1 ± 0.1*	0.4	47.1 ± 0.9§	52.9 ± 0.9§
WY-14,643	3.0 ± 0.05	1.5 ± 0.1	28.2 ± 0.07§	31.4 ± 0.6	5.2 ± 0.3	7.0 ± 0.07	22.1 ± 0.3	1.2 ± 0.2	0.4	36.6 ± 0.3	63.4 ± 0.3

Data are means ± SD of triplicate assays. ND, not detectable. *, †, ‡, and § denote significant difference from control values; $P < 0.05$, $P < 0.01$, $P < 0.005$, and $P < 0.001$, respectively.

adipocytes treated with or without 10 μmol/l troglitazone for 48 h. Lipid fraction extracted from the whole cells by chloroform was subjected to HPLC to determine the composition of total fatty acids. Total amounts of extracted fatty acids were 2,950 ± 130 μg/dish in the cells treated with troglitazone, not significantly different from those in the untreated cells (2,630 ± 260 μg/dish). Troglitazone significantly increased the composition (weight percentage) of myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) (Table 1). By contrast, it significantly decreased the composition of myristoleic acid (C14:1, Δ^9), palmitoleic acid (C16:1, Δ^9), oleic acid (C18:1, Δ^9), and linoleic acid (C18:2, $\Delta^{9,12}$) (Table 1). Thus, troglitazone decreased the composition of the unsaturated fatty acids while it increased the composition of the saturated fatty acids. Pioglitazone also elicited almost the same effects as troglitazone on the fatty acid composition in 3T3-L1 adipocytes. WY-14,643 at a concentration of 10 μmol/l had no significant effect, except that it slightly increased the composition of palmitic acid (C16:0) (Table 1).

DISCUSSION

From mouse 3T3-L1 adipocyte cDNA, we obtained one clone of which expression was downregulated by troglitazone, according to mRNA differential display method. This clone corresponded to the mouse SCD1 gene. Northern blot analysis showed that SCD1 mRNA levels were repressed in 3T3-L1 adipocytes by 10 μmol/l troglitazone. The SCD1 mRNA levels were also repressed by 10 μmol/l pioglitazone. Analysis of fatty acid composition in the whole-cell extract revealed that troglitazone, as well as pioglitazone, significantly increased the composition of saturated fatty acids (myristic, palmitic, stearic) and decreased the composition of Δ^9-*cis* desaturated fatty acids (myristoleic, palmitoleic, oleic, linoleic). SCD1 enzyme catalyzes the Δ^9-*cis* desaturation of a spectrum of methylene-interrupted fatty acyl-CoA substrates, such as myristryl-CoA, palmityl-CoA, and stearyl-CoA (15). Therefore, our results indicate that TZDs inhibit SCD1 enzyme activity in 3T3-L1 adipocytes by repressing the SCD1 mRNA expression. In the present study, TZDs failed to alter the other adipocyte-specific gene aP2 mRNA levels. In addition, TZDs had no effect on cell viability and cell morphology (data not shown). Thus, the inhibitory effects of TZDs are indicated to be specific for SCD1 gene expression.

The peroxisome proliferator WY-14,643, which has a higher affinity for PPAR-α but less of an affinity for PPAR-γ (4), showed no apparent effect on SCD1 mRNA levels in 3T3-L1 adipocytes at a concentration of 10 μmol/l. WY-14,643 had no apparent effect on fatty acid composition in these cells. Because TZDs are high-affinity ligands for PPAR-γ but not for PPAR-α (4), their effect on SCD1 gene expression is indicated to be mediated by PPAR-γ. In this regard, it is of interest to note that the functional PPAR response element (PPRE) has been found in the promoter region of the SCD1 gene (16).

There have been several findings concerning the regulation of SCD1 gene expression. During 3T3-L1 preadipocyte differentiation, SCD1 mRNA levels increase principally at the level of gene transcription in which C/EBPα is involved (17). In differentiated 3T3-L1 adipocytes, polyunsaturated fatty acids (PUFAs) repress SCD1 gene expression (18). PUFAs are found to be ligands for PPAR-α and PPAR-γ (19), and PPAR-γ but not PPAR-α is preferentially expressed in 3T3-L1 adipocytes (20). Thus, the inhibitory effects of PUFAs on SCD1 gene expression in these cells might also be mediated by PPAR-γ.

The predominant products catalyzed by SCD1 enzyme (palmitoleic acid and oleic acid) are the major constituents of membrane phospholipids and triglyceride stores in adipocytes (14). Alteration of the ratio of stearic acid to oleic acid composition, which influences cell membrane fluidity, is implicated in insulin action (21,22). Recently, it has been shown that increased SCD1 mRNA levels are associated with increased adiposity (23). Thus, the TZD-induced decrease in the SCD1 mRNA levels may reflect their pharmacological effects on insulin sensitivity and lipid metabolism. In addition, it has been recently observed that troglitazone improves insulin secretion from pancreatic β-cells (24). Since saturated fatty acids are found to stimulate insulin release in the perfused rat pancreas (25), the TZD-induced increase of the saturated fatty acids might be involved in their effects on insulin secretion.

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