Effect of Pioglitazone on Blood Proinsulin Levels in Patients with Type 2 Diabetes Mellitus

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Abstract. The objective of this study was to clarify the influence of pioglitazone (Pio) on proinsulin (PI) in patients with type 2 diabetes mellitus. The subjects were 55 patients with type 2 diabetes. Among them, 18, 18, and 19 patients were respectively treated with Pio alone (group P), gliclazide (Gli) alone (group G), or Pio plus Gli (group PG) for 12 weeks. Fasting blood samples were obtained before and after treatment and were used to measure fasting plasma glucose (FPG), HbA$_1c$, immunoreactive insulin (IRI), and PI. The levels of FPG, HbA$_1c$, and IRI showed a significant decrease after treatment with Pio in groups P and PG. Treatment with Pio also caused PI to decrease significantly (group P: from 24.7 ± 12.9 (mean ± SD) to 14.0 ± 6.2 pmol/L, p < 0.01, group PG: from 24.3 ± 11.3 to 14.4 ± 6.5 pmol/L, p < 0.01). In group G, treatment with Gli caused FPG and HbA$_1c$ to decrease significantly, but PI showed no change (21.5 ± 12.3 to 21.6 ± 10.4 pmol/L, p = n.s.). In patients with type 2 diabetes, treatment with Pio achieved an improvement of glycemic control and reduced the load on the pancreatic beta cells.

Key words: Insulin resistance, Type 2 diabetes mellitus, Thiazolidinedione derivative, Insulin secretion


TYPE 2 diabetes mellitus is considered to be characterized by insufficient insulin secretion [1] as well as insulin resistance [2]. Pioglitazone is a new oral antihyperglycemic agent from the thiazolidinedione group that exhibits an antihyperglycemic effect by reducing insulin resistance [3]. Pioglitazone has been reported to be useful for treating patients with type 2 diabetes mellitus in whom satisfactory glycemic control cannot be achieved by dietary or sulfonylurea therapy [3, 4].

Patients with type 2 diabetes mellitus are reported to have increased blood proinsulin levels [5, 6] and this is assumed to reflect the dysfunction of pancreatic beta cells [7]. However, the literature has few studies assessing the influence of pioglitazone on blood proinsulin levels.

In the present study, we administered pioglitazone to patients with type 2 diabetes mellitus and assessed the influence of this drug on blood proinsulin levels. We also administered gliclazide, a sulfonylurea, to patients with type 2 diabetes mellitus and compared the influence of treatment with gliclazide or pioglitazone on blood proinsulin levels.

Materials and Methods

The subjects were 55 patients with type 2 diabetes mellitus who were being treated at the diabetic outpatient clinic of our hospital. Pioglitazone was administered alone at a dose of 30 mg/day for 12 weeks to 18 patients (4 men and 14 women). Gliclazide was administered alone at a dose of 40 mg/day for 12 weeks to 18 other patients (5 men and 13 women). In the remaining 19 patients (5 men and 14 women), 30 mg/day of pioglitazone was administered in combination with 40 mg/day of gliclazide for 12 weeks. Fasting blood samples were collected before and af-
ter treatment and were used for the measurement of blood glucose, \( \text{HbA}_{1C} \), serum insulin, C-peptide, total cholesterol, triglycerides (TG), HDL-cholesterol, free fatty acids (FFA), and proinsulin. The blood pressure was also determined and the body mass index (BMI) was calculated before and after treatment. During the treatment period, the dietary and exercise therapy were kept constant and the dose of gliclazide was not changed. In addition, lipid-lowering agents were not administered during the treatment period. Pioglitazone was administered at a dose of 30 mg once daily after breakfast. Patients with diabetic retinopathy, nephropathy, neurological disease, arteriosclerotic disease, or hepatic dysfunction were excluded from the study. This study was approved by the Ethics Committee of Hiroshima Prefectural Hiroshima Hospital. Prior to enrollment in the study, written informed consent was obtained from all subjects participating in this study.

