Improvement by the Insulin-Sensitizing Agent, Troglitazone, of Abnormal Fibrinolysis in Type 2 Diabetes Mellitus

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This study evaluated abnormal fibrinolysis in diabetic patients in terms of the pathophysiological significance and reversibility by oral hypoglycemic agents. Forty-seven patients with type 2 diabetes mellitus were randomly treated for 4 weeks with glibenclamide (n = 23) or troglitazone (n = 24). Before and after treatment, glycemic control, steady-state plasma glucose and insulin (SSPG and SSPI, respectively), and markers of fibrinolysis (tissue plasminogen activator [tPA] and plasminogen activator inhibitor-1 [PAI-1]) were analyzed in each patient. Pretreatment plasma PAI-1 in diabetic patients, but not tPA, was well correlated with the severity of retinopathy assessed by the fluorescence technique. Four weeks of treatment with troglitazone significantly decreased hemoglobin A_{1c} (HbA_{1c}), SSPG, and PAI-1 without an alteration of tPA. The troglitazone-induced decrease in plasma PAI-1 (50.3 *v* 28.8 μmol/L; $P < .05$) was correlated with HbA_{1c} (8.80% *v* 7.21%, $r = .539$, $P < .01$) and SSPG (16.2 *v* 8.97 mmol/L, $r = .562$, $P < .01$) but not with SSPI. In contrast, treatment with glibenclamide for 4 weeks also reduced the HbA_{1c} titer to almost the same extent as troglitazone (1.38% *v* 1.59%), but did not change the plasma PAI-1 or SSPG titer. These results suggest that an abnormal fibrinolytic state, especially overproduction of PAI-1, may be a pathogenic factor in the development of diabetic complications such as retinopathy, which may be improved by correction of the insulin resistance with troglitazone.

VASCULAR COMPLICATIONS are a common factor determining morbidity and mortality in the diabetic population. The mechanisms by which hyperglycemia induces macroangiopathy and microangiopathy in diabetic patients are poorly understood, although some metabolic changes induced by hyperglycemia are proposed to mediate this process.[1-3] The fibrinolytic system may play an important role in the development of atherosclerosis and other diabetic vascular complications.[4,5] Two of the main determinants of fibrinolytic activity in the circulation are tissue plasminogen activator (tPA) and its inhibitor, plasminogen activator inhibitor-1 (PAI-1), with PAI-1 considered to be more critical for total fibrinolytic activity compared with tPA.[6] Several studies have suggested that plasma PAI-1 is elevated in diabetic patients, possibly associated with insulin resistance.[7,8] However, the precise interaction between insulin resistance and plasma PAI-1 in the diabetic population has not been clarified.

Troglitazone, a newly developed thiazolidinedione derivative, has been shown to decrease plasma glucose by enhancing insulin sensitivity in patients with type 2 diabetes.[9,10] Thus, this agent may be a useful tool to clarify the role of insulin resistance in the fibrinolytic abnormality in diabetic patients. The present study examines the action of troglitazone on abnormal fibrinolysis in comparison to glibenclamide, a sulfonylurea, to elucidate (1) the interrelationship between insulin resistance and abnormal fibrinolysis and (2) the clinical relevance of the treatment of insulin resistance for the correction of abnormal fibrinolysis in patients with type 2 diabetes mellitus.

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SUBJECTS AND METHODS

Patients

Forty-seven randomly selected patients with type 2 diabetes mellitus (22 men and 25 women) admitted for glycemic control to the Third Department of Internal Medicine, Fukushima Medical University, were enrolled in this study. Thirty healthy, nondiabetic individuals (13 men and 17 women) aged 48 to 67 years (mean, 52) were used as control subjects to evaluate basal plasma PAI-1 and tPA levels. All patients were free of liver dysfunction, hematologic diseases, thyroid dysfunction, hypertension, and infectious disease and were placed on an optimum-calorie dietary regimen (25 kcal/standardized body weight per day) containing 150 mmol sodium per day for at least 2 weeks before the study.

The study protocol was approved by the Committee for Human Research of Fukushima Medical University, and all subjects provided informed consent to participate. The study was performed in accordance with the Declaration of Helsinki and its revisions.

