Effects of Pioglitazone on Metabolic Parameters, Body Fat Distribution, and Serum Adiponectin Levels in Japanese Male Patients With Type 2 Diabetes

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The aim of this study was to evaluate the effects of pioglitazone on clinical and metabolic parameters, body fat distribution, and serum adiponectin, a recently discovered antiatherosclerotic hormone, in Japanese patients with type 2 diabetes. Ten male patients aged 40 to 66 (57.7 ± 7.4) years, who were being treated with dietary therapy alone (n = 7) or with a stable dose of sulfonylurea (n = 3), were studied at baseline and after 3 months of pioglitazone treatment (30 mg/d). Body mass index (BMI), blood pressure, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA_{1c}), serum insulin, adiponectin, and lipid profile were measured. Also, visceral adipose tissue area (VAT) and subcutaneous adipose tissue area (SAT) at the umbilical level were determined by computed tomographic (CT) scanning. Mean blood pressure (109 ± 14 to 101 ± 10 mm Hg), FPG (8.6 ± 1.4 to 7.0 ± 1.0 mmol/L), serum insulin (54 ± 11 to 30 ± 8 pmol/L, $P < .01$ for all), and HbA_{1c} (6.7 ± 0.8 to 6.1% ± 0.6%, $P = .013$) decreased significantly during 3 months of pioglitazone treatment. BMI (26.4 ± 3.2 to 27.0 ± 3.5 kg/m^2), low-density lipoprotein (LDL) cholesterol (124 ± 24 to 138 ± 24 mg/dL) and SAT (155 ± 69 to 179 ± 81cm^2, $P < .05$ for all) increased, while triglycerides and high-density lipoprotein (HDL) cholesterol did not change significantly after 3 months of pioglitazone treatment. Serum adiponectin level increased in all patients (4.8 ± 1.7 to 14.4 ± 2.1 μg/mL, $P = .005$). VAT tended to increase (165 ± 38 to 180 ± 46 cm^2) and VAT/SAT ratio tended to decrease (1.2 ± 0.3 to 1.1 ± 0.3), but these differences did not reach statistical significance. These results suggest that pioglitazone exerts good glycemic and blood pressure control despite increased BMI and SAT in Japanese male patients with type 2 diabetes. It is also suggested that pioglitazone may have an antiatherosclerotic effect by increasing serum adiponectin level.

DIABETES MELLITUS AFFECTS over 140 million people worldwide, more than 90% of them having type 2 diabetes.[1] Type 2 diabetes is characterized by impaired insulin secretion and/or insulin resistance.[2] Thiazolidinediones have been shown to ameliorate insulin resistance both in animal and clinical studies. Treatment of type 2 diabetic subjects with these drugs lowers blood glucose, glycohemoglobin, and serum insulin levels as a consequence of enhanced insulin action.[3]

We[4] and others[5,6] have demonstrated in type 2 diabetic patients that a thiazolidinedione, troglitazone, promotes subcutaneous adipose tissue accumulation, but decreases visceral fat after 3 to 6 months of treatment. Because it is recognized that the accumulation of visceral fat is associated with insulin resistance and the development of atherosclerosis,[7-9] it is very important to reduce visceral fat for the prevention of macrovascular disease.

An adipocyte-derived plasma protein, adiponectin,[10,11] which is the same as gelatin-binding protein of 28 kd (GBP28),[12-14] adheres to injured vascular walls in vitro,[15] inhibits endothelial nuclear factor kappaB (NF-κB) signaling,[16] and inhibits smooth muscle cell proliferation induced by heparin-binding epidermal growth factor (HB-EGF) and platelet-derived growth factor (PDGF).[17] Serum levels of this protein have been reported to be paradoxically low in obese subjects[11] and were decreased in type 2 diabetic subjects and even lower in diabetic patients with coronary artery disease.[18]

The purpose of the present study was to evaluate the effects of 3-month treatment with pioglitazone on (1) clinical and metabolic parameters, (2) body fat distribution, and (3) serum adiponectin level in Japanese male patients with type 2 diabetes.

SUBJECTS AND METHODS

Subjects

Ten male patients aged 40 to 66 years (57.7 ± 7.4 years), who were being treated with dietary therapy alone (n = 7) or with a stable dose of sulfonylurea (n = 3), were studied before and after 3 months of treatment with pioglitazone. Body weight, glycemic and blood pressure control, and therapy in each patient had been stable for at least 3 months before starting this study. All 10 patients had received dietary instructions for using a meal-exchange plan by nutritionists, as described previously.[4] The ideal dietary caloric intake for each patient was calculated as the ideal body weight (kg) × 25 kcal/kg. The recommended dietary composition was as follows: carbohydrate, 55% to 60%; fat, 20% to 25%; protein, 15% to 20% of total caloric intake; saturated fatty acids, less than 10%; and cholesterol, less than 300 mg/d.

