Pioglitazone Reduces Atherogenic Outcomes in Type 2 Diabetic Patients

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Aim: The aim of this study was to evaluate the anti-atherogenic outcomes of pioglitazone, a thiazolidinedione derivative, in type 2 diabetic patients.

Methods: Eight patients with poor diabetic control were treated with 15 mg of pioglitazone for 4 months. Blood samples were collected monthly, and the levels of fasting plasma glucose (FPG), HbA1c, and lipids, such as triglycerides, total cholesterol, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol, were measured. Other parameters, including immunoreactive insulin (IRI), remnant-like particle-cholesterol (RLP-C), adiponectin, plasminogen activator inhibitor type 1 (PAI-1), tumor necrosis factor (TNF)-α, leptin, brain natriuretic peptide (BNP), and high-sensitivity (hs)-C-reactive protein (CRP), were examined at the beginning and end of the study. In addition, clinically adverse side-effects were evaluated.

Results: Treatment with pioglitazone significantly decreased the levels of HbA1c, FPG, the homeostasis model assessment of insulin resistance (HOMA-IR) index, RLP-C, PAI-1, TNF-α, and hs-CRP, but not the level, IRI, lipids, or leptin. In contrast, adverse side-effects, including body weight gain, liver dysfunction and edema, were not observed during this study.

Conclusion: These results strongly suggested that treatment with pioglitazone has a greater clinical benefit for the prevention of atherosclerosis, including coronary heart diseases, without any adverse side-effects.


Key words; Diabetes mellitus, Insulin resistance, Pioglitazone, Atherosclerosis

Introduction

Multiple epidemiological and clinical studies have established that diabetes mellitus is the most crucial risk factor in the pathogenesis of atherosclerosis, including coronary heart disease (CHD) and subsequent sudden death¹⁻³. Considerable evidence supports the view that insulin resistance, its concomitant compensatory hyperinsulinemia, and the related glucose intolerance are associated with CHD⁴⁻⁷. Therefore, to prevent CHD, the treatment must include beneficial agents for insulin resistance.

Pioglitazone, a thiazolidinedione derivatives (TZDs), is a novel insulin-sensitizing agent for the treatment of insulin resistance⁸⁻¹⁰. Interestingly, in a very recent study, pioglitazone was demonstrated to reduce not only the need to add insulin but also the number of all-cause mortality, non-fatal myocardial infarction, and stroke, much more significantly than the control in patients with type 2 diabetes who have a high risk of macrovascular events¹¹. However, pioglitazone could have adverse side-effects, such as body weight gain, liver toxicity, fluid retention and its related edema, and congestive heart failure, when the agent is used at maximal dose¹⁰, ¹², ¹³. Subsequently, to reduce both the progression of diabetic macroangiopathy and the risk of undesirable adverse side-effects, low-dose pioglitazone appears to be the treatment of choice, as it seems to offer a greater clinical benefit in type 2 diabetic patients.
In this study, we therefore evaluated the efficacy of pioglitazone on the changes of atherogenic and anti-atherogenic parameters, including several types of adipocytokines, and the adverse side-effects, in type 2 diabetic patients.

**Research Design and Methods**

**Subjects**

Eight patients with poorly controlled type 2 diabetes (4 men and 4 women) were enrolled from the outpatient clinics of Yamagata University Hospital and its affiliated hospitals. Informed consent was obtained from the participants. The patients, ranging in age from 53 to 82 years, had received diagnoses of type 2 diabetes, and the mean values of their fasting plasma glucose (FPG) and HbA1c were 195.6 ± 11.8 mg/dL and 8.53 ± 0.23%, respectively. The mean duration of diabetes was 15.8 ± 2.1 years. Their height and body weight were determined to calculate their body mass index (BMI), and their levels of systolic and diastolic blood pressure and body weight were also examined each time the subjects visited the hospital. A summary of the subjects’ clinical and laboratory findings is given in Table 1.

