Original Article

Pioglitazone Decreases Plasma Angiotensin II Concentration in Type 2 Diabetes

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Aim: Adipocytes express all components of the renin-angiotensin system (RAS), and adipocyte RAS regulates adipocyte differentiation and metabolism. Plasma angiotensin II (AII) is a putative marker of adipocyte RAS production. The aim of this study was to investigate the effect of pioglitazone on plasma AII in type 2 diabetes (T2D).

Methods: Fifty Japanese subjects with T2D were randomly allocated to two groups. One group was administered pioglitazone 30 mg/day (pioglitazone group) and the other group was not given pioglitazone (control group) for 16 weeks. Lipoprotein lipase mass in preheparin serum (LPL mass) was measured as an adipocyte-derived factor and a marker of insulin sensitivity.

Results: In the pioglitazone group, the mean HbA1c decreased ($p < 0.0001$), LPL mass increased ($p < 0.0001$), and plasma AII decreased ($p = 0.0007$), whereas these parameters were unchanged in the control group. The change in plasma AII correlated negatively with the change in LPL mass ($r = -0.312$) in the pioglitazone group. In the pioglitazone group, the decrease in plasma AII was higher ($p = 0.0002$) and the increase in LPL mass tended to be higher ($p = 0.0941$) in the subgroup with higher baseline plasma AII than that with lower plasma AII.

Conclusions: The present study indicates that pioglitazone decreases plasma AII associated with an increase in LPL mass in T2D. The insulin-sensitizing effect of pioglitazone may be involved in suppressing adipocyte RAS.

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Key words; Angiotensin II, Pioglitazone, Insulin resistance, Lipoprotein lipase

Introduction

Recently, it has become evident that inhibition of the renin-angiotensin system (RAS) improves many risk factors, including hypertension, metabolic disorders, and insulin resistance, and prevents the progression of cardiovascular and chronic kidney diseases¹⁾. On the other hand, adipocytes are known to express all components of the RAS, such as angiotensinogen, renin, angiotensin-converting enzyme (ACE), and

chymase, which are required for conversion to angiotensin II (AII)^{2, 3)}. Although AII is detectable in the blood, the relationship between circulating AII and the components of RAS from adipocytes is completely unknown. We have recently reported that plasma AII is associated with body weight, showing a decline during weight loss, and is associated with insulin resistance in obese subjects with type 2 diabetes (T2D)⁴⁾. These findings suggest that AII may have a potent metabolic effect on adipose tissue and/or plasma AII may be a useful marker of RAS production from adipose tissue.

Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) is expressed at high levels in adipose tissue, where it plays a central role in adipose tissue functions⁵⁾. Pioglitazone, an oral insulin-sensitiz-

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ing thiazolidinedione agent, is used for the treatment of T2D. This drug activates nuclear PPAR- γ , leading to increased transcription of various proteins in adipocytes to improve glucose and lipid metabolism⁶⁻⁹. Clinical studies have suggested that treatment with pioglitazone in T2D subjects significantly increases lipoprotein lipase mass in preheparin serum (LPL mass)¹⁰. LPL mass reflects lipoprotein lipase (LPL) production from adipocytes¹¹, and is a marker of insulin sensitivity¹²⁻¹⁷. Our *in vitro* study has shown that AII inhibits adipogenic differentiation of 3T3-L1 cells, and the inhibitory effect is reversed by pioglitazone¹⁸. Furthermore, other investigators have reported that the thiazolidinedione agent rosiglitazone decreases both angiotensinogen protein expression in isolated human subcutaneous adipocytes and AII release from the cells¹⁹. These findings suggest that the beneficial effects of pioglitazone on glucose and lipid metabolism as well as insulin sensitivity may be due to the reduced production of adipocyte RAS components; however, the effect of pioglitazone on circulating RAS components has not been investigated in clinical study.

The aim of this study was to clarify the effect of pioglitazone on plasma AII, which may be a marker of adipocyte RAS, and various metabolic parameters in subjects with T2D.