The doses of sulfonylurea for the 3 patients were unchanged during the study period. All 10 patients received 2 tablets of pioglitazone after breakfast everyday (30 mg/d) as an additional medication. The tablets were counted every 2 weeks, and the compliance in these patients was 100% during the 3 months. It was also confirmed by questionnaire, as we described previously,[4] that the diet and the physical activity level were constant in each patient throughout the study period. Age, body mass index (BMI), and the clinical profile of the 10 male patients at the start of this study are shown in Table 1.

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Submitted April 26, 2001; accepted September 10, 2001.

Supported in part by research grants (to H.H.) from Keio University, Tokyo, Japan.

Presented in part at the 61st Annual Meeting of the American Diabetes Association, Philadelphia, PA, June 22-26, 2001.

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0026-0495/02/5103-0009$35.00/0

doi:10.1053/meta.2002.30506

Table 1. Baseline Clinical Profile of Japanese Male Patients With Type 2 Diabetes in This Study

Parameter		Range
No. of subjects	10	
Age (yr)	57.4 ± 7.4	40-66
Height (cm)	164.2 ± 5.2	156.0-171.6
Weight (kg)	71.3 ± 9.8	57.3-85.1
BMI (kg/m^2)	26.4 ± 3.2	22.4-32.5
Duration of diabetes (yr)	7.0 ± 4.5	2.0-15.0
Glucose (mmol/L)	8.6 ± 1.4	6.9-11.3
HbA_{1c} (%)	6.7 ± 0.8	5.3-8.2

NOTE. Data are n or mean ± SD.

The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after full explanation of the purpose, nature, and risk of all procedures used. The protocol was approved by the ethical review committee of the Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

Measurements

Systolic blood pressure and diastolic blood pressure were measured with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki, Japan), twice in the sitting position after resting for at least 5 minutes, as described previously.[19-21] Height, weight, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA_{1c}), serum insulin, adiponectin, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and uric acid levels were measured in the morning after an overnight fast. Plasma glucose was measured by glucose oxidase method, and HbA_{1c} was measured by a high-performance liquid chromatography method.[4] Plasma lipids and uric acid were assayed by routine automated laboratory methods.[4,19-21] Serum insulin concentration was measured by enzyme immunoassay using a commercially available kit (Tosoh, Tokyo, Japan). The insulin resistance index was assessed by homeostasis model assessment (HOMA-IR).[22,23]

Measurement of GBP28/Adiponectin by Enzyme-Linked Immunosorbent Assay

The sera were diluted by 441-fold before assay. A total of 100 μL of diluted sera, standard samples of GBP28, and quality control samples were applied to 96-well microtiter plate coated with mouse anti-GBP28 monoclonal antibody. This antibody only recognizes the native GBP28 oligomer.[12] After incubation at room temperature for 60 minutes, the wells were washed and incubated for 30 minutes with the same monoclonal antibody labeled with horseradish peroxidase. The wells were washed again and incubated for 30 minutes with tetramethylbenzidine reagent. Then, 100 μL of 0.36 N sulfuric acid solution was added to each well to stop the reaction, and the absorbance at 450 nm was measured. The dilution curve was parallel to the standard curve. The intra- and the interassay coefficients were 4.8% to 4.9% and 3.3% to 6.8%, respectively. Nineteen of 20 samples in this study were assayed simultaneously.

Measurement of Fat Distribution

Subcutaneous and visceral fat distribution were determined by measuring a −150 to −50 Hounsfield unit area using a modification of the method of computed tomographic (CT) scanning by Tokunaga et al[24] at the umbilical level. CT images were obtained both at baseline and after 3 months of treatment, which was exactly the same protocol as we reported previously.[4] V/S ratio was calculated as visceral adipose tissue area (VAT) divided by subcutaneous adipose tissue area (SAT).

Statistical Analysis

All statistical analyses were performed using the StatView program for Macintosh (version 4.5-J; SAS Institute, Cary, NC). We used the Wilcoxon signed-rank test (2-tailed) for comparisons of baseline and 3-month follow-up data and Pearson's correlation coefficients and partial correlation analyses for comparisons of changes in adiponectin and other parameters. Because serum insulin, triglycerides and adiponectin levels, and HOMA-IR were normally distributed after log-transformation in large-scale studies, we used the logarithms of data for analyses of these 4 parameters. All data are expressed as mean ± SD, and $P < .05$ was considered statistically significant.

