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Review Article

The isoxazolidine-3,5-dione hypoglycemic agent JTT-501 and other nonthiazolidinedione insulin sensitizers

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Introduction

Type II diabetes is a metabolic disorder characterized by hyperglycemia that causes chronic complications such as nephropathy, retinopathy, neuropathy and premature atherosclerosis (1) which can lead to renal failure, blindness and coronary artery disease. The U.K. Prospective Diabetes Study demonstrated that intensive blood glucose control using either sulfonylureas or insulin could substantially decrease the risk of microvascular complications in patients with type II diabetes (2). Therefore, maintaining a level of blood glucose as close to normal as possible is important in preventing diabetic complications. Hyperglycemia in type II diabetes is caused by an increase of insulin resistance and/or by impaired insulin secretion from the pancreas (3). Therefore, obese type II diabetic patients with insulin resistance, in particular, require a reduction in insulin resistance in order to achieve improved glycemic control.

Since the thiazolidinedione-based compound, ciglitazone, was developed from fibrate lipid-lowering agents by Takeda and reported to be a novel oral hypoglycemic agent that potentiated the peripheral actions of insulin (4, 5), many studies on new analogs have been carried out (6). Thiazolidinediones significantly reduce glucose, lipid and insulin levels in rodent models of type II diabetes and obesity (5, 7, 9, 10) and clinical data have also supported the efficacy of these agents in patients with type II diabetes. However, 50% of 136 patients with type II diabetes did not respond to treatment with troglitazone (400 mg/day for 12 weeks) (15), a fact that may suggest the heterogeneity of type II diabetes. Among the thiazolidinedione compounds, pioglitazone and rosiglitazone have been approved for use worldwide, while troglitazone has been approved for use in Japan and the U.S. Pioglitazone was developed as the successor to ciglitazone by Takeda (8, 9, 11), rosiglitazone was developed by SmithKline Beecham (12) and troglitazone was derived from ciglitazone by Sankyo, who replaced the lipophilic tail (methylcyclohexylmethyl ether moiety) with a vitamin E residue (13-21).

Nonthiazolidinedione insulin sensitizers

Since acidic functionality of the thiazolidine-2,4-dione ring was considered to be essential for its insulin-sensitizing activity (22), replacement of this ring was attempted by Pfizer using closely related acidic heterocyclic groups such as an oxazolidine-2,4-dione ring (23) or a 1-oxa-2,4-diazolidine-3,5-dione ring (24) (Fig. 1). However, compounds **2** and **3** were less potent than their parent compound **1**, which was one of the most potent thiazolidine-2,4-diones developed by Takeda and was 620 times more potent than ciglitazone (25). Thus, replacement of the thiazolidine-2,4-dione ring with these heterocyclic rings led to decreased activity.

Replacement of the thiazolidine-2,4-dione ring with acyclic structures was more successful, such as carbonylated hydroxyureas (24) (compound **4**, Pfizer) and α-heteroatom-substituted carboxylic acids (26-29) (SB-213068, HQL-975 and compound **5**, SmithKline Beecham, Sumitomo Metal and Glaxo Wellcome, respectively) (Fig. 1). Interestingly, SB-213068 was more potent than its parent thiazolidine-2,4-dione (BRL-48482) (27). This was an important finding because it showed that there was a structure more favorable than thiazolidinedione. It was suggested that the role of the acidic thiazolidine-2,4-dione in these compounds was played by carboxylic acid and that an appropriate substituent at the alpha-position of the carboxylic acid could alter the chemical environment around the carboxylic acid in such a way that the whole group would mimic the thiazolidine-2,4-dione ring.