Subjects and Methods

Subjects

This randomized, open study was conducted at the Center of Diabetes, Endocrinology and Metabolism, Toho University Sakura Medical Center. Fifty Japanese subjects (29 males and 21 females) with T2D who had not been treated with pioglitazone were eligible for enrollment. Subjects with diabetic retinopathy, renal dysfunction, including diabetic nephropathy, congestive heart failure, coronary heart disease, and abnormal liver dysfunction (transaminases greater than twice the upper limit of normal) were excluded. The other exclusion criterion was serious systemic diseases, such as acute/chronic inflammation and malignancies. Before pioglitazone administration, 34 subjects had been treated with sulfonylureas and 12 with metformins, which also have an insulin-sensitizing effect. The same doses of sulfonylureas were continued during pioglitazone administration, but sulfonylureas were reduced or discontinued when hypoglycemia occurred. Subjects treated with ACE inhibitor and/or angiotensin type 1 receptor blocker were excluded, because plasma AII is highly sensitive to these drugs. Randomization was performed with sequentially num-

bered, sealed envelopes using a previously determined code for the administration of pioglitazone. One group was administered pioglitazone 30 mg/day (pioglitazone group, $n=25$) and the other group was not given pioglitazone (control group, $n=25$) for 12 weeks. Clinical characteristics of the subjects are shown in **Table 1**. Dietary counseling was provided by a nutritionist and subjects were asked to avoid any change in their diet or lifestyle during the study period. The study was approved by the Ethics Committee of Toho University, and all subjects provided written informed consent prior to participation in the study.

Body weight, blood pressure and blood sampling

Body weight was measured after an overnight fast. Blood pressure was measured in a sitting position after 5-minute rest in the morning. Blood samples were taken in the morning after 12 hours of fasting and 30-minute rest in the supine position. For plasma AII and hemoglobin A1c (HbA1c) measurements, blood was collected into an EDTA tube. Serum was separated within 1 hour for LPL mass, glucose, lipoprotein and other measurements. Samples for plasma AII and LPL mass measurement were frozen at -80°C until assay.

Plasma AII assay

Plasma AII was measured by a radioimmunoassay (RIA) (Mitsubishi Chemical Medience Corporation, Tokyo, Japan)²⁰. In brief, 0.5 mL plasma sample was collected into a syringe containing 2.5 mL EDTA and prechilled ethanol. The sample was centrifuged and extracted. After purification, immunoreactive AII was measured in duplicate by a radioimmunoassay using anti-AII antiserum followed by iodinated AII (^{125}I -AII) and goat anti-rabbit antiserum as the second antibody.

LPL mass assay

LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody against bovine milk LPL, as described by Kobayashi *et al.*²¹. A commercial kit (Sekisui Medical Co. Ltd, Tokyo, Japan) was used in this study. In this assay system, linearity was observed from 5 to 400 ng/mL. The within-run coefficient of variation (Cv) was 2.8%, and between-day Cv was 4.3%.

Statistical Analysis

Data are expressed as the mean $\pm$ S.D. SPSS 15.0 software (SPSS Inc., Chicago, Ill, USA) was used for all statistical analysis. Student's *t*-test was performed to

Table 1. Various parameters at baseline (week 0) and at the end of the study (week 16) in 50 patients with type 2 diabetes

	Pioglitazone group (n=25)			Control group (n=25)			Comparison at baseline
	Week 0	Week 16	<i>p</i> value	Week 0	Week 16	<i>p</i> value	<i>p</i> value*
Age (years)	63.7±12.6	-	-	61.4±12.3	-	-	NS
Gender (Male/Female)	14/11	-	-	14/11	-	-	-
Body height (m)	1.06±0.07	-	-	1.61±0.08	-	-	NS
Body weight (kg)	61.4±7.8	62.9±8.6	<0.0001	61.6±12.8	61.3±13.6	NS	NS
Body mass index (kg/m ²)	24.0±2.5	24.6±2.7	<0.0001	23.6±3.6	23.4±3.8	NS	NS
Systolic blood pressure (mmHg)	131.9±12.7	126.1±11.3	NS	133.4±12.2	127.1±14.5	NS	NS
Diastolic blood pressure (mmHg)	75.0±9.2	75.1±7.2	NS	78.1±7.2	75.9±9.9	NS	NS
Fasting blood glucose (mg/dL)	177.0±84.1	164.2±70.8	NS	159.2±67.1	164.4±46.4	NS	NS
HbA1c (%)	8.17±0.87	7.54±0.96	<0.0001	7.65±0.87	7.59±0.91	NS	NS
Total cholesterol (mg/dL)	197.1±39.4	200.8±41.4	NS	203.9±37.8	201.2±54.4	NS	NS
Triglyceride (mg/dL)	149.4±89.2	151.7±80.9	NS	158.9±118.3	154.0±122.3	NS	NS
HDL-cholesterol (mg/dL)	54.4±17.2	56.8±19.7	NS	54.6±14.6	53.2±15.5	NS	NS
LDL-cholesterol (mg/dL)	116.0±30.7	116.2±30.9	NS	119.4±32.7	118.3±37.7	NS	NS
Preheparin LPL mass (ng/mL)	58.6±20.7	68.3±21.3	<0.0001	53.4±19.2	49.6±18.2	NS	NS
Angiotensin II (pg/dL)	11.96±6.05	8.68±4.60	0.0007	9.73±3.41	11.40±2.64	NS	NS

