Short-term Low-dosage Pioglitazone Treatment Improves Vascular Dysfunction in Patients with Type 2 Diabetes

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Abstract. Endothelial dysfunction is an early marker of atherosclerosis. Pioglitazone is commonly used in the treatment of type 2 diabetes and has vascular protective effects beyond its hypoglycemic ones. We investigated the vascular effects of short-term, low-dosage pioglitazone in patients with type 2 diabetes. The study included 15 subjects with type 2 diabetes with normoalbuminuria (age, 60.7 ± 11.9 years; body mass index [BMI], 23.9 ± 3.3 kg/m²). The patients received pioglitazone at 15 mg daily for 4 weeks. BMI, systolic and diastolic blood pressure, laboratory parameters (fasting plasma glucose, insulin, lipid profile, high-sensitive C-reactive protein [hsCRP], and adiponectin) were assessed at baseline and after treatment. The forearm blood flow (FBF) was measured during reactive hyperemia by strain-gauge plethysmography. Short-term, low-dosage pioglitazone did not improve glycemic control or insulin sensitivity. However, the peak FBF and flow debt repayment (FDR) were markedly improved. There was no correlation of the improvement of peak FBF and FDR with the observed changes of metabolic parameters. However, the increment of adiponectin and decrement of hsCRP were well correlated with the improvement of peak FBF. These results indicate that short-term low-dosage pioglitazone may improve vascular function via increasing adiponectin expression and decreasing low-grade inflammation in type 2 diabetic patients.

Key words: Pioglitazone, Forearm blood flow, Strain-gauge plethysmography, Reactive hyperemia

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Materials and Methods

Subjects

We enrolled 15 patients (5 men and 10 women) with type 2 diabetes who were in follow up at the Outpatient Clinic of Shiga University Hospital; they were consecutively recruited to this study between April 2005 and July 2006, and all were normoalbuminuric subjects (albumin execution rate was 10.4 ± 7.1 µg/min, mean ± standard deviation [SD]). Four patients were treated with diet therapy alone, and 11 patients were treated with oral hypoglycemic drugs. As the oral hypoglycemic drug, 9 patients were taking sulfonylurea, 4 patients were taking α-glucosidase inhibitors, and 1 patient was taking nateglinide. There were 7 hypertensive patients. Two patients were taking angiotensin II receptor blocker, 7 patients were taking calcium channel blockers, 2 patients were taking angiotensin converting enzyme inhibitors, and 3 patients were taking diuretics. Five hypercholesterolemic patients were being treated with statins. The prescribed dosages of these drugs, which are known to influence endothelial functions, were not changed during the study period. Menstruation cycle may affect vascular function, and two of the female patients were pre-menopausal. Thus, we measured vascular function at the same menstruation stages in these subjects.

The patients received pioglitazone at 15 mg daily for 4 weeks, and BMI, systolic and diastolic blood pressure, and laboratory parameters were assessed at baseline and after treatment. The forearm blood flow (FBF) was also measured during reactive hyperemia (RH) by strain-gauge plethysmography at baseline and after treatment.

The study protocol was approved by the Ethics Committee of the Shiga University of Medical Science, and all subjects gave informed consent.

Assessment of vascular functions by strain-gauge plethysmography

The vascular function of each patient was examined under overnight fasting condition or after at least 4 h of fasting. Each subject was kept in a supine position throughout this part of the study. After 30 min in the supine position, the basal FBF was measured using a mercury-filled Silastic (Dow Corning, Midland, Michigan, USA) strain-gauge plethysmograph (EC-5R, D. E. Hokanson, Inc., Issaquah, Washington, USA) as described [12, 13]. The effect of RH on FBF was measured as described elsewhere [14, 15]. To induce RH, FBF was occluded by inflating the cuff on the right upper arm to a pressure of 200 mmHg for 5 min. After release of the cuff, FBF was measured for 180 sec. The peak FBF response [12] and total reactive hyperemic flow (flow debt repayment [FDR]) [16] during RH were used to assess the resistance of vessel endothelial function. Percent peak FBF, as an index of the peak FBF response, was obtained by calculation of the increment of peak FBF divided by mean basal FBF. The coefficient of variation (CV) of measurements for basal FBF in these subjects at baseline was 10.9 ± 5.6%.