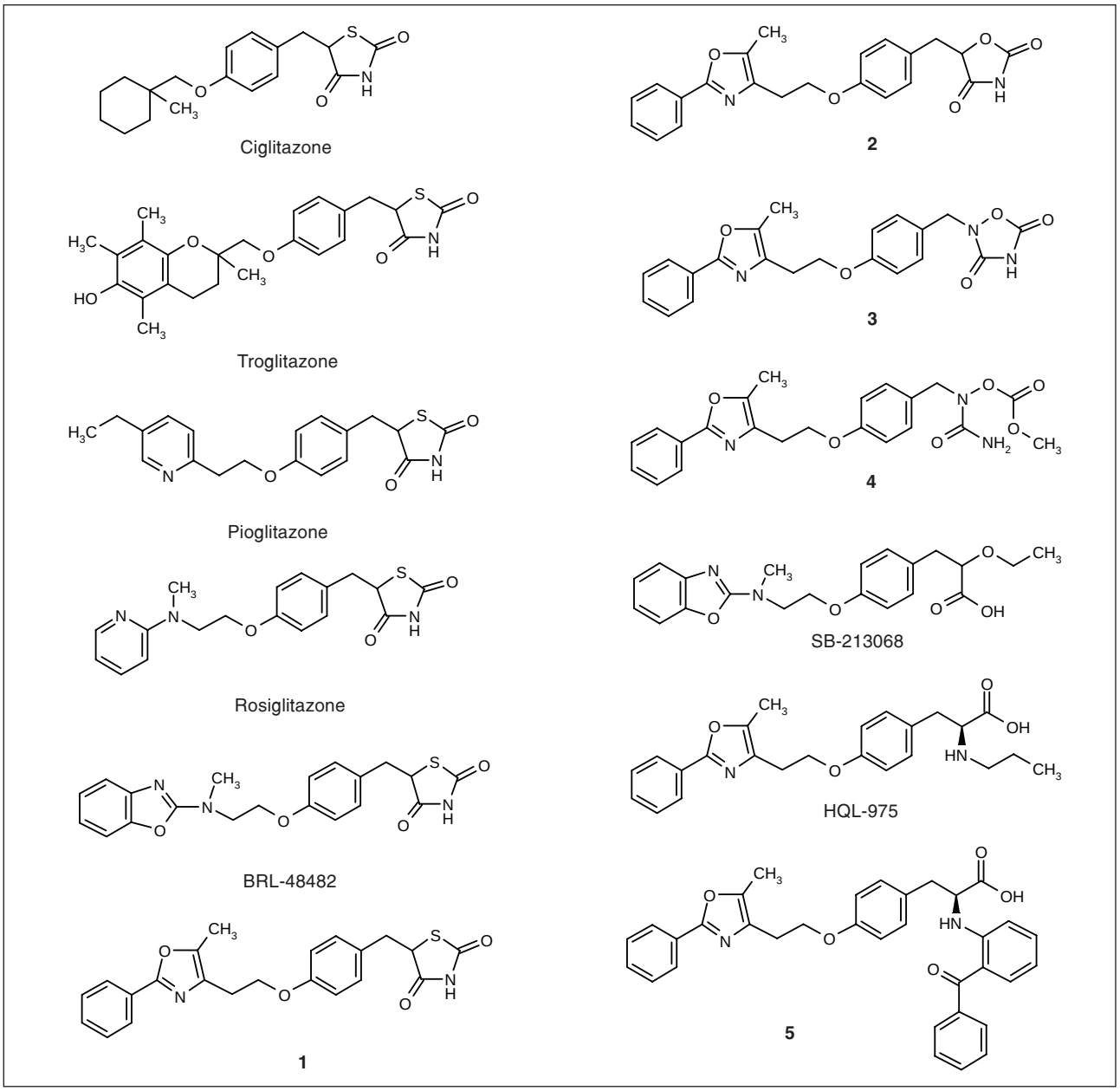


Fig. 1. Structures of insulin sensitizers.

