Troglitazone Prevents the Rise in Visceral Adiposity and Improves Fatty Liver Associated With Sulfonylurea Therapy—A Randomized Controlled Trial

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Monotherapy with sulfonylurea may result in the exhaustion of pancreatic β-cell function, fat accumulation, and dyslipidemia. We examined the possibility of dose reduction by administering sulfonylurea together with troglitazone, and investigated changes in insulin secretion and fat deposition. Seventy-eight patients with type 2 diabetes adequately controlled with glibenclamide were randomly allocated to a troglitazone (400 mg/d)-added group (n = 40) or a control group without placebo (n = 38) and monitored for 24 weeks. The daily dose of glibenclamide was adjusted to maintain stable HbA_{1c} levels. Fat accumulation to the liver and thigh muscle were measured in mean Hounsfield units determined on computed tomography (CT) scan. Visceral fat accumulation (V), subcutaneous fat accumulation (S), and the V/S ratio were also determined by CT scan. The daily dose of glibenclamide and serum fasting insulin level in the troglitazone-added group significantly decreased (from 4.05 ± 2.50 mg/d to 1.84 ± 1.65 mg/d and from 8.47 ± 4.62 μU/mL to 6.49 ± 3.28 μU/mL, respectively) during the observation period compared with the control group ($P < .01$ and $P < .01$, respectively). Serum triglyceride and homeostasis model insulin resistance index (HOMA-R) in the troglitazone-added group decreased significantly in comparison to the control group ($P < .05$ and $P < .01$, respectively). The mean Hounsfield units of liver significantly decreased in the control group compared with the troglitazone-added group ($P < .05$). Visceral fat area and the V/S ratio significantly increased in the control group compared with the troglitazone-added group ($P < .01$ and $P < .01$, respectively). Glibenclamide monotherapy resulted in fat accumulation accompanied by dyslipidemia. An alternate conclusion is that troglitazone reversed type 2 diabetes (not sulfonylurea)-associated fat accumulation. The addition of troglitazone decreased daily doses of glibenclamide, preserved fasting insulin secretion, improved fat accumulation in liver, and prevented dyslipidemia.

TROGLITAZONE, a thiazolidinedione derivative, has been reported to have a novel antidiabetic effect by improving insulin resistance and hyperglycemia in type 2 diabetic patients. As an added benefit, troglitazone can also improve blood lipid levels in these patients. Many studies suggest that the main mechanism of action of troglitazone at the organ level is enhanced glucose uptake into the skeletal muscle and inhibition of excessive hepatic glucose production.[1]

In vitro experimental systems have demonstrated that a thiazolidinedione derivative promotes the differentiation of preadipose cells into fat cells. Peroxisome proliferation-activated receptor-γ (PPAR-γ) plays an important role in the process of preadipocyte differentiation. Thiazolidinediones are ligands for the activation of PPAR-γ.[2] Sulfonylureas are widely prescribed to patients with type 2 diabetes. Sulfonylureas stimulate insulin secretion from pancreatic β cells, and body weight gain has been reported in patients with type 2 diabetes treated with sulfonylureas.

The results of the United Kingdom Prospective Diabetes Study (UKPDS) revealed no significant increases in cardiovascular complications in type 2 diabetic patients during sulfonylurea treatment. However, body weight gain over a 10-year period was about 6 kg.[3] Any weight gain may further aggravate insulin resistance and cause compensatory hyperinsulinemia. It has been reported that hyperinsulinemia not only facilitates fat synthesis/deposition, but is also a risk factor for cardiovascular complications.[4] In type 2 diabetic patients, who already have multiple risk factors for cardiovascular complications, weight gain accompanied by increases in fat accumulation in the visceral fatty tissue, skeletal muscles, and liver may adversely affect long-term prognoses.[5-7]

Preservation of the ability to secrete endogenous insulin is an important issue in type 2 diabetic patients. Deterioration of β-cell function is an important component of type 2 diabetes. However, only a few clinical reports on a decrease in endogenous insulin secretion due to excessive stimulation of insulin secretion by a sulfonylurea have been published.[8] In consideration of the excessive load imposed on the remaining β cells by insulin secretion, such load with endogenous insulin secretion should be reduced as much as possible. Improvement of hyperinsulinemia also decreases the risk of cardiovascular complications.[4]

We examined the possibility of reducing the treatment dose of sulfonylurea for type 2 diabetic patients by administering it together with troglitazone, and examined the changes in insulin secretion and fat deposition and distribution that took place under this combination therapy.