*Comparison between pioglitazone group and control group at baseline

Data are expressed as the mean ± SD.

NS, not significant; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase.

determine whether differences in levels between groups at baseline, week 16, and during the study were statistically significant. The paired *t*-test was performed to determine whether differences between levels at baseline and week 16 in each group were statistically significant. A *p* value less than 0.05 was considered significant.

Results

Table 1 shows the clinical variables of the 50 subjects with T2D in the study. There were no significant differences in all parameters between pioglitazone and control groups at baseline. None of the subjects developed marked edema or other adverse effects, such as cardiac failure, during this study.

Changes in body weight, blood pressure, and glucose and lipid parameters after treatment

First, we confirmed the effect of pioglitazone on body weight, blood pressure, and glucose and lipid parameters. In the pioglitazone group, the mean BMI increased by 2.5% ($p < 0.0001$), HbA1c decreased by 13.3% ($p < 0.0001$) and LPL mass increased by 16.6% ($p < 0.0001$) after 16 weeks of treatment (**Table 1**). Blood pressure, and fasting blood glucose and lipid parameters were unchanged. In contrast, all parameters were unchanged in the control group. The in-

crease in BMI, increase in LPL mass and decrease in HbA1c in the pioglitazone group were significant ($p < 0.0001$ for all) when comparing changes in pioglitazone and control groups (**Table 2**).

Change in plasma AII and the relationship between plasma AII and various parameters

The mean plasma AII at baseline was 10.75 ± 5.41 pg/dL in all subjects. In the pioglitazone group, the mean plasma AII decreased by 20.8% ($p = 0.0007$) after 16 weeks of treatment, whereas no change was observed in the control group (**Table 1**). The decrease in AII in the pioglitazone group was significant ($p < 0.0001$ for all) when comparing changes in pioglitazone and control groups (**Table 2**). No significant difference was shown in changes in plasma AII between subjects treated with and without metformins (data not shown).

We also examined the correlation of the change in plasma AII with the changes in body weight, blood pressure, glucose and lipid parameters in the pioglitazone group (**Table 2**). The change in plasma AII correlated negatively with the change in LPL mass ($r = -0.312$, $p = 0.0389$) (**Fig. 1**). Although the mean BMI and HbA1c changed significantly in the pioglitazone group, the changes in these parameters did not correlate with the change in plasma AII.

Table 2. Changes in various parameters in pioglitazone and control groups, and their correlation with change in plasma angiotensin II in pioglitazone group

	Pioglitazone group (<i>n</i> = 25)	Control group (<i>n</i> = 25)	<i>p</i> value	Correlation with the change in angiotensin II in pioglitazone group (<i>n</i> = 25)	
				<i>r</i>	<i>p</i> value
ΔBody weight (kg)	1.53 ± 1.42	-0.10 ± 1.43	0.0002	0.176	NS
ΔBody mass index (kg/m ²)	0.60 ± 0.53	-0.05 ± 0.51	<0.0001	0.166	NS
ΔSystolic blood pressure (mmHg)	-0.11 ± 14.25	-5.58 ± 9.79	NS	0.106	NS
ΔDiastolic blood pressure (mmHg)	-1.11 ± 5.62	-2.08 ± 8.41	NS	0.365	NS
ΔFasting blood glucose (mg/dL)	-12.7 ± 70.5	6.4 ± 58.9	NS	-0.296	NS
ΔHbA1c (%)	-0.62 ± 0.40	-0.05 ± 0.47	<0.0001	0.005	NS
ΔTotal cholesterol (mg/dL)	3.76 ± 32.36	-4.17 ± 26.95	NS	0.084	NS
ΔTriglyceride (mg/dL)	2.28 ± 58.48	-6.00 ± 35.89	NS	0.126	NS
ΔHDL-cholesterol (mg/dL)	2.44 ± 11.00	-1.08 ± 4.93	NS	0.204	NS
ΔLDL-cholesterol (mg/dL)	0.24 ± 19.82	-2.54 ± 18.65	NS	0.081	NS
ΔPreheparin LPL mass (ng/mL)	8.64 ± 8.66	-3.78 ± 6.79	<0.0001	-0.312	0.0389
ΔAngiotensin II (pg/dL)	-3.28 ± 2.95	2.67 ± 3.16	<0.0001	-	-