JTT-501 and its acyclic derivatives

An acidic heterocyclic isoxazolidine-3,5-dione ring was designed as a bioisostere of the thiazolidine-2,4-dione ring (30) (Fig. 2). The isoxazolidinedione compound (JTT-501) achieved a 25% reduction in blood glucose at an oral dose of 38 mg/kg in genetically diabetic KKAy mice as a result of improving their insulin resistance. JTT-501 is the first nonthiazolidinedione insulin sensitizer that has advanced to clinical assessment and is currently undergoing evaluation in phase II/III trials. Further studies showed that derivatives of JTT-501, acyclic 1,3-dicarbonyl compounds, also possessed insulin-sensitizing activity. Among these compounds, the

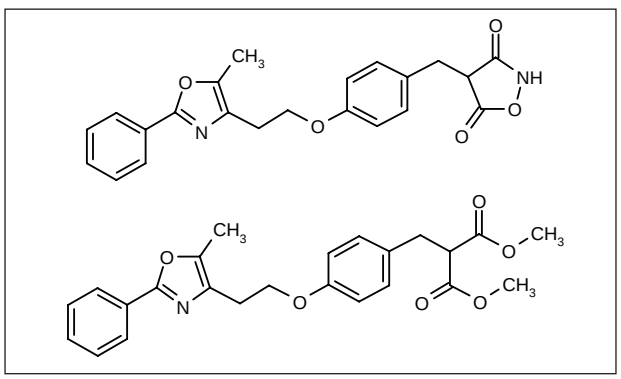


Fig. 2. Structures of JTT-501 (top) and JTP-20993 (bottom).

Scheme 1: Preparation of 1,3-Dicarbonyl Compounds

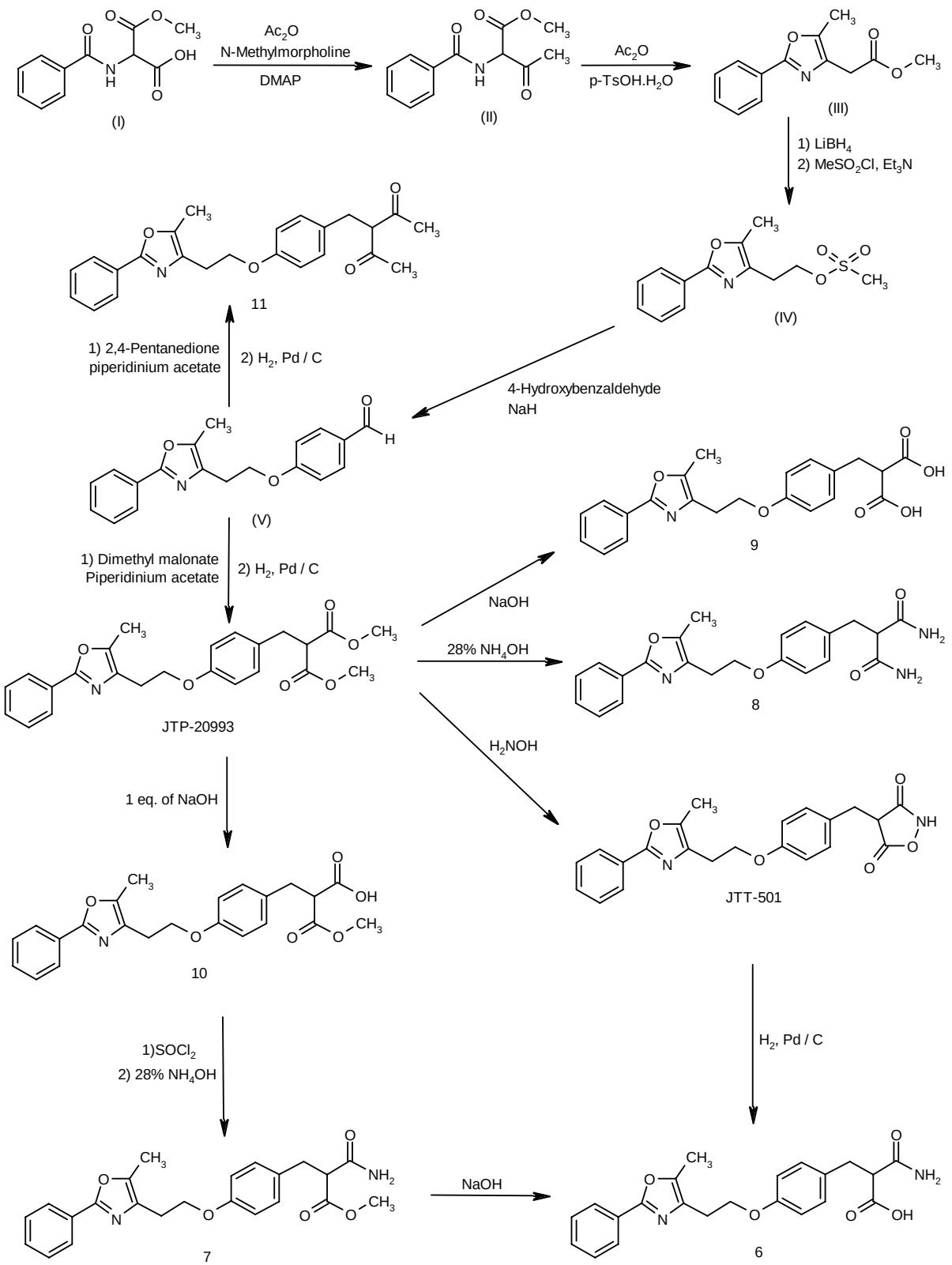
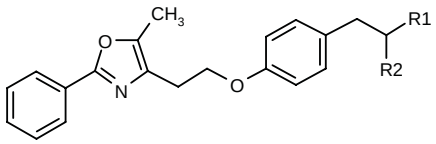


