Pioglitazone Improves Endothelial Function with Increased Adiponectin and High-density Lipoprotein Cholesterol Levels in Type 2 Diabetes

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Abstract. Endothelial dysfunction is considered to be an early event in the development of atherosclerosis. The present study was undertaken to evaluate endothelial function and biochemical markers in type 2 diabetes mellitus (T2DM) patients before and after treatment with or without pioglitazone (PIO). Forty-one T2DM patients without macroangiopathy were randomized to treatment with (n=20) or without (control, n=21) PIO for 12 weeks. Endothelial function was assessed by flow-mediated vasodilation (FMD) using a high-resolution ultrasound method before and after treatment. After treatment, HbA1c levels equally decreased in both groups, but PIO-treated group had significantly increased high-density lipoprotein cholesterol (HDL-C) levels, and decreased triglyceride, fasting insulin levels and HOMA-R. After treatment, increases in %FMD, plasma HDL-C and adiponectin (APN) levels were significantly greater in PIO-treated group than those in control group. Changes of %FMD showed significant positive correlations with those of plasma APN and HDL-C levels. In conclusion, the present study showed that treatment of T2DM improved endothelial function with greater increases in %FMD, APN and HDL-C levels in PIO-treated group than those in control group, suggesting the beneficial effect of PIO on endothelial function in T2DM.

Key words: Thiazolidinediones, Flow mediated vasodilation, Adiponectin

ENDOTHELIAL dysfunction is considered as an early event in the development of atherosclerosis [1]. Accumulating lines of evidence suggest that patients with type 2 diabetes mellitus (T2DM) have impaired endothelial function, thereby leading to the development of atherosclerosis, and finally to cardiovascular events [2, 3, 4].

Measurement of brachial flow-mediated vasodilation (FMD) due to reactive hyperemia-induced endothelial nitric oxide (NO) release by non-invasive ultrasound technique is currently used as a reliable method for evaluating endothelial function [1, 5]. On the other hand, nitroglycerin-mediated vasodilation (NMD) representing endothelium-independent vasodilation [5], is simultaneously determined to ascertain that impaired FMD is not due to vascular smooth muscle dysfunction [1]. This technique is currently considered as one of the standard tests for assessment of conduit artery endothelial function based on its validation, many clinical trials, and a potential predictor for cardiovascular events [1, 6, 7, 8].

Adiponectin (APN), an adipocyte-derived hormone, has been reported to improve glucose metabolism and protect the endothelium from early atherosclerotic events [9]. Since plasma APN levels are reduced in T2DM [10], hypoadiponectinemia may cause endothelial dysfunction in T2DM. Dyslipidemia associated with T2DM also induces endothelial dysfunction, whose correction restores endothelial function [8, 11, 12].

Pioglitazone (PIO), an agonist for peroxisome proliferator-activated receptor γ (PPARγ), is currently and widely used for treatment of T2DM as an insulin sensitizer. Besides affecting the glucose metabolism, it
has been reported that PIO increases circulating APN levels [13], improves lipid profile [14] and endothelial function [15] in T2DM. However, it remains unknown whether the changes of circulating APN and lipid profile may contribute to endothelial function during PIO treatment in T2DM. In this study, we studied vascular endothelial function and biochemical markers in T2DM patients before and after treatment with or without PIO.

### Subjects and Methods

#### Study population and design

Forty-one consecutive patients with T2DM without past history of stroke, coronary artery disease, or arteriosclerosis obliterans were recruited; 33 patients were on oral anti-diabetic agents, including sulfonylurea (n=16), biguanides (n=6), α-glucosidase inhibitor (n=13), insulin (n=5), and the remaining 9 received no medication. Other medications included angiotensin II receptor blockers (n=12), calcium channel blockers (n=12), and HMG-CoA reductase inhibitors (n=10). This study protocol was approved by the Ethics Committee of our institution. Each subject was given informed consent before enrollment in this study.

In PIO-treated group, PIO (average dose: 18mg/day) was added for glycemic control, and maintained in same dose during the study period. In control group, various oral anti-diabetic drugs other than PIO, including sulfonylurea (n=4), biguanide (n=4), and α-GI (n=7), alone or in combination, were started; among them, 3 had no additional medications, 3 had increased dose, and 2 had decreased dose of the same drugs during the study period. All patients were instructed to continue diet (25-30 kCal/kg, 50-60% carbohydrate, 20-30% lipid, and approximately 20% protein) and exercise therapy during the study. After 12-week treatment, the second measurement was undertaken.