RESULTS

Effects of 3-Month Treatment With Pioglitazone on Clinical and Metabolic Parameters

As shown in Table 2, systolic blood pressure, diastolic blood pressure, FPG, HbA_{1c}, serum insulin, and HOMA-IR decreased significantly ($P < .05$) after 3 months of pioglitazone treatment. BMI and LDL cholesterol increased, while triglycerides, HDL cholesterol, and uric acid did not change significantly.

Effect of 3-Month Treatment With Pioglitazone on Body Fat Distribution

Data on fat distribution are shown in Table 3. SAT increased significantly ($P = .037$). VAT tended to increase and V/S ratio tended to decrease, but these differences did not reach statistical significance.

Effect of 3-Month Treatment With Pioglitazone on Serum Adiponectin Level

The serum adiponectin level increased in all the patients (from 4.8 ± 1.7 to 14.4 ± 2.1 μg/mL, $P = .005$) (Fig 1). Furthermore, we examined correlations of percent changes in 3

Table 2. Effects of 3-Month Treatment With Pioglitazone on Clinical and Metabolic Parameters in Japanese Male Patients With Type 2 Diabetes

Parameter	Baseline	3-Month Follow-up	Percent Change in 3 Months
No. of subjects	10	10	
BMI (kg/m^2)	26.4 ± 3.2	27.0 ± 3.5*	2.1 ± 2.2
Systolic blood pressure (mm Hg)	149 ± 18	138 ± 15†	−7.0 ± 4.9
Diastolic blood pressure (mm Hg)	89 ± 13	83 ± 9*	−6.3 ± 6.9
Glucose (mmol/L)	8.6 ± 1.4	7.0 ± 1.0†	−18.2 ± 9.3
HbA_{1c} (%)	6.7 ± 0.8	6.1 ± 0.6*	−9.0 ± 8.8
Insulin (pmol/L) (log)	54 ± 11	30 ± 8†	−23.7 ± 13.5
HOMA-IR (log)	3.4 ± 1.9	1.6 ± 1.3*	−62.6 ± 22.6
Total cholesterol (mg/dL)	208 ± 26	218 ± 35	4.8 ± 10.9
Triglycerides (mg/dL) (log)	134 ± 2	127 ± 2	−1.0 ± 6.3
HDL cholesterol (mg/dL)	51.7 ± 9.4	52.1 ± 7.3	2.0 ± 11.2
LDL cholesterol (mg/dL)	124 ± 24	138 ± 24*	12.3 ± 12.8
Uric acid (mg/dL)	5.9 ± 1.1	5.6 ± 1.3	−5.0 ± 9.5

NOTE. Data are n or mean ± SD.
*$P < .05$ and †$P < .01$ by Wilcoxon test.

Table 3. Effects of 3-Month Treatment With Pioglitazone on Body Fat Distribution in Japanese Male Patients With Type 2 Diabetes

Parameter	Baseline	3-Month Follow-up	Percent Change in 3 Months
No. of subjects	10	10	
Visceral adipose tissue area (cm^2)	165 ± 38	180 ± 46	11.3 ± 21.3
Subcutaneous adipose tissue area (cm^2)	155 ± 69	179 ± 81*	16.1 ± 18.7
V/S ratio	1.2 ± 0.3	1.1 ± 0.3	−4.0 ± 11.2

NOTE. Data are n or mean ± SD.
*$P < .05$ by Wilcoxon test.

months between adiponectin and other parameters shown in Tables 2 and 3. Only the percent change in SAT tended to correlate with percent change in adiponectin level ($r = -.628$, $P = .051$). When the absolute changes were used in the analysis, only SAT change was negatively correlated with the change in adiponectin level ($r = -.758$, $P = .009$). However, the other parameters were not correlated, even after adjustment for change in BMI or SAT.

DISCUSSION

Effects of Pioglitazone on Metabolic Parameters

The beneficial effects on glycemic and blood pressure control in this study were the same as with troglitazone,[4] even when compared with control dietary therapy. Concerning thiazolidinedione effects on blood pressure, troglitazone was reported to reduce blood pressure in insulin-resistant animals[25] and in clinical studies.[26]

Regarding the effects on LDL cholesterol, 3-month treatment with pioglitazone significantly increased LDL cholesterol in the present study despite the improvement of blood pressure and insulin resistance. Although the mechanism(s) are unclear, treatment with troglitazone[3] or rosiglitazone[27] has been associated with an increase in LDL cholesterol. Further studies will be needed to clarify these issues, including basic and longitudinal studies in different age, sex, and ethnic groups.