Protocols

Our preliminary study indicated that 400 mg/d troglitazone and 2.5 mg/d glibenclamide possess almost equal potency in terms of hypoglycemic action in type 2 diabetic patients. The chemical structure of the agents is shown in Fig 1. Thus, we performed a randomized group study in which 200 mg troglitazone twice daily orally after breakfast and dinner or 2.5 mg glibenclamide once daily orally before breakfast were prescribed for 24 and 23 patients, respectively, for 4 weeks. Subjects were randomly assigned to one of two treatment groups according to the randomization code. There were no significant differences between troglitazone and glibenclamide groups in age, sex, body mass index (BMI), waist to hip ratio, serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), diabetic complications, and duration of diabetes (Table 1). The severity of diabetic retinopathy was classified by 3 grades according to the conventional classification using a fluorescent technique: no retinopathy, simple retinopathy, and proliferative retinopathy. The stage of diabetic nephropathy was assessed by the grade of microalbuminuria (20 to 200 μg/min for urinary albumin excretion rate or 30 to 300 mg/d for urinary albumin excretion) and/or overt and consistent proteinuria (>0.3 g/d for total protein).

At the beginning and end of the 4-week treatment period, blood

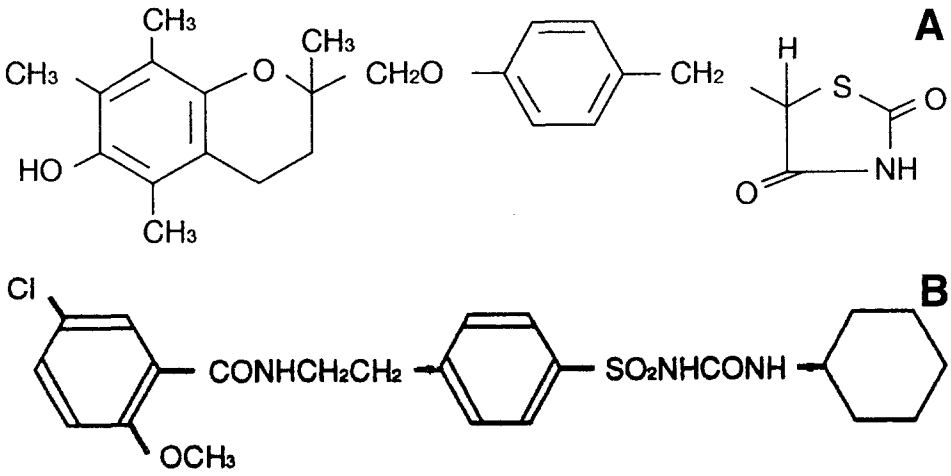

Fig 1. Chemical structure of (A) troglitazone and (B) glibenclamide.

samples were obtained from the cubital vein in the morning after an overnight fast for the determination of hemoglobin A_{1c} (HbA_{1c}), TG, TC, HDL-C, tPA, and PAI-1 in serum samples. Blood for the determination of tPA and PAI-1 was citrated, immediately cooled in icewater, and then centrifuged at 3,000× *g* at 4°C for 15 minutes, and the resultant plasma was stored at −20°C until tPA and PAI-1 determinations. Under the conditions we used, the measurement of the two variables was stable for at least 3 months after sample collection. Plasma glucose was assayed enzymatically using an autoanalyzer (Glucose Auto and Stat, GA-1122). HbA_{1c} was determined using an autoanalyzer (HLC-723GHb) coupled with high-performance liquid chromatography. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), TG, and TC were determined enzymatically using an autoanalyzer (Cobas Fara; Baxter, Chicago, IL). The HDL-C level was measured using commercially available kits (HDL-L-2 DAIICHI) and an autoanalyzer (Cobas Fara). Plasma PAI-1 and tPA antigen levels were measured by specific and sensitive enzyme-linked immunosorbent assay methods using kits from Biopool, Umeå, Sweden (TintElize and Imulyse-5).

