Effect of Troglitazone on Microalbuminuria in Patients With Incipient Diabetic Nephropathy

EIICHI IMANO, MD
TSUTOMU KANDA, MD
YOSHIHISA NAKATANI, MD
TSUTOMU NISHIDA, MD
KATSUMI ARAI, MD
MASAAKI MOTOMURA, MD
YOSHITAKA KAJIMOTO, MD
YOSHIMITSU YAMASAKI, MD
MASATSUGU HORI, MD

OBJECTIVE — Although some studies have suggested a direct action of troglitazone on vascular cells, its effects on diabetic vascular diseases have not been reported. We therefore investigated the effect of troglitazone on microalbuminuria in patients with incipient diabetic nephropathy.

RESEARCH DESIGN AND METHODS — A total of 30 patients with type 2 diabetes associated with microalbuminuria (urinary albumin-to-creatinine ratio [ACR] [milligrams per gram creatinine] ranging from 30 to 300 mg/g creatinine) were studied. They were randomly divided into two groups: patients treated with metformin (500 mg/day, $n = 13$) or with troglitazone (400 mg/day, $n = 17$) for 12 weeks. ACR, lipid profile, blood pressure, glycated hemoglobin, and plasma glucose during meal-load tests were measured every 4 weeks.

RESULTS — Anthropometric indices (BMI and percent fat), lipid profile, and blood pressure did not change with either treatment. Fasting and postmeal glucose levels decreased similarly in the two groups. Decrements in glycated hemoglobin were greater in the metformin group at 4 and 8 weeks after the initiation of treatment ($P < 0.05$). Troglitazone reduced ACR (median [25–75th percentiles]) from 70 (49–195) to 40 (31–90) mg/g creatinine at 4 weeks ($P = 0.021$) and maintained these reduced levels throughout the treatment period (8 weeks: 35 [26–68], $P = 0.007$; 12 weeks: 43 [26–103], $P = 0.047$). Metformin did not change ACR throughout the 12 weeks.

CONCLUSIONS — Troglitazone ameliorated microalbuminuria in diabetic nephropathy. Furthermore, our findings suggest that troglitazone has some effects on vascular cells other than lowering plasma glucose levels. Troglitazone might be useful for diabetic angiopathy, including nephropathy and coronary artery disease.

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Diabetic nephropathy is a serious health issue in many countries. The significance of urinary albumin excretion, a predictor of diabetic nephropathy, in type 1 diabetes has been well established. Furthermore, in patients with type 2 diabetes, overt diabetic nephropathy has developed with microalbuminuria. In fact, the existence of microalbuminuria in type 2 diabetic subjects suggests an increased risk for advanced diabetic nephropathy (1,2). The significance of microalbuminuria was also supported by the fact that patients with microalbuminuria show a higher incidence of cardiovascular death (1,3). Therefore, patients with microalbuminuria should be identified (4) for appropriate therapy, e.g., strict glycemic control (5,6) and/or the administration of inhibitors of ACE (7).

Troglitazone, a newly developed thiazolidinedione derivative, has been shown to decrease plasma glucose levels by enhancing insulin sensitivity in patients with type 2 diabetes (8,9). Several reports also suggested that troglitazone could ameliorate the lipid profile (8,9) and reduce blood pressure (9,10). Because insulin resistance plays a major role in the pathogenesis of atherosclerotic diseases (11,12) and hypertension (12), as well as type 2 diabetes, many patients would be candidates for treatment with troglitazone (10).

Recently, other effects of thiazolidinediones that seem to be independent of the lowering of the plasma glucose level have been reported. One study showed a vasodepressor effect due to inhibition of calcium uptake by vascular smooth muscle cells (13). Animal experiments have demonstrated that troglitazone could inhibit the hyperplasia of vascular smooth muscle and intimal cells (14). These results showing direct vascular effects of thiazolidinediones prompted us to investigate the possible beneficial effects of troglitazone on diabetic vascular complications. We therefore studied the effect of troglitazone on urinary albumin excretion in patients with incipient diabetic nephropathy. Metformin, another type of oral antidiabetic drug that acts primarily on the liver, was used as a control.