Data are expressed as the mean ± SD. NS, not significant; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, Lipoprotein lipase.

Fig. 1. Relationship between change in plasma AII and LPL mass in pioglitazone group (25 subjects with T2D)

The scatter plot with regression line shows the correlation between changes in plasma AII and LPL mass after 16 weeks of pioglitazone treatment.

Changes in various parameters in subjects with high plasma AII concentration

To clarify the influence of plasma AII on the metabolic effect of pioglitazone, we divided the subjects in the pioglitazone group into two subgroups according to the mean plasma AII at baseline (11.96 pg/dL); lower AII (plasma AII < 12 pg/dL, *n* = 17) and higher AII (plasma AII ≥ 12 pg/dL, *n* = 8). There were

no significant differences in various parameters, except for plasma AII, between pioglitazone and control groups at baseline (Table 3). The mean plasma AII levels at baseline were 9.22 pg/dL in the lower AII subgroup and 19.00 pg/dL in the higher AII subgroup.

The decrease of fasting blood glucose in the high AII group was significant higher than in the low AII subgroup (*p* = 0.0401, data not shown). In the higher AII subgroup, the decrease in plasma AII was greater (*p* = 0.0002) (Fig. 2A) and the increase in LPL mass tended to be greater (*p* = 0.0941) (Fig. 2B) than in the lower AII subgroup. There were no differences in the changes in other parameters between higher and lower AII subgroups.

Discussion

The aim of this study was to investigate the effect of pioglitazone on plasma AII, a marker of RAS production from adipose tissue. First, we confirmed the effects of pioglitazone on body weight, blood pressure, and glucose and lipid metabolism. This study showed that pioglitazone treatment for 16 weeks increased BMI and decreased HbA1c. These results are consistent with previous reports on Japanese subjects with T2D^{10, 22}. Numerous studies have revealed that pioglitazone is typically associated with weight gain and an increased risk of edema. None of the subjects developed marked edema during this study; therefore, we considered that this study had high reliability to evaluate the effect of pioglitazone on adipose tissue

Table 3. Comparison of various parameters between in lower and higher AII subgroups at baseline

	Lower AII subgroup (n = 17)	Higher AII subgroup (n = 8)	<i>p</i> value
Age (years)	63.5 ± 12.7	64.4 ± 13.4	NS
Body height (m)	1.61 ± 0.07	1.58 ± 0.05	NS
Body weight (kg)	63.2 ± 8.0	57.9 ± 6.0	NS
Body mass index (kg/m ²)	24.4 ± 2.6	23.2 ± 1.7	NS
Systolic blood pressure (mmHg)	132.1 ± 14.6	131.5 ± 8.6	NS
Diastolic blood pressure (mmHg)	75.8 ± 8.0	74.6 ± 7.9	NS
Fasting blood glucose (mg/dL)	180.2 ± 81.7	171.2 ± 93.7	NS
HbA1c (%)	8.27 ± 0.95	7.97 ± 0.57	NS
Total cholesterol (mg/dL)	202.0 ± 41.9	184.4 ± 30.9	NS
Triglyceride (mg/dL)	153.0 ± 63.1	141.3 ± 51.7	NS
HDL-cholesterol (mg/dL)	51.5 ± 14.3	61.8 ± 22.7	NS
LDL-cholesterol (mg/dL)	118.6 ± 31.6	111.4 ± 17.7	NS
Preheparin LPL mass (ng/mL)	56.8 ± 18.8	62.1 ± 26.1	NS
Angiotensin II (pg/dL)	9.22 ± 1.31	19.00 ± 7.83	< 0.0001

Data are expressed as the mean ± SD.

NS, not significant; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase.