MATERIALS AND METHODS

We performed a pilot study to determine the sample size. Fifty outpatients with type 2 diabetes treated with glibenclamide at the Kawaguchi Municipal Medical Center were examined to determine the mean daily dose of glibenclamide, which was 3.9 ± 3.066 mg/d. Although this dose may be low compared with the dose in North America, the maximum dose of glibenclamide was restricted to 10 mg/d by a Japanese regulation. A primary outcome measure is dose reduction of glibenclamide from 4.0 mg/d to 2.0 mg/d. To ensure an 80% chance of finding a significant difference using a 2-sided significance test would require a sample size of 37 patients in each group. Seventy-eight outpatients with type 2 diabetes adequately controlled

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(HbA_{1c}, 6.782% ± 0.605%) with glibenclamide at the Kawaguchi Municipal Medical Center were randomly allocated to a troglitazone (400 mg/d)-added group (n = 40) and a control group without placebo (n = 38) by envelope method and monitored for 24 weeks. All patients provided informed consent and were enrolled from November 1997 through May 1998. All had received dietary supervision in the past. At the start of the open trial, patients were instructed to continue on the same diet therapy as before. The daily dose of glibenclamide was adjusted to maintain HbA_{1c} at baseline level. The daily dose of glibenclamide was decreased every 2 months by 2.5 mg/d or 1.25 mg/d if the HbA_{1c} level decreased by 0.5% or 0.25%, respectively.

Fat accumulation in the liver, psoas muscle, and thigh muscle was evaluated by mean Hounsfield units of liver (LmHU), psoas muscle (PmHU) at the umbilicus level, and thigh muscle (TmHU) at the mid-femoral level by abdominal computed tomography (CT) scan.[6,7] Body fat distribution was determined by CT imaging according to the procedure of Tokunaga et al.[5] Most of the radiographs covered a window close to 500 HU and centered at approximately -40 HU. The subcutaneous fat layer was clearly defined between the skin and muscle in the range from about -40 to -140 HU. The intraperitoneal part had the same density as the subcutaneous fat layer and was considered the visceral fat area. The total cross-cut area, subcutaneous fat area, muscle plus bone, intraperitoneal area, and visceral fat area were respectively measured at the umbilicus level in all subjects. Each area (cm^2) was measured by tracing object contours on the picture of each individual scan using the computerized planimetric method. The ratio of the intra-abdominal visceral fat area (V) to subcutaneous fat area (S), a value adopted as an index of the relative amount of visceral fat, was calculated. Total body fat composition (%fat) was evaluated by the bioelectrical impedance analysis method.[9] Fasting immunoreactive insulin (IRI) and plasma C-peptide immunoreactivity (CPR) were measured in both groups before and after the study. The homeostasis model insulin resistance index (HOMA-R) (fasting glucose [mmol/L] × fasting insulin [mU/L]/22.5) was used for simple assessment of insulin sensitivity.[10] The blood glucose level was determined by the hexokinase method, IRI by the enzyme-linked immunosorbent assay (ELISA) method, HbA_{1c} by high-performance liquid chromatography (HPLC) (Shionogi, Settsu, Osaka, Japan), and lipid and hepatic function by enzymatic methods.

All values are the means ±SD. A repeated-measures ANOVA was applied to detect significant differences and interactions between groups. *P* values less than .05 were considered to be statistically significant. All analyses were completed on an intention-to-treat basis.