RESEARCH DESIGN AND METHODS

Patients

We studied 30 patients with type 2 diabetes associated with microalbuminuria attending the outpatient clinic of our hospital. The inclusion criteria were as follows: *1*) no history of ketoacidosis, 2) treatment by diet alone or in combination with sulfonylureas (gliclazide or glibenclamide), 3) fasting C-peptide >0.33 mmol/l, 4) glycated hemo-

From the Department of Gastroenterology and Metabolic Disease (E.I., T.K., Y.N., T.N., K.A., M.M.), Osaka Prefectural General Hospital, Mandai-higashi, Sumiyoshi-ku; and the First Department of Medicine (Y.K., Y.Y., M.H.), Osaka University School of Medicine, Osaka, Japan.

Address correspondence and reprint requests to Eiichi Imano, MD, Department of Gastroenterology and Metabolic Disease, Osaka Prefectural General Hospital, 3-1-56 Mandai-higashi, Sumiyoshi-ku, Osaka, 558-8558, Japan.

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Abbreviations: ACR, urinary albumin-to-creatinine ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Baseline clinical characteristics of the study patients*

	Troglitazone	Metformin	*P* value
n	17	13	—
Age (years)	62 ± 13	66 ± 8	0.384
Sex (M/F)	5/12	3/10	0.697
Diabetic duration (years)	10 ± 3	10 ± 3	0.863
Treatment (diet/sulfonylureas)	4/13	6/7	0.181
Use of ACE inhibitors	6	3	0.377
BMI (kg/m²)	25.2 ± 3.9	24.8 ± 4.5	0.827
Fat (%)	26.1 ± 9.2	24.3 ± 5.0	0.507

Data are *n* or means ± SD. *P* values are by Student's unpaired *t* test or Fisher's exact probability test.

globin >7.5%, 5) microalbuminuria:spot urinary albumin corrected with creatinine (urinary albumin-to-creatinine ratio [ACR]) ranging from 30 to 300 mg/g creatinine (15,16). Patients with liver disease, showing an elevation of serum alanine aminotransferase levels >1.5 times the normal upper limit, were excluded from the study. Patients with hematuria or casturia or those who had a known history of nondiabetic renal diseases were also excluded. Before the study, its nature, purpose, and possible risks were explained to all patients, and voluntary informed consent was obtained. The study was approved by the institutional ethics committee.

Study design

The patients were randomly assigned into two groups: those treated with metformin 500 mg/day (*n* = 13) and those treated with troglitazone 400 mg/day (*n* = 17). They received the designated drug for 12 weeks. For those being treated with sulfonylureas and ACE inhibitors, the drug types and doses were kept constant throughout the study. The background characteristics of the patients are shown in Table 1.

Height and weight were recorded, and BMI was calculated. The percent fat was measured by bioelectrical impedance analysis using the Tanita TBF-102 Body Fat Analyzer (Tanita, Tokyo). Its reproducibility has been reported elsewhere (17). Supine blood pressure was recorded every 4 weeks with a standard clinical sphygmomanometer after 10 min of rest. Blood was withdrawn every 4 weeks after overnight fasting for analysis of total cholesterol, HDL cholesterol, triglyceride, insulin, C-peptides, complete blood count, and liver function tests by standard laboratory technique. The levels of glycated hemoglobin were measured with high-performance liquid chromatography with a reference value of 3.5–6.5%. A 2-h meal-load test (the meal of 428 kcal was consumed at 9:00 A.M.) was performed at every visit, and glucose and insulin responses were evaluated.

Spot urine was collected every 4 weeks. After the pretest urination (8:30–10:00 A.M.), patients were asked to avoid exercise for 1 h, and the accumulated urine was measured for albumin by radioimmunoassay. Urinary creatinine concentration was also measured with the modified Jaffe method, and ACR was calculated. Subjects with urinary tract infections were excluded by testing.