#### Measurement of physiological parameters

Flow-mediated vasodilation (FMD) of brachial artery was measured by the ultrasound method using SONOS 5500 (Philips Medical Systems, Andover, MA, USA) equipped with an 11-MHz ultrasound probe as described (16). Percent flow-mediated vasodilation (%FMD) was expressed as the %increase in the maximal diameter from the baseline after cuff deflation within 120 seconds. Percent NTG-mediated vasodilation (%NMD) was expressed as the %increase in the diameter 3 min after administration of NTG (0.3 mg). Inter- and intra-observer variabilities for brachial diameter measurements revealed 0.05 ± 0.02 mm and 0.04 ± 0.02 mm, respectively [16].

Carotid intima media thickness (IMT) was measured by ultrasound (SONOS 5500) as described [16]. B-mode scan was obtained with electronic calipers to 10 mm proximal to the common carotid artery (CCA)-bulbus junction; three points of each right and left CCA were measured and averaged.

#### Measurement of biochemical parameters

Blood samples were withdrawn from antecubital vein after an overnight fasting for at least 12 h. Hemoglobin A1c (HbA1c) levels were measured by latex agglutination, and plasma glucose levels by a glucose oxidase method. Serum immunoreactive insulin (IRI) and lipids were measured by immunoradiometric assay and enzymatic method, respectively. Plasma APN and highly-sensitive C-reactive protein (hsCRP) were measured by ELISA (Fujirebio Inc, Japan) and latex-enhanced immunonephelometric assays (Mitsubishi Chemical Medience, Japan), respectively.

#### Statistical analysis

Data are expressed as means ± standard deviation (SD). All variables were checked for normal distribution by the Kolmogorov-Smirnov one-sample test for goodness of fit. The χ² test was used for comparing proportions of subjects in the two groups. Comparison between groups was made using non-paired t test. Differences between before- and after-treatment values were analyzed using paired t test. Linear relationships between two continuous variables were tested by Pearson’s correlation coefficient. P values less than 0.05 were considered significant. All statistical analyses were performed using Windows software Prism 5.0 (GraphPad Software, CA, USA).

### Results

The clinical characteristics of T2DM patients treated with or without PIO are shown in Table 1. There
were no significant differences of gender, age, current smoking, and medication between PIO-treated and control group. Body mass index (BMI), blood pressure, lipid profile, HbA1c, FPG, IRI, HOMA-R, APN, hsCRP, carotid IMT, %FMD, and %NMD were similar between the two groups.

Table 2 shows the changes of various physiological and biochemical parameters in PIO-treated and control group after 12-week treatment. HbA1c levels significantly \((p < 0.05)\) decreased in both groups. PIO treatment significantly \((p < 0.05)\) increased HDL-C and decreased TG levels, whereas they did not change in control group. Fasting IRI and HOMA-R levels in PIO-treated group were significantly \((p < 0.05)\) decreased, but not in the control group. Plasma APN levels were significantly \((p < 0.05)\) increased in both groups; they were significantly \((p < 0.05)\) greater in PIO-treated group than in control group. Plasma
gesting that treatment of T2DM patients selectively improved endothelial function without affecting vascular smooth muscle function. In comparison with other anti-diabetic treatments, PIO-treated patients had greater increases in %FMD, APN and HDL-C levels, suggesting that PIO could have more beneficial effects on not only circulating APN and HDL-C, but also en-

Table 2. Changes of various parameters in T2DM patients before and after treatment with or without pioglitazone (PIO).

<table>
<thead>
<tr>
<th></th>
<th>PIO-treated</th>
<th>Control</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2±4.3</td>
<td>26.5±4.2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128±13</td>
<td>124±11</td>
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<tr>
<td>DBP (mmHg)</td>
<td>77±11</td>
<td>78±9</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>123±24</td>
<td>119±22</td>
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<tr>
<td>HDL-C (mg/dL)</td>
<td>44±14</td>
<td>54±17***</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>141±62</td>
<td>114±52**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.0±1.1</td>
<td>7.3±1.0***</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>162±28</td>
<td>148±31</td>
</tr>
<tr>
<td>IRI (μU/mL)</td>
<td>6.2±3.0 (n=18)</td>
<td>4.5±2.0*** (n=18)</td>
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<tr>
<td>HOMA-R</td>
<td>2.4±1.2 (n=18)</td>
<td>1.6±0.8** (n=18)</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>5.3±2.3</td>
<td>11.8±4.8***</td>
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<tr>
<td>hsCRP (mg/dL)</td>
<td>0.11±0.15</td>
<td>0.12±0.13</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.93±0.27</td>
<td>0.90±0.28</td>
</tr>
<tr>
<td>%FMD (%)</td>
<td>5.50±1.58</td>
<td>7.45±2.23***</td>
</tr>
<tr>
<td>%NMD (%)</td>
<td>17.9±4.3</td>
<td>18.3±2.6</td>
</tr>
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Data are means ± SD. *p < 0.05, **p < 0.01 and ***p < 0.001 as compared with before treatment in each group. *p < 0.05 and †p < 0.01 as compared with after treatment in control group.