Insulin sensitivity was estimated in all diabetic patients by the steady-state plasma glucose (SSPG) method using Octreotide, a somatostatin analog (Novartis Pharma, Basel, Switzerland) as originally described by Harano et al.[11] Briefly, intravenous catheters were placed in both arms after the subjects fasted overnight. Blood was sampled from one arm for determination of plasma glucose and insulin every 30 minutes until 120 minutes from the start of the examination, and the contralateral arm was used for administration of the test substance; Octreotide in a solution containing 0.5% human serum albumin was administered at 30 μg/h to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at 50 mU/kg/h and 6 mg/kg/min, respectively. Plasma glucose and insulin reached plateau levels in 90 and 30 minutes, respectively. We defined the plasma glucose and insulin value at 120 minutes of infusion as the SSPG and steady-state plasma insulin (SSPI), respectively. Under these conditions, SSPG was shown to be inversely correlated with insulin sensitivity as previously reported.[11]

Table 1. Clinical Profile of the Patients Before Treatment With Troglitazone or Glibenclamide

Parameter	Troglitazone	Glibenclamide
No. of subjects	24	23
Age (yr)	51.3 ± 2.1	52.5 ± 2.4
Sex ratio (male/female)	12/12	10/13
BMI (kg/m²)	22.9 ± 0.6	21.5 ± 0.5
Waist to hip ratio	1.14 ± 0.05	1.13 ± 0.05
Duration of diabetes (yr)	9.6 ± 1.6	9.3 ± 1.5
Serum TG (mmol/L)	0.89 ± 0.01	0.92 ± 0.02
TC (mmol/L)	5.13 ± 0.01	5.28 ± 0.01
HDL-C (mmol/L)	1.83 ± 0.15	1.79 ± 0.08
Diabetic complications (n)		
Retinopathy (no/simple/proliferative)	5/16/3	6/15/2
Nephropathy (no/microalbuminuria/ overt proteinuria)	5/17/2	8/13/2

NOTE. Values are the mean ± SE.

Statistical Analysis

The results are expressed as the mean ± SE. Statistical analysis of the data was performed by a multiple-comparison procedure and Student's *t*

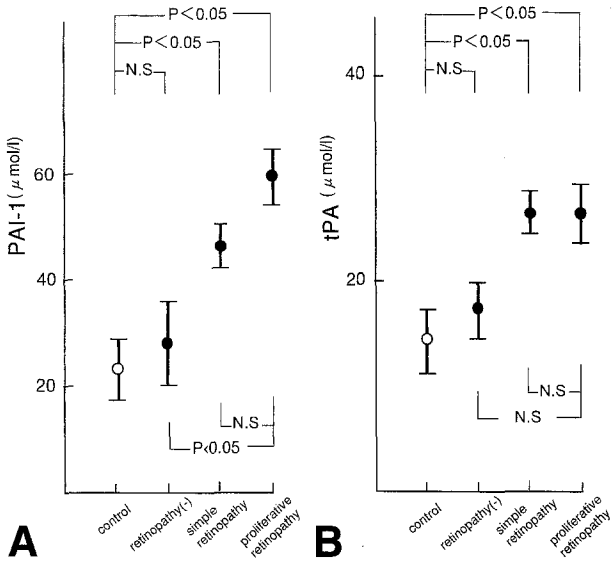

Fig 2. Plasma PAI-1 (A) and tPA (B) levels in diabetic patients with 3 grades of retinopathy (no retinopathy, simple retinopathy, and proliferative retinopathy) and control subjects. (A) Plasma PAI-1 was significantly elevated in all diabetic groups *v* control subjects and was increased significantly in correlation with the grade of retinopathy. (B) Plasma tPA was elevated in diabetic patients with simple or proliferative retinopathy *v* controls and diabetic patients without retinopathy. However, there was no difference between patients with simple retinopathy and proliferative retinopathy. NS, nonsignificant.