Statistical analysis

Data are given as means ± SD, unless otherwise stated. The values of ACR were transformed into $\log_{10}$ for calculations because of their skewed distribution. Comparisons at different time points were made after accounting for the baseline value with the use of two-way ANOVA for repeated measures. Comparisons in metabolic measures before and 12 weeks after the treatments were done by Student's two-tailed *t* test. Proportions were compared by Fisher's exact probability test. A value of $P < 0.05$ was considered significant.

RESULTS

Patients

Baseline clinical characteristics and metabolic values before each treatment were compared (Tables 1 and 2). There were no significant differences in any of the variables between the two groups.

Anthropometric parameters

BMI did not change throughout the 12-week treatment in the troglitazone group (25.2 ± 3.9 vs. 25.3 ± 3.5 kg/m²) or the metformin group (24.8 ± 4.5 vs. 24.3 ± 5.1

Table 2—*Metabolic measurements before and 12 weeks after the administration of troglitazone or metformin in patients with incipient diabetic nephropathy*

	Troglitazone			Metformin		
	Before	After	*P* value	Before	After	*P* value
Fasting plasma glucose (mmol/l)	11.2 ± 2.5	9.1 ± 2.8	0.003	10.4 ± 2.3	9.5 ± 2.7	0.012
Postmeal plasma glucose (mmol/l)	14.2 ± 3.0	11.9 ± 3.8	0.017	13.3 ± 2.4	12.6 ± 3.3	0.330
Fasting plasma insulin (pmol/l)	42.6 ± 23.4	33.0 ± 13.2	0.035	40.2 ± 18.6	37.8 ± 23.4	0.455
Postmeal plasma insulin (pmol/l)	99.0 ± 56.4	92.4 ± 58.2	0.414	96.0 ± 36.5	84.0 ± 48.0	0.681
Fasting plasma C-peptide (nmol/l)	0.76 ± 0.26	0.76 ± 0.23	0.970	0.79 ± 0.43	0.96 ± 0.50	0.254
Glycated hemoglobin (%)	8.9 ± 1.0	8.3 ± 1.4	0.010	8.8 ± 0.8	7.9 ± 0.8	0.001
Total cholesterol (mmol/l)	5.54 ± 0.81	5.77 ± 0.96	0.228	5.33 ± 1.01	5.36 ± 0.78	0.809
Triglyceride (mmol/l)	1.90 ± 0.82	1.81 ± 0.78	0.589	1.34 ± 0.76	1.25 ± 0.56	0.523
HDL cholesterol (mmol/l)	1.35 ± 0.31	1.38 ± 0.26	0.631	1.43 ± 0.29	1.51 ± 0.39	0.351
Systolic blood pressure (mmHg)	148 ± 19	145 ± 20	0.731	147 ± 17	148 ± 20	0.836
Diastolic blood pressure (mmHg)	80 ± 23	80 ± 8	0.966	71 ± 8	76 ± 10	0.200

Data are means ± SD. *P* values are by Student's paired *t* test.