Table 1. Clinical Profiles of Patients

	Troglitazone-Added Group	Control Group
No. of patients	40	38
Gender		
Male	27	28
Female	13	12
Age (yr)	59.425 ± 8.676	58.421 ± 9.220
Body mass index (kg/m^2)	25.274 ± 3.867	24.822 ± 3.816
%Fat	27.617 ± 7.732	30.874 ± 14.257
Duration (yr)	7.128 ± 6.820	7.866 ± 6.917
Dose of glibenclamide (mg/d)	4.052 ± 2.499	3.651 ± 2.717
HbA_{1c} (%)	6.862 ± 0.568	6.701 ± 0.639
Fasting plasma glucose (mg/dL)	146.974 ± 33.368	146.184 ± 30.791
Fasting IRI (μU/mL)	8.470 ± 4.620	6.863 ± 2.952
Fasting CPR (ng/mL)	2.594 ± 1.230	2.122 ± 0.790
HOMA-R	3.357 ± 2.939	2.535 ± 1.355
Aspartate aminotransferase (IU/L)	24.000 ± 8.373	22.026 ± 6.537
Alanine aminotransferase (IU/L)	26.175 ± 12.782	26.447 ± 15.335
Cholineesterase (U/mL)	6.093 ± 1.176	5.847 ± 1.201
γ-Glutamyltransferase (IU/mL)	31.100 ± 23.302	23.789 ± 16.017
Total cholesterol (mg/dL)	197.125 ± 38.737	202.421 ± 32.599
Triglyceride (mg/dL)	133.846 ± 77.211	124.026 ± 60.472
HDL cholesterol (mg/dL)	53.744 ± 10.615	56.216 ± 14.591
Apolipoprotein A I (mg/dL)	126.684 ± 19.566	125.632 ± 24.275
Apolipoprotein B (mg/dL)	103.162 ± 24.633	95.184 ± 21.217
Apolipoprotein B/A I	0.836 ± 0.247	0.793 ± 0.258
Blood pressure		
Systolic (mm Hg)	144.162 ± 15.939	139.921 ± 12.667
Diastolic (mm Hg)	84.920 ± 9.832	81.974 ± 7.027
Visceral fat area (cm^2)	126.624 ± 57.551	112.393 ± 51.584
Subcutaneous fat area (cm^2)	145.176 ± 73.815	152.366 ± 97.327
V/S ratio	0.960 ± 0.370	0.874 ± 0.416
Mean Hounsfield unit		
Liver	55.199 ± 9.762	55.478 ± 7.314
Psoas muscle	47.708 ± 5.634	48.792 ± 5.262
Thigh muscle	46.849 ± 7.897	46.765 ± 4.819

RESULTS

All patients completed the trial. Their baseline characteristics are compared in Table 1. The groups were similar in clinical profiles. Fasting plasma glucose, fasting plasma CPR, and HOMA (resistance) were slightly higher in troglitazone-added group, but not to a significant degree. Mean fasting plasma glucose levels in both groups before treatment were somewhat greater than the published guidelines, which recommend fasting plasma glucose levels be maintained at less than 125 mg/dL.[11]

Interactions between the 2 groups were tested by repeated-measures ANOVA (Table 2). There were no significant differences in body mass index, %fat, HbA_{1c}, and fasting plasma glucose between the groups during the observation period. In the troglitazone-added group, daily dose of glibenclamide, serum fasting IRI, fasting CPR, alanine aminotransferase, γ-glutamyltransferase, triglyceride, and HOMA-R decreased significantly ($P < .01$, $P < .01$, $P < .01$, $P < .01$, $P < .01$ $P < .05$, and $P < .01$, respectively) after 24 weeks in comparison to values in the control group. Serum high-density lipoprotein cholesterol in the troglitazone-added group significantly ($P < .05$) increased after 24 weeks compared with that in the control group. There were no significant changes in atherogenic apolipoprotein index (apolipoprotein B/AI) in the troglitazone-added group during the observation period. The index in the control group increased during the same period, although the interaction was not statistically significant. LmHU decreased significantly in the control group compared with the troglitazone-added group ($P < .05$). A low mean Hounsfield unit suggests fat deposition into the tissue.[7,12] PmHU and TmHU decreased in both groups. We found that visceral fat area and the V/S ratio significantly decreased in the troglitazone-added group compared with the control group ($P < .01$ and $P < .01$, respectively), although subcutaneous fat area increased in both groups during the observation period (Table 2).