**Protocol and Measurement of Laboratory Parameters**

The subjects were administered 15 mg of pioglitazone once a day in the morning for 4 months. Blood samples were collected monthly, and the levels of FPG, HbA1c, triglycerides (TG), total cholesterol (T-Chol), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) were measured. Other parameters, including immunoreactive insulin (IRI), remnant-like particle-cholesterol (RLP-C), adiponectin, plasminogen activator inhibitor type 1 (PAI-1), tumor necrosis factor (TNF)-α, leptin, and high-sensitivity (hs)-C-reactive protein (CRP), were examined at the beginning (Month 0) and the end (Month 4) of the study. The values of FPG and IRI were also used to estimate the homeostasis model assessment insulin resistance (HOMA-IR) index. In addition, compliance with the agent, including clinically adverse side-effects, was carefully checked, and the following parameters were measured each month: glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) for hepatic dysfunction; and plasma brain natriuretic peptide (BNP) for fluid retention, peripheral edema, and congestive heart failure. Furthermore, to evaluate the occurrence of edema by the agent, the edema index was measured using a body composition analyzer, InBody 3.0® (Biospace, Tokyo, Japan), at the beginning (Month 0) and end (Month 4) of the study. All subjects were instructed to maintain the same caloric intake and level of physical activity, and the dose and/or type of anti-hypertensive or anti-hyperlipidemic, if used, or anti-diabetic agents was not changed throughout the study.

**Statistical Analysis**

The results are expressed as the mean ± SEM. Statistical significance was first estimated by one-way analysis of variance (ANOVA) for comparison. When a significant effect was found, the results were further compared with the Bonferroni multiple range test, and the difference was considered significant at p < 0.05.

**Results**

**Effect of Pioglitazone on Glucose Metabolism**

As shown in Fig. 1, by treatment with pioglitazone, the levels of both FPG and HbA1c significantly decreased after Month 2 in FPG and Month 3 in

### Table 1. Baseline characteristics and laboratory findings of patients with type 2 diabetes

| Number (M/F) | 8 (4/4) |
| Age (years) | 65.3 ± 4.9 |
| DM Duration (years) | 15.8 ± 2.1 |
| BMI (kg/m²) | 25.8 ± 1.1 |
| Sys BP (mmHg) | 126.0 ± 5.0 |
| Dia BP (mmHg) | 67.5 ± 2.2 |
| FPG (mg/dL) | 195.6 ± 11.8 |
| HbA1c (%) | 8.53 ± 0.23 |
| IRI (µU/mL) | 12.1 ± 2.1 |
| TG (mg/dL) | 134.4 ± 16.7 |
| T-Chol (mg/dL) | 201.8 ± 6.9 |
| LDL-C (mg/dL) | 117.6 ± 6.4 |
| HDL-C (mg/dL) | 57.3 ± 3.1 |
| GOT (IU) | 30.6 ± 3.2 |
| GPT (IU) | 30.1 ± 4.2 |
| LDH (IU) | 393.4 ± 34.8 |
| BNP (pg/mL) | 31.7 ± 6.7 |
| Edema index*(%) | 0.346 ± 0.005 |

Values are the mean ± SEM. DM, diabetes mellitus; BMI, body mass index; Sys BP, systolic blood pressure; Dia BP, diastolic blood pressure; FPG, fasting plasma glucose; IRI, immunoreactive insulin; TG, triglycerides; T-Chol, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactate dehydrogenase; BNP, brain natriuretic peptide.

*recommended normal range; < 0.350
HbA1c compared with Month 0. The reduction of HbA1c continued until the end of the study. At the end of the study, the levels of FPG and HbA1c were 155.9 ± 8.3 mg/dL and 7.29 ± 0.19%, respectively (p < 0.05). In addition, treatment with pioglitazone significantly reduced the level of the HOMA-IR index at the end of this study (p < 0.05). The level of IRI also slightly, but not significantly, decreased with the agent (Table 2).