Blood glucose, total cholesterol, TG, and FFA were measured by enzymatic methods, \( \text{HbA}_{1C} \) was measured by HPLC, and HDL-cholesterol was measured by the selective inhibition method. Insulin was determined using an immunoradiometric assay kit (Dainabot, Tokyo, Japan). The assay showed no cross-reaction with proinsulin or C-peptide. C-peptide was measured using a radioimmunoassay kit (Daiichi Radioisotope, Tokyo, Japan). Proinsulin was also determined using a radioimmunoassay kit (Linco Research, St. Charles, MO, U.S.A.). The assay showed no cross-reaction with insulin or C-peptide, but showed a cross-reaction with a metabolite of proinsulin. The proinsulin/insulin ratio was calculated as a molar ratio (PI/Pl + IRI, Pl=proinsulin, IRI=immunoreactive insulin).

Numerical data are reported as the mean ± standard deviation. The significance of differences between two groups was determined by Wilcoxon's signed-rank test, while the significance of differences between 3 groups was determined by one-way factorial ANOVA and the multiple comparison test. Pairs of variables were analyzed by Spearman's rank correlation test. The level of significance was set at \( p < 0.05 \).

**Results**

Table 1 compares various pretreatment data between the pioglitazone, gliclazide, and pioglitazone plus gliclazide groups. There were no differences of

| Table 1. Clinical characteristics of the subjects before and after treatment |
|---------------------------------|---------------------------------|---------------------------------|
|                                | Pioglitazone Group              | Gliclazide Group               | Combined Group                  |
| Number of patients             | 18                              | 18                              | 19                              |
| (Male/Female)                  | (4/14)                          | (5/13)                          | (5/14)                          |
| Age (years)                    | 63.4 ± 9.5                      | 61.2 ± 8.8                      | 64.1 ± 5.4                      |
|                                | before                          | after                           | before                          | after                           |
| FPG (mg/dl)                    | 175.1 ± 34.4                    | 128.7 ± 26.0*                   | 180.2 ± 41.3                    | 135.5 ± 27.7*                   | 173.2 ± 31.1                    | 120.2 ± 23.8*                   |
| \( \text{HbA}_{1C} \) (%)      | 8.6 ± 1.6                       | 7.5 ± 1.3*                      | 8.2 ± 0.8                       | 7.0 ± 0.7*                      | 8.6 ± 0.8                       | 7.3 ± 0.8*                      |
| IRI (\( \mu \)U/ml)           | 8.2 ± 2.6                       | 6.6 ± 3.9*                      | 7.1 ± 3.3                       | 8.9 ± 5.5*                      | 7.7 ± 4.2                       | 5.3 ± 2.8*                      |
| CP (ng/ml)                     | 1.7 ± 0.7                       | 1.3 ± 0.5*                      | 2.1 ± 0.8                       | 2.1 ± 0.8                       | 1.8 ± 0.8                       | 1.3 ± 0.3*                      |
| T. Chol (mg/dl)                | 209.7 ± 24.0                    | 206.0 ± 30.9*                   | 210.7 ± 28.2                    | 206.1 ± 29.4                    | 211.0 ± 34.5                    | 210.4 ± 27.8                    |
| TG (mg/dl)                     | 137.4 ± 51.0                    | 106.2 ± 55.1*                   | 161.3 ± 91.8                    | 153.5 ± 86.6                    | 146.2 ± 81.7                    | 98.6 ± 51.5*                    |
| HDL-C (mg/dl)                  | 55.6 ± 10.2                     | 63.3 ± 14.3*                    | 55.2 ± 10.7                     | 53.4 ± 9.7                      | 56.3 ± 10.6                     | 68.2 ± 12.4*                    |
| FFA (\( \mu \)Eq/l)           | 617.4 ± 178.4                   | 516.8 ± 136.6*                  | 592.3 ± 130.0                   | 617.4 ± 215.7                   | 498.9 ± 173.2*                  | 438.6 ± 171.6                   |
| SBP (mmHg)                     | 141.7 ± 12.5                    | 142.6 ± 11.4                    | 142.7 ± 13.4                    | 142.7 ± 10.9                    | 142.7 ± 13.4                    | 143.2 ± 12.9                    |
| DBP (mmHg)                     | 87.9 ± 3.1                      | 88.0 ± 4.1                      | 85.8 ± 6.6                      | 86.0 ± 4.0                      | 86.4 ± 5.3                      | 86.9 ± 5.7                      |
| BMI (kg/m²/m²)                 | 27.8 ± 3.2                      | 28.4 ± 3.4*                     | 26.7 ± 3.3                      | 27.0 ± 3.1                      | 26.7 ± 2.6                      | 27.5 ± 2.8*                     |
| PI/Pl+IRI (\%)                | 29.1 ± 9.6                      | 24.7 ± 8.7                      | 30.0 ± 10.5                     | 27.0 ± 11.1                     | 31.7 ± 9.1                      | 28.5 ± 11.0                     |