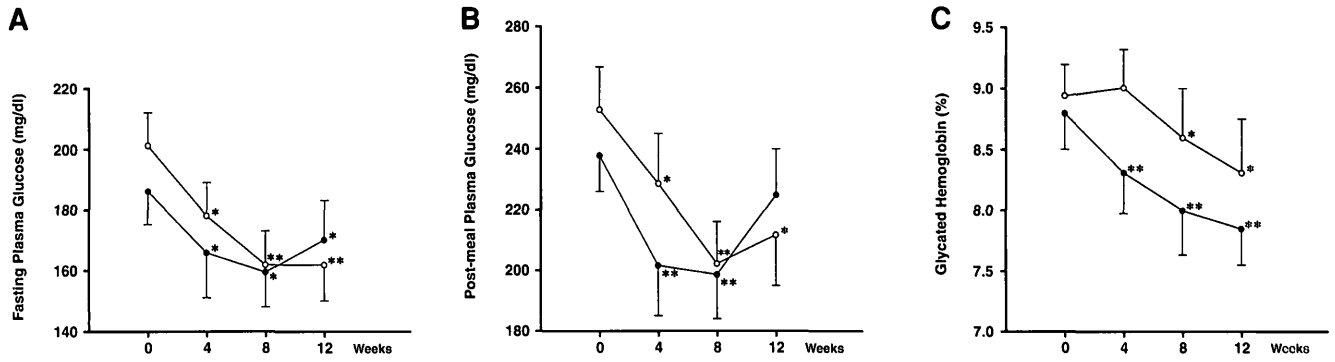

Figure 1—*Changes in glycemic parameters in patients treated with troglitazone or metformin.* A: *Fasting plasma glucose concentrations;* B: *2-h postmeal plasma glucose concentrations;* C: *glycated hemoglobin levels.* ○, *troglitazone* (n = *17*); ●, *metformin* (n = *13*). *Statistically significant change from week 0:* *P < *0.05*, **P < *0.01*. *Values are means ± SEM.*

kg/m²). Similarly, percent fat did not change in the troglitazone group (26.1 ± 9.2 vs. 26.7 ± 9.4%) or the metformin group (24.3 ± 5.0 vs. 24.4 ± 9.8%).

Plasma glucose and insulin

Changes in fasting and postmeal plasma glucose and glycated hemoglobin levels are illustrated in Fig. 1. Troglitazone significantly reduced the fasting plasma glucose level from 11.2 ± 2.5 mmol/l (week 0) to 9.9 ± 2.5 mmol/l (4 weeks; $P < 0.05$). The reduced level was maintained throughout the treatment (9.1 ± 2.2 mmol/l at 8 weeks [$P < 0.01$] and 9.1 ± 2.8 mmol/l at 12 weeks [$P < 0.01$]). Comparable reductions in fasting plasma glucose level were observed in the patients treated with metformin (Fig. 1*A*). Plasma glucose levels 2 h after test-meal ingestion decreased from 14.2 ± 3.0 mmol/l (week 0) to 12.8 ± 3.8 mmol/l (4 weeks; $P < 0.05$), 11.3 ± 3.4 mmol/l (8 weeks; $P < 0.01$), and 11.9 ± 3.8 mmol/l (12 weeks; $P < 0.05$) in the troglitazone group. Similar changes were noted in the metformin group, except that the value rose in 12 weeks and did not differ from the pretreatment level (Fig. 1*B*). Fasting and postmeal plasma insulin levels did not change throughout the study, except that the fasting insulin level was significantly reduced at 12 weeks in the troglitazone group (Table 2). Fasting plasma C-peptide level did not change significantly over time in either group.

Glycated hemoglobin

The glycated hemoglobin level significantly decreased, from 8.9 ± 1.0 to 8.5 ± 1.2% at 8 weeks ($P < 0.05$) and 8.2 ± 1.4% at 12 weeks ($P < 0.05$) in the troglitazone group (Fig. 1*C*). Metformin also reduced the glycated hemoglobin level from 8.8 ± 0.8 to 8.3 ± 1.0% at 4 weeks ($P < 0.01$), 8.0 ± 1.0% at 8 weeks ($P < 0.01$), and 7.9 ± 0.8% at 12 weeks ($P < 0.01$). Decrements in glycated hemoglobin at 4 and 8 weeks were greater in the metformin group than in the troglitazone group.

Plasma lipid levels and blood pressure

Neither treatment changed the concentrations of total cholesterol, triglyceride, or HDL cholesterol (Table 2). Systolic and diastolic blood pressures measured every 4 weeks were also unchanged in both groups (Table 2).