Table 2. Comparison of Status in Patients of Troglitazone-Added Group and Control Group

	Troglitazone-Added Group		Control Group		
	0 wk	24 wk	0 wk	24 wk	*P* Value*
Body mass index (kg/m^2)	25.274 ± 3.867	25.230 ± 3.880	24.822 ± 3.816	25.565 ± 4.693	NS
%Fat	27.617 ± 7.732	27.183 ± 7.656	30.874 ± 14.257	27.162 ± 9.216	NS
Dose of glibenclamide (mg/d)	4.052 ± 2.499	1.844 ± 1.650	3.651 ± 2.717	3.784 ± 3.194	<.01
HbA_{1c} (%)	6.862 ± 0.568	6.823 ± 0.966	6.701 ± 0.639	6.895 ± 0.764	NS
Fasting plasma glucose (mg/dL)	146.974 ± 33.368	137.000 ± 30.650	146.184 ± 30.791	139.324 ± 28.533	NS
Fasting IRI (μU/mL)	8.470 ± 4.620	6.492 ± 3.275	6.863 ± 2.952	7.637 ± 3.525	<.01
Fasting CPR (ng/mL)	2.594 ± 1.230	1.905 ± 0.841	2.122 ± 0.790	2.308 ± 1.515	<.01
HOMA-R	3.357 ± 2.939	2.315 ± 1.725	2.535 ± 1.355	2.703 ± 1.459	<.01
Aspartate aminotransferase (IU/L)	24.000 ± 8.373	23.050 ± 7.706	22.026 ± 6.537	23.474 ± 7.986	NS
Alanine aminotransferase (IU/L)	26.175 ± 12.782	21.026 ± 12.938	26.447 ± 15.335	30.395 ± 23.671	<.01
Cholineesterase (U/mL)	6.093 ± 1.176	5.941 ± 1.269	5.847 ± 1.201	5.759 ± 1.230	NS
γ-Glutamyltransferase (IU/mL)	31.100 ± 23.302	20.200 ± 18.432	23.789 ± 16.017	26.368 ± 17.232	<.01
Total cholesterol (mg/dL)	197.125 ± 38.737	208.650 ± 25.260	202.421 ± 32.599	201.632 ± 26.201	NS
Triglyceride (mg/dL)	133.846 ± 77.211	111.026 ± 50.069	124.026 ± 60.472	131.395 ± 84.298	<.05
HDL cholesterol (mg/dL)	53.744 ± 10.615	61.025 ± 15.266	56.216 ± 14.591	57.342 ± 16.743	<.05
Apolipoprotein A I (mg/dL)	126.684 ± 19.566	137.425 ± 18.656	125.632 ± 24.275	138.079 ± 21.635	NS
Apolipoprotein B (mg/dL)	103.162 ± 24.633	104.103 ± 18.496	95.184 ± 21.217	102.000 ± 16.778	NS
Apolipoprotein B/A I	0.836 ± 0.247	0.844 ± 0.231	0.793 ± 0.258	0.849 ± 0.234	NS
Blood pressure					
Systolic (mm Hg)	144.162 ± 15.939	145.250 ± 12.215	139.921 ± 12.667	143.265 ± 18.323	NS
Diastolic (mm Hg)	84.920 ± 9.832	84.075 ± 7.862	81.974 ± 7.027	83.853 ± 12.896	NS
Visceral fat area (cm^2)	126.624 ± 57.551	113.734 ± 57.953 $\Delta = -10.2\%$	112.393 ± 51.584	126.124 ± 54.293 $\Delta = +12.2\%$	<.01
Subcutaneous fat area (cm^2)	145.176 ± 73.815	168.615 ± 107.467 $\Delta = +16.1\%$	152.366 ± 97.327	168.241 ± 112.316 $\Delta = +10.4\%$	NS
V/S ratio (by CT)	0.960 ± 0.370	0.796 ± 0.370	0.874 ± 0.416	0.912 ± 0.439	<.01
Mean Hounsfield unit					
Liver	55.199 ± 9.762	53.362 ± 6.771	55.478 ± 7.314	50.294 ± 9.276	<.05
Psoas muscle	47.708 ± 5.634	45.204 ± 10.239	48.792 ± 5.262	44.760 ± 4.453	NS
Thigh muscle	46.849 ± 7.897	41.851 ± 6.755	46.765 ± 4.819	43.500 ± 4.802	NS

* *P* values were evaluated for the variances for treatment by time interaction by a repeated-measures ANOVA. NS, not significant.