Table I: Effect on triglyceride accumulation in 3T3-L1 cells.



	R_1	R_2	EC_{50} (nM)
JTT-501	-CO-NH-O-CO-		110
JTP-20993	-COOMe	-COOMe	0.059
6	$-CONH_2$	-COOH	130
7	$-CONH_2$	-COOMe	1.3
8	$-CONH_2$	$-CONH_2$	1.4
9	-COOH	-COOH	4.4
10	-COOMe	-COOH	0.087
11	-COMe	-COMe	110
1	-CO-NH-CO-S-		0.45
Troglitazone			130
Pioglitazone			160

Effective concentration for 50% enhancement of insulin-induced triglyceride accumulation in 3T3-L1 cells.

dimethylmalonate JTP-20993 was found to be the optimum compound and was selected as a successor to JTT-501.

Chemistry

Scheme 1 shows the preparation of 1,3-dicarbonyl compounds containing isoxazolidine-3,5-dione. Acetylation of *N*-benzoyl-L-aspartic acid β-methyl ester (I) with acetic anhydride and subsequent decarboxylation gave the 4-oxovalerate (II). The methyl oxazolylacetate (III) was prepared by cyclization of the 4-oxovalerate (II). Then (III) was reduced with lithium borohydride and converted to a methylsulfonyl ester (IV). The aldehyde (V) was synthesized by coupling (IV) and 4-hydroxybenzaldehyde. Knoevenagel condensation between (V) and dimethyl malonate, followed by subsequent catalytic hydrogenation with 5% palladium on carbon, gave the dimethyl malonate (JTP-20993). The 1,3-diketone **11** was prepared from (V) and 2,4-pentanedione using the same procedure. Isoxazolidine-3,5-dione (JTT-501) was synthesized by coupling JTP-20993 and hydroxylamine. Hydrolysis of JTP-20993 with more than two equivalents of sodium hydroxide gave malonic acid **9**. The diamide **8** was prepared from JTP-20993 with ammonia using strongly basic catalysis. Partial hydrolysis of JTP-20993 with one equivalent of sodium hydroxide gave the half ester **10**. The Schotten-Baumann reaction between an acid chloride of the half ester **10** and ammonia gave the amide ester **7**. The malonic amide **6** was prepared by hydrolysis of the amide ester **7** or reduction of JTT-501 with 5% palladium on carbon as the catalyst.