ACR

Figure 2 shows the effect of troglitazone (Fig. 2*A*) or metformin (Fig. 2*B*) on urinary albumin excretion. Data for ACR at 12 weeks after metformin administration were not available for three patients. The ACR levels at week 0 (pretreatment) were not different between the groups. Troglitazone treatment significantly reduced ACR (median [25–75th percentiles], milligrams per gram creatinine) from 70 (49–195) to 40 (31–90) at 4 weeks ($P = 0.021$), 35 (26–68) at 8 weeks ($P = 0.007$), and 43 (26–103) at 12 weeks ($P = 0.047$). The beneficial effect of troglitazone was more evident in those whose ACR levels were rel-

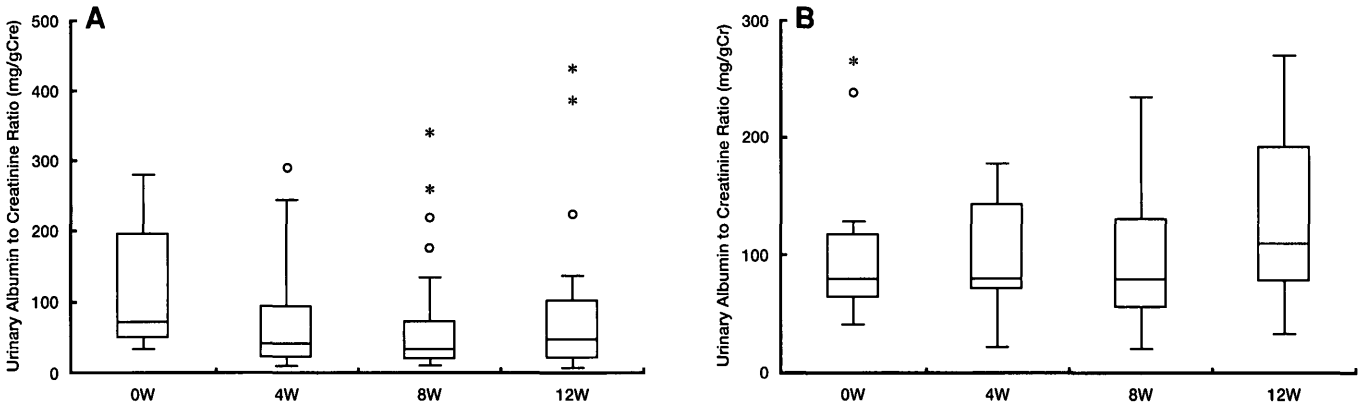

Figure 2—*Effect of troglitazone* (A) *and metformin* (B) *on ACR. The bottom and top of the box are 25th and 75th percentiles, respectively. The line in the box represents the median value. Circles indicate outliers and asterisks indicate extreme outliers. W, weeks.*

atively low: of the 13 patients with pretreatment ACR levels of 30–200 mg/g creatinine, ACR levels declined in 12 patients at 4 weeks, in 13 (all patients) at 8 weeks, and in 11 at 12 weeks. In contrast, in patients with pretreatment ACR levels of 200–300 mg/g creatinine, ACR levels declined in only 2 of 4 patients throughout the study period. Metformin treatment did not change the ACR during the 12-week treatment: 79 (64–117) at week 0, 78 (70–134) at 4 weeks, 77 (54–129) at 8 weeks, and 108 (78–186) at 12 weeks.

Safety

All 30 patients tolerated the treatments well, and no serious adverse events occurred. No patients experienced hypoglycemic episodes, and none dropped out of the study. Of the troglitazone-treated patients, a transient increase in lactate dehydrogenase was found in three. Liver function tests did not show any significant abnormalities in the two groups. A mild decrease in hemoglobin level of ~1 g/dl was found in three patients in the troglitazone group, but the hemoglobin levels remained at >12 g/dl.