Biochemical measurements

Venous blood samples were obtained in overnight fasting state. For postprandial examination, blood samples were also obtained 30 min before strain-gauge plethysmography. Routine chemical methods were used to determine the serum concentrations of total cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, and high-sensitive C-reactive protein (hsCRP). The plasma adiponectin concentration was measured as described elsewhere [17]. The hsCRP and adiponectin were measured by SRL Inc. (Tokyo, Japan). The CVs of these measurements were within 10–15%. Homeostasis model assessment of insulin resistance (HOMA-IR), an insulin resistance index, was calculated as described elsewhere [18].

Statistical analysis

Values are expressed as the mean ± SD. Two-tailed unpaired Student’s t test was used to compare means. Relationships between variables were estimated with simple regression analysis. A P-value of <0.05 was considered statistically significant. Analyses were processed using the JMP IN 5.1 software package (SAS Institute Inc., Cary, North Carolina, USA).

Results

Effect of pioglitazone treatment on metabolic parameters

The metabolic characteristics of the 15 diabetic subjects measured in the overnight fasting state at baseline
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The effects of pioglitazone at 15 mg daily for 4 weeks on metabolic parameters are also summarized in Table 1. After treatment with pioglitazone, levels of HbA1c, lipid profiles, and HOMA-IR were unchanged from those at baseline, even though fasting plasma glucose was decreased. Adiponectin levels were increased by pioglitazone treatment. On the other hand, levels of hsCRP were significantly decreased by pioglitazone treatment.

**Effect of pioglitazone treatment on vascular function**

The time courses of % peak FBF before and after pioglitazone treatments are shown in Figure 1. Because it has been reported that hyperglycemia influences vascular function [19–21], we thus assessed the relationship among % peak FBF, FDR, and plasma glucose levels under overnight fasting and 4-h fasted conditions. At baseline, we found a strong relationship between % peak FBF and FDR (r = 0.859, P < 0.001), but we did not find any relationship between % peak FBF and plasma glucose levels (r = 0.472, P < 0.104). After pioglitazone treatment, peak FBF was markedly increased, although basal FBF values were comparable. The peak FBF response (% peak) and total FDR were also increased (Table 2). Furthermore, we found a strong relationship between % peak FBF and plasma glucose levels (r = 0.639, P < 0.014) after pioglitazone treatment. However, the increments in % peak FBF were not correlated with changes in plasma glucose levels (r = 0.072, P < 0.799) (Fig. 2).

Furthermore, the % peak FBF was not shown to correlate with any metabolic parameters when plotted against any plasma biochemical parameters except glucose levels. The FDR was also not shown to correlate with any metabolic parameters. However, we found that increment in adiponectin was well correlated with increment in % peak FBF (r = 0.642, P < 0.010) and that decrement of hsCRP level was well correlated with % peak FBF (r = −0.724, P < 0.005), as shown in Table 1.
Figure 2. On the other hand, there was no relationship between changes in FDR and adiponectin ($r = 0.184$, $P = 0.512$), or between changes in FDR and hsCRP ($r = -0.004$, $P = 0.989$).

**Discussion**

Among the major findings of this study, fasting and postprandial vascular function as assessed by changes in FBF during reactive hyperemia by strain-gauge plethysmography were improved by pioglitazone treatment in patients with type 2 diabetes. Second, short-term low-dosage pioglitazone did not improve insulin sensitivity or metabolic controls, as reflected in terms of HOMA-IR or HbA1c, respectively. Finally, improvement of vascular dysfunction was correlated with changes in adiponectin and hsCRP levels but not with any other metabolic parameters, such as plasma glucose and lipid levels.