Structure-activity relationships

Because acidity of the thiazolidine-2,4-dione moiety is considered essential for its insulin-sensitizing activity (22), isoxazolidine-3,5-dione (JTT-501) was designed as a more acidic heterocyclic compound than thiazolidine-2,4-dione. The pK_a values of thiazolidinedione and isoxazolidinedione were reported to be 6.82 (31) and 1.86 (32), respectively. Insulin-sensitizing activity was evaluated by the effect on insulin-regulated differentiation, which was monitored from the accumulation of triglycerides in 3T3-L1 cells (33). The triglyceride level was measured after incubation of these cells with insulin (10 ng/ml) and test compounds for 4 days. JTT-501 caused a 50% increase in triglyceride accumulation in 3T3-L1 cells at a concentration of 110 nM (Table I). Although JTT-501 was less potent than its parent thiazolidinedione **1**, it was equipotent as pioglitazone and troglitazone.

The main metabolite in humans was identified as malonic amide **6** created by reductive cleavage of JTT-501. The *in vitro* insulin-sensitizing activity of **6** was as potent as that of JTT-501 (Table I). However, the nonacidic amide ester **7** was 100 times more potent than either JTT-501 or **6**. Therefore, the necessity for acidity of these compounds was questioned and the 1,3-dicarbonyl structure, which was common to all three compounds (JTT-501, **6** and **7**), seemed to be more important.

The nonacidic diamide **8** also showed equipotent activity with the amide ester **7**. The dicarboxylic acid **9**, with a pK_a value of < 3, was less potent than the nonacidic compounds **7** and **8**, indicating that acidic functionality of the 1,3-dicarbonyl moiety was not essential. The diester (JTP-20993) and the half ester **10** were more potent than the amide ester **7**, the diamide **8** and the dicarboxylic acid **9**. The 1,3-diketone **11** showed decreased potency relative to the dicarboxyl compounds, which may have been attributable to the presence of keto-enol tautomerism. The keto content of **11** was 37% as measured by proton NMR. Among the 1,3-dicarbonyl compounds, the dimethyl malonate (JTP-20993) and monomethyl malonate **10** showed the highest potency *in vitro*, indicating that a methyl ester combined with another methyl ester or a carboxylic acid was the optimum structure. Since JTP-20993 is partially hydrolyzed to **10** *in vitro* and both JTP-20993 and **10** are probably hydrolyzed to the less potent dicarboxylic acid **9**, it is difficult to decide which is the best combination, *i.e.*, methyl ester plus methyl ester or methyl ester plus carboxylic acid. JTP-20993 caused a 50% increase in triglyceride accumulation in 3T3-L1 cells at a concentration of 59 pM and was about 7 times more potent than the corresponding thiazolidinedione **1**. Therefore, the dimethyl malonate structure was concluded to be superior to the thiazolidinedione structure.

This study revealed the potent antihyperglycemic activity of a series of 1,3-dicarbonyl compounds which could replace the thiazolidine-2,4-dione structure. The cyclic 1,3-dicarbonyl derivative JTT-501 has advanced to clinical development. In addition, the acyclic 1,3-dicarbonyl derivative JTP-20993, which was one of the optimum compounds, has been selected as a successor to JTT-501.

Table II: Hypoglycemic effect in KKAy mice.

	ED_{25} (mg/kg/day)	ED_{50} (mg/kg/day)	Ref.
JTT-501	38	238	20, 34
JTP-20993	0.011	0.17	30
9	0.44	2.7	30
10	0.024	0.12	30
Troglitazone	170	>323	30, 34
Pioglitazone	4.6	30	30, 34

Effective dose for a 25% and 50% decrease of blood glucose in genetically diabetic and obese KKAy mice.

Table III: Insulin-lowering and triglyceride-lowering effect in KKAy mice

	ED_{50}(mg/kg/day		
	Insulin	Triglyceride	Ref.
JTT-501	234	206	34
Troglitazone	>323	>323	34
Pioglitazone	31	>108	34

Effective dose for 50% decrease of blood insulin and triglyceride levels in generically diabetic and obese KKAy mice.