**Effect of Pioglitazone on Lipid Metabolism**

Although pioglitazone gradually decreased the levels of TG, T-Cho and LDL-C, and slightly elevated the level of HDL-C, we could not observe any change in these parameters at the end of the study (Fig. 2). The agent significantly decreased the level of TG from Month 2 compared to Month 0, and the level had decreased to 73% at the end of the study.

Almost identical to the result of TG, pioglitazone significantly reduced the RLP-C level at the end of the study and the value reduced to 73% of that in Month 0 (Table 2).

**Effect of Pioglitazone on the Production of Adipocytokines**

Treatment with pioglitazone remarkably reduced the levels of PAI-1 and TNF-α in Month 4 compared with Month 0. The level of adiponectin was slightly, but not significantly, elevated by treatment with the agent (Table 2). In contrast, the level of leptin was not significantly changed by the agent.

Notably, as shown in Fig. 3, pioglitazone significantly decreased the relative change of the hs-CRP in Month 4, and reached 61.4 ± 7.1% of Month 0.

**Effect of Pioglitazone on the Other Parameters**

Throughout the study, we did not observe clinically adverse effects of pioglitazone, including systolic and diastolic blood pressure, body weight, or liver dysfunction (Fig. 4A, B, C, D). In addition, the occurrence of edema did not clinically appear and the plasma BNP level and edema index were not changed by treatment with pioglitazone (Fig. 4E, F).
Discussion

In this study, we clearly showed that treatment with pioglitazone reduced the atherogenic outcomes, including the HOMA-IR index, RLP-C, PAI-1, TNF-α, and hs-CRP, in addition to improving essential glycemic control. These favorable results strongly suggest that treatment with pioglitazone might be one of the most promising anti-atherogenic therapies for type 2 diabetic patients who have a high risk of macrovascular events.

First, we examined the effect of pioglitazone on glucose metabolism in the subjects. In this study, pioglitazone, even at 15 mg, significantly decreased the levels of FPG after Month 2 and of HbA1c after Month 3 (Fig. 1). In addition, pioglitazone remarkably reduced the HOMA-IR index value and, partly, the IRI level (Table 2), indicating that 15 mg of pioglitazone could clinically ameliorate insulin resistance in type 2 diabetic patients, even for a short duration.

Extensive studies have indicated that, as potential risk factors for the development of insulin resistance and atherosclerosis, the abnormalities of lipid metabolism and multiple adipocytokines produced by adipose tissue are thought to contribute to the progression of insulin resistance and its related diabetic macroangiopathy. Recent intervention trials have shown that insulin-sensitizing agents, including metformin and other TZDs, affect dyslipidemia and the abnormal secretion of adipocytokines, such as adiponectin, in diabetic patients; however, whether insulin-sensitizing agents such as pioglitazone could affect the levels of these potential risk factors for diabetic macroangiopathy remains undetermined.

As for the atherogenic risk factors, RLP-C has recently been identified as an independent risk factor of CHD and is strongly associated with the progression of atherosclerosis, since remnants, such as RLP-C, are easily accumulated by macrophages. In addition, it has been revealed that increased PAI-1 reduced fibrinolytic activity and that TNF-α plays an important role in inflammation as well as decreasing the tyrosine kinase activity of the insulin receptor. It is likely that the elevation of these parameters might result in the development of diabetic macroangiopathy through the upregulation of CRP production.