Vascular endothelium maintains vascular homeostasis, and endothelial cell dysfunction precedes atherosclerosis and consecutive cardiovascular events [22]. Endothelium-dependent vasodilation in the peripheral circulation can serve as a useful biomarker of atherosclerosis [23], and abnormality of endothelium-dependent vasodilation has been demonstrated in type 2 diabetic patients [3–5]. However, the measurement of flow-mediated dilatation (FMD) following intraarterial infusion of vasoactive agents such as acetylcholine and L-NMMA is invasive and time consuming [24, 25]. Furthermore, although FMD is a non-invasive means of detecting endothelial dysfunction, it has low reproducibility [26]. Thus, we assessed vascular function by measurement of FBF during RH by strain-gauge plethysmography in the present study.

Insulin resistance is associated with vascular endothelial function [6]. Furthermore, pioglitazone treatment was shown to improve endothelial function and simultaneously improve insulin resistance in both non-diabetic and diabetic patients [9–11]. Thus, it is difficult to distinguish the effect of improved insulin resistance on its vascular effect. In the present study, at low dose and with short-term treatment, pioglitazone did not improve either HOMA-IR values or HbA1c values. However, we found that pioglitazone improved vascular dysfunction, suggesting that this effect may not be due to its enhancing insulin sensitivity and hypoglycemic effect. We also found that improvement of vascular dysfunction was correlated with improvements of adiponectin and hsCRP levels but not with improvement of any other metabolic parameters, such as plasma glucose and lipid levels. It has been reported that hypoadiponectinemia is closely linked to endothelial dysfunction [27], and adiponectin levels are discussed as cardiovascular risk factors in Japanese men with type 2 diabetes [28]. Furthermore, pioglitazone increases circulating adiponectin levels and subsequently reduces tumor necrosis factor-α levels [29], and it inhibits carotid arterial wall thickness in type 2
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Moreover, it is reported that the antiatherogenic effect of pioglitazone in type 2 diabetic patients is unrelated to the responsiveness to its antidiabetic effect [31]. Thus, pioglitazone improves vascular function at multiple steps.

Martens et al. recently reported that pioglitazone treatment decreased hsCRP levels and increased adiponectin levels [32]. However, they did not find any relationship between vascular function and adiponectin concentration. Regarding this discrepancy with our findings, we note that they assessed vascular endothelial function by using FMD, whereas we measured peak FBF and FDR during RH by strain-gauge plethysmography. Although % peak of FBF is reported to be correlated with acetylcholine-induced vasodilation [15], it is considered to be a combination marker of shear stress and local metabolic factors at the early phase of RH. Supporting this idea, the FMD method failed to detect any response, while the FBF method did, in a study of peripheral vasodilatation after a fatty meal [33]. On the other hand, FDR is reported to be a relatively nitric oxide (NO)-dependent marker at the mid- to late phase of RH [18]. However, we did not find any relationship between improvement of FDR and that of adiponectin. Thus, adiponectin may affect vasculature tissues such as smooth muscle rather than endothelium [34]. Furthermore, it has been reported that NO-independent vasodilation is impaired in diabetic subjects [4, 5]. Thus, pioglitazone may improve both endothelial and non-endothelial function, although it also improves vascular endothelial functions assessed by FMD [8, 9].

Study limitation

The present study was not a double-blind, randomized, placebo study. Furthermore, the number of experimental subjects was not large. Thus, we were not completely able to rule out the possibility of insulin sensitizing and hypoglycemic effects of pioglitazone on vascular function. Further study is needed to confirm the present conclusion.

In summary, short-term, low-dosage pioglitazone treatment was shown to improve vascular function in patients with type 2 diabetes. Thus, pioglitazone is useful for diabetic patients owing to its pleiotropic effects including vascular effects. Thus, pioglitazone might be a promising oral therapy in preventing atherosclerosis seen in patients with type 2 diabetes mellitus.

References