hsCRP levels and carotid IMT did not change in either group. %FMD significantly (p < 0.05) increased in both groups, whereas %NMD did not change (Fig. 1).

Table 3 shows univariate correlations between the changes of %FMD and those of various physiological and biochemical parameters after 12-week treatment with or without PIO. In total subjects, the changes of %FMD showed significant (p < 0.05) correlations with those of plasma APN (r = 0.32) and HDL-C levels (r = 0.32) (Fig. 2). There were significant (p < 0.01) correlations between the changes of %FMD and those of plasma APN levels in PIO-treated group, although there were no significant correlations between the changes of %FMD and HDL-C levels in either PIO-treated or control group. The changes of %FMD showed no significant correlations with those of blood pressure, BMI, FPG, IRI or HbA1c.

Discussion

The present study clearly demonstrates that treatment of T2DM patients improved endothelial function, and that the increase in FMD correlated with those of plasma APN and HDL-C levels. In contrast, %NMD did not change by treatment with or without PIO.
Among them, APN, a collagen-like protein produced specifically by adipose tissue and abundantly present in the circulation, is decreased in T2DM patients [19]. It has been reported that plasma APN levels correlate with %FMD in humans [19]. It has been shown that APN directly stimulates nitric oxide production by activation of endothelial nitric oxide synthase (eNOS) [20]. Furthermore, troglitazone treatment increases plasma APN levels in patients with the metabolic syndrome [21], which correlated with acet-
ylcholine-induced vasodilation. In the present study, there was a positive correlation between the changes of %FMD and those of APN in PIO-treated group, but not in control group, suggesting that PIO treatment improved endothelial function due to increased circulating APN levels. In addition, our data with a greater increase of plasma APN levels after PIO treatment than any other treatments are consistent with those from the previous studies using several PPARγ agonists [13, 22, 23]. Taken together, it is suggested that increased circulating APN by PIO treatment could improve endothelial function partly via enhancement of NO production.

We failed to demonstrate significant correlations between the changes of %FMD and those of HDL-C in either PIO-treated or control group, possibly due to small sample size. However, the changes of %FMD in total subjects positively correlated with those of HDL-C, suggesting that treatment of T2DM could improve endothelial function partly via increased circulating HDL-C levels. In fact, it has been shown that HDL-C stimulates eNOS-phosphorylation to enhance NO production in endothelial cells in vitro [24], and that intravenous recombinant HDL-C infusion restores endothelium-dependent vasodilation in patients with dyslipidemia by increasing NO bioavailability in vivo [11, 25].

PPARγ agonists have also been reported to improve endothelial function via activation of endothelial PPARγ [26, 27]. PPARγ activation has been shown to decrease the expression of adhesion molecules that induce endothelial inflammation by adherence of monocytes to the endothelium [27, 28, 29]. Furthermore, rosiglitazone has been shown to directly enhance NO production in cultured endothelial cells via PPARγ-dependent mechanism [30]. These findings suggest that PPARγ agonists could directly improve endothelial function by decreasing local inflammation and increasing NO production. Thus, PPARγ activation in endothelium could also contribute to improve endothelial function in PIO-treated group, independently of circulating APN and HDL-C.

A limitation of the present study is the relatively small sample size and the lack of a placebo-controlled group. It was difficult to perform multivariate analysis to determine the independent effect of APN and HDL-C on endothelial function due to such a small sample size. Further study using larger sample size and placebo-controlled group should be done.

In conclusion, the present study has demonstrated that treatment with PIO for T2DM patients increases plasma APN and HDL-C levels and improves FMD, suggesting that PPARγ agonists improve endothelial function, possibly via increase in circulating APN and HDL-C levels.

Acknowledgments

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