CONCLUSION

In the present study, the patients satisfactorily followed diet therapy during the 24-week observation period, and no significant weight gain was observed in either group, unlike in the UKPDS. However, a single treatment with a sulfonylurea increased fat depositions in the liver and visceral adipose tissue, which was accompanied by worsened serum triglyceride level. Who can definitely say that fat deposition and lipid metabolism disturbance have no adverse effects on long-term prognosis over 20 or 30 years?[13,14] In the troglitazone-added group, fat deposition in the liver was decreased, and no worsening of serum triglyceride level was observed. However, troglitazone failed to significantly inhibit lipid depositions in the skeletal muscle and subcutaneous adipose tissue, which are target organs of troglitazone. Because inhibition of endogenous insulin secretion by troglitazone was observed from a relatively early period after the start of administration, it is inferred that a decrease in blood insulin level over a long period of time resulted in the inhibition of fat deposition in the liver. Insulin action in skeletal muscle and adipose tissue is enhanced by troglitazone, offsetting the inhibition of fat depositions in the skeletal muscle and subcutaneous adipose tissue. However, there is another possibility. Fat deposition in the liver may be inhibited by the decrease in free fatty acid level in portal vein by troglitazone[15] or its direct effect on hepatocytes.[16] Although we measured aspartate aminotransferase and alanine aminotransferase levels every month to detect liver dysfunction, there was no liver damage in the troglitazone-added group during the observation period. Long-term troglitazone treatment improved fatty liver. Based on the results of the present study, it can be hypothesized that the inhibition of fat deposition in the liver by troglitazone is a mechanism of beneficial action of troglitazone for glucose and lipid metabolism in type 2 diabetic patients. Mori et al reported not only a selective increase in subcutaneous fat accumulation, but also an increase in visceral fat accumulation by adding troglitazone to sulfonylurea therapy.[17] Differing from their report, we observed no selective increase in visceral fat accumulation after adding troglitazone to sulfonylurea therapy. In the present study, a marked decrease in blood insulin level was successfully achieved while the dose of sulfonylurea was reduced with the purpose of maintaining HbA_{1c} at a certain level. This could offset the visceral fat accumulation, resulting in a significant decrease in V/S ratio, in addition to a decrease in visceral fat. Differences in visceral fat accumulation with troglitazone may also be related to the length of treatment. However, the subcutaneous fat area and the body weight increased in obese women with type 2 diabetes, although troglitazone sufficiently controlled their blood glucose

levels. The difference in the response of men and women to the troglitazone-glibenclamide combination may due to the difference in the area of subcutaneous fat at the baseline. At baseline, women had a large subcutaneous area compared with men in the troglitazone-treated group. A hormonal difference and/or genetic difference of gender may also contribute to the dissimilarity. Prescribing troglitazone for type 2 diabetic patients who cannot control food intake and who cannot exercise well may result in the secondary failure because the capacity of fat deposition is not infinite.

With regard to the preservation of endogenous insulin secretion, the results of our clinical study clearly indicate that the addition of troglitazone to sulfonylurea therapy can reduce the dose of sulfonylurea, even in type 2 diabetic patients whose blood glucose levels have been satisfactorily controlled with the sulfonylurea alone, and that troglitazone is expected to decrease the load on β cells by insulin secretion and to preserve the ability of insulin secretion.[18] Although the UKPDS data show that the slopes for decline in β-cell function are equivalent between metformin and sulfonylurea, metformin treatment shifted the slope upwards and to the right. This, and our data, suggest that insulin-preserving antidiabetic agents such as metformin and thiazolidinedione may delay β-cell failure compared with single-agent sulfonylurea treatment. Most Japanese patients with type 2 diabetes genetically have a low secretory capacity of insulin to compensate for insulin resistance and hyperglycemia. Therefore, a slight deterioration of hyperinsulinemia and insulin resistance from a single treatment with sulfonylurea may result in an overload of insulin secretion.[19,20] Since these results were obtained from Japanese type 2 diabetic patients with mild obesity, larger effects might expected in type 2 diabetic patients with severe obesity in North Europe and North America.

The sale of troglitazone was voluntarily discontinued in the United States. There have not been any death cases caused by troglitazone in Japan since the warning of possible liver damage.

Further fundamental and clinical studies are necessary to verify the above findings and hypotheses regarding thiazolidiedione.

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