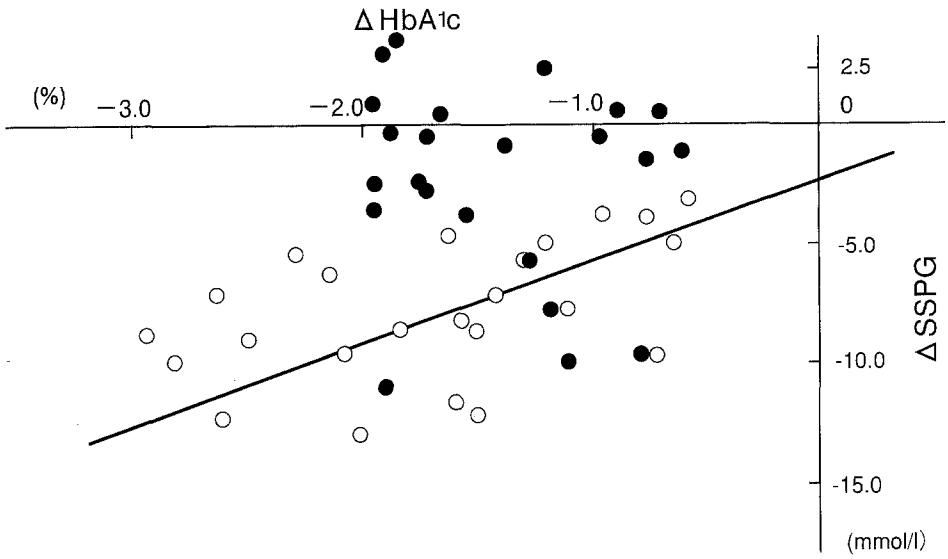

Fig 3. Correlation between changes in SSPG and in HbA_{1c} in diabetic patients treated with troglitazone (○) or glibenclamide (●). A significant positive correlation was observed between the 2 variables in patients treated with troglitazone ($r = .539$, $P < .01$), but not in those treated with glibenclamide.

test (paired and unpaired samples), if appropriate. Differences at a P level less than .05 were considered significant.

RESULTS

Plasma PAI-1 was significantly increased in all of the diabetic patients compared with the nondiabetic control subjects and increased significantly in correlation with the grade of retinopathy in the diabetic patient (Fig 2A). However, plasma tPA was not well correlated with the grade of retinopathy in diabetic patients, although it was significantly higher in diabetic patients with retinopathy versus the control subjects and diabetic patients without retinopathy (Fig 2B). HbA1c, PAI-1, tPA, SSPG, SSPI, ALT, and AST levels before treatment were not significantly different between the two treatment groups. After 4 weeks of treatment with glibenclamide, good glycemic control was achieved in all of the patients as judged from the decrease in the HbA_{1c} level. In patients treated with troglitazone for 4 weeks, glycemic control remained poor in 4 patients ($HbA_{1c} > 8\%$), improved in 15 ($HbA_{1c} < 8\%$ but $>6.5\%$), and was very good in 5 ($HbA_{1c} \leqq 6.5\%$). The reduction of the HbA_{1c} level in patients after treatment with troglitazone (1.59% ± 0.16%) was essentially equal to that obtained after treatment with glibenclamide (1.38% ± 0.10%). A significant reduction in the plasma PAI-1 level was found only in the troglitazone group. In contrast, no significant differences in plasma tPA and SSPI were observed before and after treatment in any of the patient groups. An improvement of the HbA_{1c} level and of SSPG was well correlated in the troglitazone group ($r = .539$, $P < .01$), but not in the glibenclamide group (Fig 3). In addition, a positive correlation was observed between the changes in SSPG and in PAI-1 ($r = .562$, $P < .01$) in the troglitazone group. However, in the glibenclamide group, the decrease in SSPG was only 14% and was not correlated with the decrease in plasma PAI-1 (Fig 4). No significant changes in TG, TC, HDL-C (data not shown), ALT, and AST (Table 2) were observed before and after treatment with the medications.

DISCUSSION

We first confirmed that the plasma concentration of PAI-1, but not tPA, is well correlated with the severity of retinopathy in Japanese type 2 diabetic patients.[12] Thus, it is suggested that