FPG: fasting plasma glucose; \( \text{HbA}_{1C} \): hemoglobin \( \text{A}_{1C} \); IRI: immunoreactive insulin; CP: C-peptide; T. Chol: total cholesterol; TG: triglycerides; HDL-C: HDL-cholesterol; FFA: free fatty acids; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; PI: proinsulin; Combined Group: pioglitazone + gliclazide; *p<0.01 vs. before treatment, **p<0.05 vs. before treatment, *p<0.05 vs. pioglitazone group. Values are mean ± SD.
fasting blood glucose or HbA1C between the three groups, nor was there any difference of glycemic control before treatment between the three groups. There were also no significant differences of age, blood pressure, BMI, serum levels of insulin, C-peptide, total cholesterol, TG, or HDL-cholesterol between the three groups. Furthermore, there was no significant difference of blood proinsulin levels before treatment between the three groups. FFA levels were significantly lower (p<0.05) in the pioglitazone plus gliclazide group than in the pioglitazone group.

Table 1 shows the results of various tests performed before and after pioglitazone monotherapy. After treatment with pioglitazone, fasting blood glucose, HbA1C, TG, and FFA showed a significant decrease, as did insulin and C-peptide, while HDL-cholesterol and BMI showed a significant increase. After treatment with pioglitazone, blood proinsulin levels showed a significant decrease (24.7 ± 12.9 to 14.0 ± 6.2 pmol/L, p<0.01) (Fig. 1, left).

Table 1 also shows the results of various tests before and after gliclazide monotherapy. After treatment with gliclazide, fasting blood glucose and HbA1C showed a significant decrease, while the insulin level showed a significant increase. Blood proinsulin levels showed no significant change (from 21.5 ± 12.3 to 21.6 ± 10.4 pmol/L, p=n.s.) before and after treatment with gliclazide (Fig. 1, center).

Furthermore, Table 1 shows the results of various tests before and after combination therapy with gliclazide plus pioglitazone. After combination therapy, fasting blood glucose, HbA1C, and TG showed a significant decrease, as did insulin and C-peptide, while HDL-cholesterol and BMI showed a significant increase. Blood proinsulin levels also showed a significant decrease (24.3 ± 11.3 to 14.4 ± 6.5 pmol/L, p<0.01) after combination therapy (Fig. 1, right).

In the pioglitazone monotherapy group, a significant positive correlation (r = 0.539, p<0.05) was noted between the change of blood proinsulin levels and the change of HbA1C (Fig. 2). No significant correlation was noted between these two parameters in the gliclazide monotherapy group (r = 0.020, ns). In the pioglitazone plus gliclazide group, no significant correlation was noted between these two parameters (r = −0.104, ns). In each group, the proinsulin/insulin ratio showed no significant change after treatment (Table 1). There were no patients who developed hepatic dysfunction after treatment with pioglitazone. Edema was noted in 3 patients.

![Graphs showing changes in proinsulin levels after treatment with pioglitazone, gliclazide, or a combination of the two drugs.](image)

**Fig. 1.** Changes of proinsulin after treatment with pioglitazone, gliclazide, or a combination of the two drugs.
who received pioglitazone, but it was mild and treatment could be continued in all of them.

**Discussion**

Blood proinsulin level has been reported to show an increase in patients with type 2 diabetes mellitus [5, 6] and this has been suggested to reflect pancreatic beta cell dysfunction [7]. The present study showed that blood proinsulin levels were decreased by pioglitazone therapy in patients with type 2 diabetes mellitus. Dietary therapy has also been reported to decrease blood proinsulin levels in patients with type 2 diabetes [8, 9]. Additionally, it has been reported that blood proinsulin levels were decreased after the administration of metformin in type 2 diabetics [10]. Furthermore, we have found that troglitazone, another thiazolidine-group drug for improving insulin resistance, could decrease blood proinsulin levels in patients with type 2 diabetes [11], and Prigeon et al. [12] obtained similar findings.