Effect of Pioglitazone on Body Fat Distribution

It was obvious in this study that SAT significantly increased after a 3-month treatment with pioglitazone, which was the same as with troglitazone.[4-6] Because pioglitazone also acts on peroxisome proliferator-activated receptor (PPAR)γ, a master regulator of adipocytes, it is rational that pioglitazone, as well as troglitazone, increased SAT. In contrast, VAT did not decrease, but rather increased in this study, which was different from the observation with troglitazone by our group[4] and others.[5,6] The reason for this difference is not clear, but may be due to a structural difference, such as vitamin E structure in troglitazone and/or the functional potency of PPARα, etc. In Caucasians, Miyazaki et al[28] reported a decline in VAT and V/S ratio after 4 months of 45 mg/d pioglitazone treatment in 13 patients with type 2 diabetes (9 male and 4 female; BMI, 29.0 ± 1.2 kg/m^2).

Effects of Pioglitazone on Serum Adiponectin Level

Adiponectin levels have been reported to be low in obesity[11] and were significantly elevated by vigorous weight reduction in both diabetic and nondiabetic subjects.[18] We speculate that the mechanisms of increased adiponectin level by pioglitazone despite increased BMI may be: (1) secondary to changes in adiposity, but it seems unlikely considering that SAT increased, but VAT did not decrease in this study. (2) Secondary to changes in glucose control, but it also seems unlikely considering that the change in adiponectin did not correlate with the percent change in FPG, HbA_{1c}, or HOMA-IR in this study. (3) Direct stimulation of adipocytes, although we do not know so far if there is PPAR response element in the promotor region of adiponectin or not. (4) Via decreasing tumor necrosis factor (TNF)-α levels or action, because TNF-α is reported to reduce the expression and secretion of adiponectin in human preadipocytes.[29] It was unfortunate that we could not measure plasma or tissue TNF-α levels before and after pioglitazone treatment. It has, however, been demonstrated in human and animal studies that pioglitazone decreases plasma and tissue TNF-α concentrations.[30,31]

Adiponectin has been reported in in vitro studies to attenuate TNF-α–induced expression of adhesion molecules on endothelial cells,[32] adhere to injured vascular walls,[15] inhibit endothelial NF-κB signaling,[16] and inhibit smooth muscle cell proliferation.[17] Therefore, it is possible that an increase in adiponectin levels might be one of the mechanisms by which pioglitazone exerts beneficial effects to prevent atherosclerosis in type 2 diabetic patients, who are at a high risk to develop macrovascular disease. However, further basic

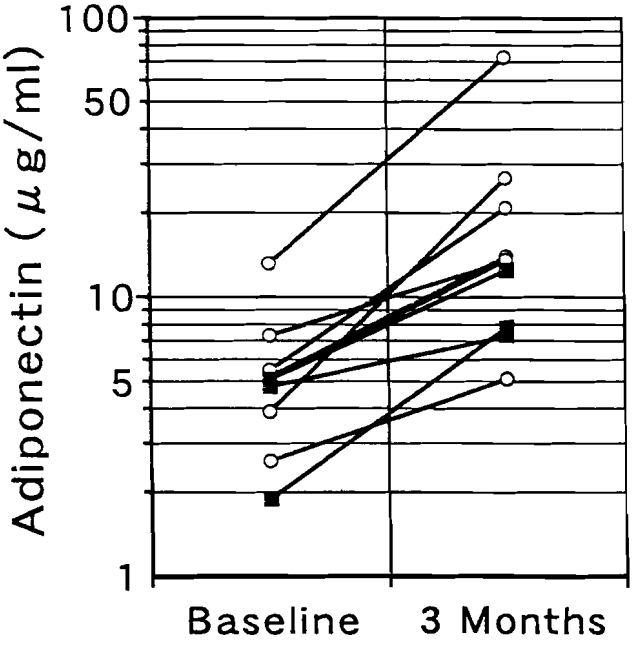

Fig 1. Changes in serum adiponectin level by 3-month treatment with pioglitazone (30 mg/d) in 10 Japanese male patients with type 2 diabetes. ○—○, Dietary therapy plus pioglitazone (n = 7); ■—■, dietary therapy, sulfonylurea plus pioglitazone (n = 3).

and long-term clinical studies will be needed to clarify these issues.

In conclusion, we suggest that pioglitazone exerts good glycemic and blood pressure control despite increased BMI and SAT in Japanese male patients with type 2 diabetes. It is also suggested that pioglitazone may have an antiatherosclerotic effect by increasing serum adiponectin levels.

ACKNOWLEDGMENT

We thank Dr Wendy Gray for editing the manuscript.

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