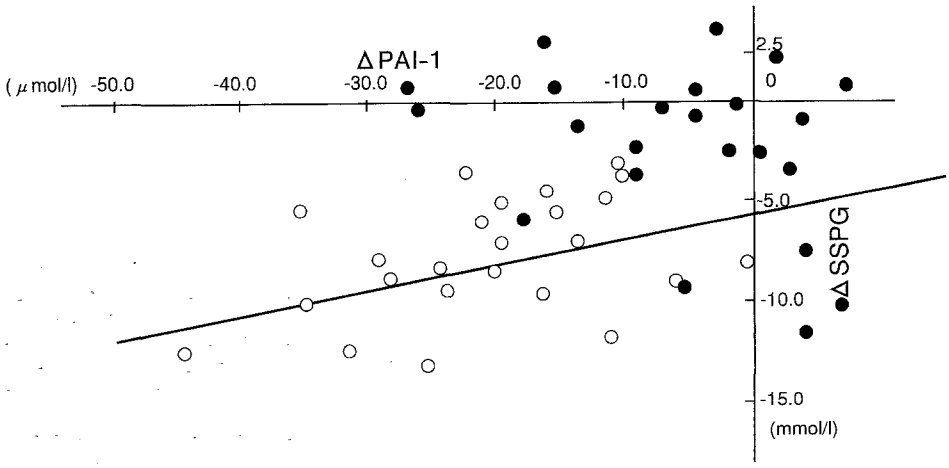

Fig 4. Correlation between changes in SSPG and in PAI-1 (ΔPAI-1) in diabetic patients treated with troglitazone (○) or glibenclamide (●). A significant positive correlation was observed between these 2 variables in those treated with troglitazone ($r = .562$, $P < .01$), but not in those treated with glibenclamide.

Table 2. Effect of Treatment With the Hypoglycemic Agents Troglitazone and Glibenclamide on Glycemic Control, Parameters of Fibrinolysis, Insulin Sensitivity, and Liver Function

Variable	Troglitazone (n = 24)		Glibenclamide (n = 23)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
HbA_{1c} (%)	8.80 ± 0.32	7.21 ± 0.30*	8.62 ± 0.27	7.24 ± 0.16*
PAI-1 (μmol/L)	50.3 ± 2.1	28.8 ± 1.6†	46.8 ± 1.8	40.2 ± 2.7
tPA (μmol/L)	23.3 ± 1.3	18.9 ± 0.79	29.2 ± 1.6	25.6 ± 1.3
SSPG (mmol/L)	16.2 ± 0.41	8.97 ± 0.38†	15.6 ± 0.51	13.4 ± 0.72
SSPI (pmol/L)	1,307 ± 81.1	1,378 ± 137.5	1,087 ± 68.1	1,173 ± 117.1
AST (IU/L)	20.1 ± 1.3	19.3 ± 1.3	19.8 ± 1.2	14.8 ± 0.9
ALT (IU/L)	17.4 ± 1.2	16.7 ± 0.7	20.5 ± 1.3	16.5 ± 1.0

*$P < .05$ *v* before treatment.

†$P < .01$ *v* before treatment.

PAI-1, but not tPA, may have a pathogenic potential for diabetic microangiopathy as previously suggested.[13,14]

Next, we demonstrated that glycemic control with troglitazone, but not glibenclamide, resulted in a significant decrease of SSPG and plasma PAI-1 without changes in the plasma lipoprotein profile and liver function. An early report that treatment of nondiabetic obese patients with metformin decreased the plasma PAI-1 level[15] supports the presence of a biological interrelationship between an increase in the PAI-1 level and insulin resistance. Our results clearly show that the decrease of plasma PAI-1 in diabetic patients treated with troglitazone was correlated with a decrease in the SSPG level, although the exact mechanism underlying this relationship remains to be explored. In diabetes mellitus, insulin resistance is associated with a high concentration of plasma insulin and its precursors (proinsulin and split proinsulin). Insulin[8,16] and proinsulin[17] were reported to enhance PAI-1 release from endothelial cells or hepatocytes in parallel with increased expression of PAI-1 mRNA. Thus, it may be possible that hyperinsulinemia enhanced PAI-1 expression in our patients. However, our data clearly show that SSPI was not changed, in contrast to plasma PAI-1, by treatment with either troglitazone or glibenclamide. In contrast, endothelial dysfunction in relation to insulin resistance seems to play a pathogenic role, instead of hyperinsulinemia, in the elevation of plasma PAI-1 in our diabetic patients, as we recently reported that troglitazone improves endothelial cell function such as the overexpression of endothelin-1 and PAI-1 in human umbilical endothelial cells in culture.[18,19]

In conclusion, this study demonstrates that the improvement of insulin resistance with troglitazone decreases the plasma PAI-1 level, which may be beneficial for the prevention of diabetic vascular complications.

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