The mechanism by which pioglitazone causes a decrease of proinsulin is not clear. However, the improvement of insulin resistance by pioglitazone would lead to a decrease of the load on pancreatic beta cells, and this may have been the reason proinsulin levels were decreased. Since dietary therapy [8, 9], administration of metformin [10], and administration of troglitazone [11, 12], which improve insulin resistance, have all been reported to decrease the blood proinsulin level, improvement of insulin resistance may also be related to the decrease of proinsulin by pioglitazone therapy.

In this study, a significant correlation was noted between the improvement of HbA1C by pioglitazone and the decrease of proinsulin. This result suggests the possibility that the improvement of glycemic control is closely related to the decrease of proinsulin. In pancreatic beta cells, 32/33 split proinsulin is synthesized from intact proinsulin by endopeptidase (PC3). It is then converted to des 31/32 proinsulin and finally secreted as insulin and C-peptide [13]. The activity of PC3, a proinsulin convertase, has been reported to increase in hyperglycemia [14]. When glycemic control improves, the synthesis of intact proinsulin is initially lowered and then the activity of PC3 decreases, resulting in inhibition of the conversion of intact proinsulin to 32/33 split proinsulin. Accordingly, it seems that the blood proinsulin level decreased due to the improvement of glycemic control by pioglitazone. On the other hand, no significant correlation was noted between the improvement of HbA1C by pioglitazone and the decrease of insulin. Therefore, proinsulin was influenced more strongly than insulin by the improvement of HbA1C.

In this study, blood proinsulin levels showed no change after treatment with gliclazide. We have also reported previously that there was no change of the blood proinsulin level after gliclazide therapy [11]. Similarly, Davies et al. [15] reported that blood proinsulin levels showed no change or were increased when a sulfonylurea was administered to patients with type 2 diabetes. As a result of the improvement of glycemic control by gliclazide, PC3 activity should have decreased and the conversion of proinsulin to 32/33 split proinsulin should have been inhibited. However, the combined level of intact proinsulin plus 32/33 split proinsulin apparently showed no change, presumably because the synthesis of intact proinsulin was promoted by gliclazide.

The possibility cannot be ruled out that pioglitazone acted directly on pancreatic beta cells to improve their function, resulting in a decrease of the blood proinsulin level. It has been reported that the insulin content of pancreatic beta cells was increased when troglitazone was administered to rats after 90% pancreatectomy [16]. In addition, insulin secretion from rat pancreatic islets has been reported to in-
crease with troglitazone treatment [17]. Furthermore, it has been reported that a marked increase of granules and other structural changes occurred in pancreatic beta cells of db/db mice that were treated with troglitazone [18]. Since these changes were caused by troglitazone, it is possible that pioglitazone, which is another thiazolidinedione-group drug, also has a direct effect on the functioning of pancreatic beta cells. In this study, FFA and TG were both decreased significantly after administration of pioglitazone. High blood FFA levels and hypertriglyceridemia have been reported to directly cause damage to pancreatic beta cells and inhibit insulin secretion [19]. Therefore, it seems possible that the decrease of FFA and TG with pioglitazone therapy may have a secondary influence on the decrease of blood proinsulin levels by reducing the damage to pancreatic beta cells.

It has been reported that not only the blood proinsulin level but also the proinsulin/insulin ratio is high in patients with type 2 diabetes [12]. This ratio is considered to be an indicator of the function of pancreatic beta cells, since beta cell dysfunction impairs the processing of proinsulin and leads to an increase of the proinsulin/insulin ratio. In this study, the proinsulin/insulin ratio showed no change after administration of pioglitazone, so there seems little likelihood that beta cell activity was altered by pioglitazone. On the other hand, pioglitazone was able to improve insulin resistance, presumably because it decreased blood proinsulin levels by reducing the load on pancreatic beta cells. Troglitazone [12] and rosiglitazone [20] have been reported to significantly decrease the proinsulin/insulin ratio in patients with type 2 diabetes, but the ratio showed no significant change after administration of pioglitazone in the present study. Since the cause of this difference is still unclear, it will be necessary to conduct further studies in a larger number of patients for clarification.

In conclusion, this study showed that administration of pioglitazone to patients with type 2 diabetes could not only improve glycemic control but also decrease the blood proinsulin level. It is possible that these beneficial effects of pioglitazone therapy could prevent a decrease of pancreatic beta function during long-term treatment. Accordingly, pioglitazone is considered to be useful for the treatment of patients with type 2 diabetes mellitus.

References