CONCLUSIONS — Troglitazone is a newly developed antidiabetic drug, classified as a thiazolidinedione, that enhances insulin sensitivity, and thereby reduces the plasma glucose level (8–10). Beneficial effects have also been reported for the lipid profile (8,9) and for blood pressure (9,10). It was not known, however, whether thiazolidinediones exert preventive effects against diabetic vascular complications. In this study, 12 weeks of treatment with troglitazone and metformin produced similar changes in blood glucose. Although ACR was unchanged in the metformin group, troglitazone reduced ACR. This effect was more evident in patients with moderate degree of microalbuminuria (ACR 30–200 mg/g creatinine). These results suggest that troglitazone can reduce microalbuminuria in patients with incipient diabetic nephropathy. The decrease in ACR in incipient diabetic nephropathy may have practical implications. First, microalbuminuria has been shown to be a predictor of advanced diabetic nephropathy (1,2). Overt diabetic nephropathy develops in some, but not all, patients with type 2 diabetes and microalbuminuria. Reduction in microalbuminuria by strict glycemic control (5,6) or the administration of ACE inhibitors (7) is believed to prevent or retard the progression of renal disease into overt diabetic nephropathy. Although conducted in a relatively short time span, this study suggests that troglitazone is useful for this purpose. Second, a more important implication is that microalbuminuria may be a marker of generalized vascular disease and increased vascular permeability (18). Indeed, microalbuminuria has been identified as a predictor of cardiovascular mortality in patients with type 2 diabetes (1,3). Therefore, troglitazone could also be useful for a variety of vascular diseases, e.g., coronary artery disease. Although a long-term study is needed, troglitazone seems to have potential usefulness for diabetic angiopathy, including diabetic nephropathy and various vascular diseases.

The patients in this study were not very insulin resistant, as evidenced by their anthropometric data (BMI and percent fat) and plasma insulin levels. Taking into account the fact that the primary site of action of troglitazone is peripheral tissue (muscle and adipose tissue), but that of metformin is the liver, the effect of troglitazone may become milder than that of metformin when the patients become thin. This may be why a 400 mg daily dose of troglitazone was as effective as a relatively low dose of metformin (500 mg/day) that was recommended as a starting dose (19); the decreases in plasma glucose level in the two groups were comparable, and the level of glycated hemoglobin decreased more remarkably in the metformin group. Despite this, only troglitazone reduced microalbuminuria. The decrease in ACR by troglitazone, therefore, does not appear to be due to improved glycemic control. Furthermore, troglitazone did not change the blood pressure or lipid profile measured every 4 weeks. Blood pressure was recorded only by clinical blood pressure recording, and not by ambulatory blood pressure measurement. Subtle changes in blood pressure might occur in the patients. Nevertheless, we suggest that the relevance of these known effects of troglitazone was unlikely to reduce ACR.

Just how troglitazone reduced microalbuminuria could not be determined from this study. Fujii et al. (20) showed that troglitazone prevented the progression of albuminuria in streptozocin-induced diabetic rats. Because these rats were insulinopenic, and no changes in blood glucose were observed in troglitazone-treated rats, this effect did not seem to be mediated by the improvement of glycemic control. Furthermore, recent reports have suggested that thiazolidinediones have direct effects on vascular cells other than their effect of lowering plasma glucose levels. Buchanan et al. (13) showed that pioglitazone, another thiazolidinedione derivative, exerts a vasodepressor effect by inhibiting the calcium uptake by vascular smooth muscle cells. In vitro and animal experiments have demonstrated that troglitazone inhibits the growth of vascular smooth muscle and the intimal hyperplasia (14). We speculate that the amelioration of microalbuminuria in the study was mediated by these direct vascular effects of troglitazone.

In conclusion, we have revealed that troglitazone treatment for 12 weeks reduced microalbuminuria in patients with incipient diabetic nephropathy. This phenomenon was not observed in patients treated with metformin, although similar reductions in plasma glucose and glycated hemoglobin levels were observed. Direct effects of troglitazone on vascular cells may be considered. Troglitazone may have direct therapeutic potential for diabetic angiopathy, such as diabetic nephropathy and coronary artery disease, in addition to its effect of lowering plasma glucose levels. The present study provides rationale for long-term studies to look for the proposed beneficial effects of troglitazone.

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