Subsequently, we evaluated the effects of pioglitazone on the levels of lipids and several adipocytokines. In this study, treatment with pioglitazone significantly decreased the levels of RLP-C, PAI-1, and TNF-α (Table 2). In addition, pioglitazone markedly decreased the relative change of the hs-CRP, a strong predictor of CVD events, at the end of the study.
(Fig. 3). Therefore, reduction of these atherogenic parameters by the agent might be beneficial for the prevention of atherosclerosis, including CHD, due to its anti-inflammatory effect, in diabetic patients. However, different from other reported studies\(^\text{11-20}\), we could not observe any significant change in other parameters, including TG, T-Cholesterol (T-Cho), LDL-C, HDL-C, leptin, or adiponectin, although the value of TG decreased to 73% (Fig. 2A) and that of adiponectin increased to 162.8% at the end of the study (Table 2). Indeed, even in this study, treatment with pioglitazone significantly decreased the level of TG in Month 2. The precise mechanism(s) is not clear, but it is likely that the discrepancy may result from the different types of study protocols used; i.e., our study had a low dose of pioglitazone and a small number of subjects, while other studies had more than 30 mg of pioglitazone and a large number of subjects.

On the other hand, different from the result of pioglitazone\(^\text{11}\), the most recent study reported that rosiglitazone, another TZD, is associated with a significant increase in the risk of myocardial infarction, with an increase in the risk of death from cardiovascular causes\(^\text{28}\). Although the precise mechanism(s) remains unclear, it is likely that the discrepancy may result from the adverse effects of the agent on serum lipids. Goldberg et al. showed that pioglitazone and rosiglitazone have significantly different effects on plasma lipids, independent of glycemic control or comorbid lipid-lowering or other anti-hyperglycemic therapy; i.e., pioglitazone can significantly decrease the levels of TG, LDL-C, particle concentration and increase the levels of HDL-C and LDL-C particle size compared with rosiglitazone\(^\text{24}\). In this study, although not significant, we could observe the tendency of the reduction of TG and LDL-C, and the elevation of HDL-C (Fig. 2A, C, D).

Furthermore, in this study, we further evaluated the effect of pioglitazone on clinically adverse side-effects, including blood pressure, liver dysfunction, body weight gain, or occurrence of edema; however, we did not observe any change of these parameters, including symptoms or laboratory findings. Diabetes mellitus itself has the potential to induce sodium retention and volume expansion, eventually affecting the cardiac hormone systems. In addition, several studies have reported that TZDs, such as pioglitazone, further induced the appearance of edema and subsequent congestive heart failure in diabetic patients\(^\text{29, 30}\); however, we and other investigators showed that reduction of the insulin amount by pioglitazone might, at least, be beneficial to decrease the plasma BNP level\(^\text{15, 31}\). In this study, to evaluate the occurrence of edema by pioglitazone, we measured the edema index using a body composition analyzer, InBody 3.0\(^\text{10}\). This device can measure various parts of the body more accurately by using segmental multi-frequency bioelectrical impedance analysis, and also evaluate the body composition of muscle-fat distribution, protein, bone, and intracellular and extracellular fluid levels\(^\text{32, 33}\). The InBody 3.0\(^\text{10}\) is now widely used to assess weight management, in exercise science, and the clinical diagnosis of obesity and/or nutritional assessment after gastrectomy\(^\text{34, 35}\); however, in this study, pioglitazone did not affect the level of the edema index, suggesting that the agent did not induce adverse extracellular fluid retention (Fig. 4F).

In conclusion, in this study, we have demonstrated that treatment with pioglitazone could safely ameliorate glucose metabolism with a striking reduction of the atherogenic outcome. These results provide new insights into the potential mechanisms of the pleiotropic effects of pioglitazone whereby the treatment could have clinical benefits and contribute to the prevention of atherosclerosis, including CHD, especially in type 2 diabetic patients.

References

2) Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, and Brunzell JD: Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure. JAMA, 1998; 280:140-146
30) Benbow A, Stewart M, and Yeoman G: Thiazolidinediones for Type 2 diabetes: All glitazones may exacerbate heart failure. BMJ, 2001; 322:236
32) Cha K, Chertow GM, Gonzalez J, Lazarus JM, and Wilmore DW: Multifrequency bioelectrical impedance estimates the distribution of body water. J Appl Physiol,
1995; 79:1316-1319