Fig. 2. Changes in plasma AII (A) and LPL mass (B) in subjects with higher and lower plasma AII concentration

Subjects in the pioglitazone group were divided into two subgroups according to baseline plasma AII concentration: lower AII (<12 pg/dL, white bars) and higher AII ($\geq$ 12 pg/dL, black bars). Bars show the data of changes in plasma AII and LPL mass after 16 weeks of pioglitazone treatment.

metabolism.

In our previous report, based on the findings that plasma AII is associated with body weight, visceral fat area, adipocyte-derived factors and insulin resistance, and that AII decreases during weight loss in obese subjects with T2D, we concluded that plasma AII may reflect the production of RAS components from adipose tissue⁴. In this study, pioglitazone treatment decreased plasma AII markedly by 20%. This is the first report of the effect of thiazolidinediones on circu-

lating RAS components in a clinical study, although an in vitro study has shown that rosiglitazone down-regulates RAS expression and production in human adipocytes¹⁹. Some studies have reported the relationship between PPAR-gamma and RAS in adipocytes²³; however, the mechanism by which pioglitazone down-regulates RAS in adipocytes is still unknown. Therefore, to investigate the relationship between plasma AII and adipocyte function, we also measured an adipocyte-derived factor, LPL mass, together with plasma A

II. On the other hand, angiotensinogen is synthesized primarily by the liver, and is secreted into the circulation, where it is cleaved by renin, which is produced mainly by juxtaglomerular cells, to angiotensin I. Angiotensin I is subsequently converted to AII by ACE from the lungs and endothelial cells^{3, 24}; therefore, plasma AII may be regulated by not only adipocytes, but also many cells and organs. A limitation of this study is the origin of plasma AII. Recent studies have shown that intrarenal AII levels may contribute to the development of hypertension; however, intrarenal AII levels have no association with circulating Ang II levels^{25, 26}. No report has mentioned the relationship among the liver, lung and plasma AII; however, we emphasize that plasma AII may reflect the production of RAS components from adipose tissue, since plasma AII is associated with body fat composition and adipocyte-derived factors.

We and other investigators have reported that the LPL mass reflects LPL production, particularly in adipocytes, and that low LPL mass reflects increased fat accumulation and insulin resistance^{11-15, 27, 28}. In a previous diet-induced weight loss study, we also showed that increased LPL mass is associated with decreased plasma AII⁴. Our *in vitro* study also showed that the addition of AII peptide to the culture medium suppresses LPL activity and expression in 3T3-L1 cells¹⁸. In adipocytes, the LPL gene is regarded as a target gene of PPAR- γ ^{11, 29}; therefore, AII may down-regulate LPL expression and production through the suppression of PPAR- γ in adipocytes. On the other hand, pioglitazone treatment in this study increased the LPL mass, consistent with a previous report¹⁰. It is obvious that pioglitazone upregulates LPL mass through the activation of PPAR- γ in adipocytes and increases insulin sensitivity. The salient findings of this study are a negative correlation between the change in LPL mass and plasma AII, and the tendency toward a greater LPL mass-enhancing effect of pioglitazone in subjects with higher plasma AII. Therefore, we assume that pioglitazone may have two types of PPAR- γ -enhancing mechanisms; a direct effect on PPAR- γ and an indirect effect by suppressing RAS in adipocytes, and both mechanisms consequently increase LPL mass.

In diabetes, RAS is thought to play an important role in the regulation of hemodynamics and also in the development of complications, such as diabetic nephropathy³⁰; however, the plasma AII level in T2D has not been fully investigated. We have reported that fasting blood glucose and HbA1c do not correlate with plasma AII in subjects with T2D⁴. Nicola *et al.*³¹ have reported that plasma AII is lower in non-compli-

cated T2Ds compared to healthy controls, and higher in T2Ds with nephropathy compared to non-complicated T2Ds. In this study, although pioglitazone decreased HbA1c significantly, the decrease in HbA1c did not correlate with the decrease in plasma AII; therefore, the effect of pioglitazone on decreasing plasma AII may be independent of its glucose-lowering effect.

In summary, we investigated the effect of pioglitazone on plasma AII, a putative marker of adipocytes RAS, and various metabolic parameters in subjects with T2D. Pioglitazone treatment for 16 weeks resulted in a decrease in plasma AII correlating with an increase in LPL mass, which is a marker of LPL production from adipocytes and insulin sensitivity. These results suggest that the insulin-sensitizing effect of pioglitazone may be involved in suppressing RAS in adipocytes.

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