Effects on hyperglycemia, hyperinsulinemia and hypertriglyceridemia

In vivo insulin-sensitizing activity was evaluated by the effects on hyperglycemia, hyperinsulinemia and hypertriglyceridemia in genetically diabetic KKAy mice (34). The KKAy mouse is a model of type II diabetes that features all three conditions (35). The test compounds were mixed into the powdered animal diet and were administered for 4 days. The oral ED_{25} values of JTT-501, troglitazone and pioglitazone were 38, 170 and 4.6 mg/kg/day, respectively (Table II). The *in vivo* hypoglycemic activity of JTT-501 was about 4 times greater than that of troglitazone and 1/8 of that of pioglitazone. The oral ED_{25} values of JTP-20993 and compound **10** were 0.011 and 0.024 mg/kg/day, respectively, while the oral ED_{50} values were 0.17 and 0.12 mg/kg/day, respectively. This dispersion of the *in vivo* data may be attributed to the unstable ester structure which is readily hydrolyzed *in vivo* by esterases. The oral ED_{50} values of JTT-501 for glucose-, insulin- and triglyceride-lowering activity were 238, 234 and 206, respectively, and thus these actions were equipotent. On the other hand, the oral ED_{50} values of pioglitazone for glucose-, insulin- and triglyceride-lowering activity were 30, 31 and > 108, respectively, indicating that its effect on serum triglycerides was less potent than its effects on serum glucose and serum insulin (Table III). The fact that the triglyceride-lowering activity did not parallel the hypoglycemic activity indicates that these activities may be mediated by different pathways.

Effect on peroxisome proliferator-activated receptors

Some interesting reports on the molecular mechanism of the insulin-sensitizing action of thiazolidinediones have recently been published (36, 37). The Glaxo group showed that thiazolidinedione was an agonist for the peroxisome proliferation-activated receptor-γ (PPAR-γ), an orphan member of the nuclear hormone receptor superfamily that is expressed in high levels in adipocytes. Since PPAR-γ is a central regulator of adipocyte gene expression and differentiation, the authors proposed that it was a molecular target for the adipogenic effect of thiazolidinediones.

The activation of both PPAR-γ and PPAR-α by various compounds was evaluated using the reporter gene assay. NIH/3T3 cells were transfected with a receptor plasmid for the chimera of the PPAR-γ or PPAR-α ligand-binding domain and the CAL4 DNA-binding domain, together with a receptor plasmid containing a GAL4-responsive promoter driving the expression of luciferase. These cells were cultured for 2 days with the test compounds and luciferase activity was measured as PPAR activation. JTT-501 activated PPAR-γ (EC 5-fold = 0.28 μM), and this action was probably related to the compound's antidiabetic effect. The thiazolidinediones troglitazone (EC 5-fold = 0.24 μM) and pioglitazone (EC 5-fold = 0.16 μM) had similar effects. On the other hand, JTT-501 had a more potent activating effect on PPAR-α (EC 5-fold = 5.4 μM) as compared to troglitazone (no effect) and pioglitazone (EC 5-fold = 25 μM). PPAR-α is reported to be related to lipid metabolism and; therefore the triglyceride-lowering activity of JTT-501 may be enhanced by its PPAR α-activating effect.

Conclusions

There is a great need to improve glycemic control in type II diabetes in order to prevent diabetic complications. The thiazolidinedione insulin sensitizers have contributed to the improvement of glycemic control in type II diabetic patients with insulin resistance. However, there are many nonresponders to thiazolidinediones and these drugs have no effect on glucosamine-induced insulin resistance (38). Therefore, the development of different types of insulin sensitizers is desirable. Further investigation of nonthiazolidinediones, including JTT-501, may lead to a second generation of insulin sensitizers with different biological characteristics from those of the thiazolidinediones.

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