Pioglitazone Restores Endothelial Function in Patients with Type 2 Diabetes Treated with Insulin

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ABSTRACT

The primary aim of this study was to evaluate the effect of pioglitazone on endothelial function, as assessed by flow-mediated dilatation (FMD) nitroglycerine-induced dilatation (NID) in patients with type 2 diabetes mellitus treated with insulin. A randomized double-blind placebo-controlled trial involved 20 patients with insulin-treated type 2 diabetes. Patients received either pioglitazone 30 mg or placebo for 4 months. FMD, NID, and HbA1c were measured before and after 4 months of treatment. HbA1c decreased from 10.0 (± 2.3) to 8.4 (± 2.0) in the pioglitazone group, a statistically significant improvement in glycemic control (p = 0.018). HbA1c was unchanged in the placebo group (p = 0.477). Endothelial function as assessed by FMD significantly improved from 10.1 (± 4.0)% to 14.6 (± 6.2)% in the pioglitazone group (p = 0.036) as compared to the placebo group (p = 0.705). There was a trend towards improvement in the NID in the pioglitazone group (from 13.3 ± 8.0% to 18.9 ± 5.4%; p = 0.056). In insulin-treated patients with type 2 diabetes, the addition of pioglitazone improves endothelial function, as measured by FMD. Addition of pioglitazone to insulin in type 2 diabetes patients may favorably impact vascular function in diabetes, even after many years of insulin resistance and hyperglycemia.

INTRODUCTION

CARDIOVASCULAR DISEASES, compared with those without diabetes, disproportionately affect people with type 2 diabetes. Furthermore, patients with diabetes have not benefited from the advances in the management of coronary heart disease (CHD) and/or its risk factors that have resulted in a decrease in mortality for CHD patients without diabetes.1 Some of this excess risk relates to an increased prevalence of established risk factors, such as obesity, dyslipidemia, and hypertension, in persons with diabetes. Nevertheless, these traditional risk factors do not fully explain the excess risk for CHD associated with diabetes.2 Therefore, other nontraditional risk factors may be important in people with diabetes, and include, among others insulin resistance, endothelial dysfunction, inflammation, and hyperhomocysteinemia.3,4 Insulin sensitizers have a variety of effects on many of these risk factors including increasing production of nitric oxide in the endothelium.5 If these abnormalities are

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associated with insulin resistance, then treatment with insulin sensitizers should improve endothelial function.

The thiazolidinediones are activators of peroxisome proliferator activator receptor-gamma (PPAR-γ). They enhance insulin action at peripheral tissues and reduce insulin resistance, thereby indicating that they can improve some of the cardiovascular risk factors associated with insulin resistance. Previous studies with thiazolidinediones have demonstrated improvement in endothelial function in both obese subjects without type 2 diabetes and in those with recently diagnosed type 2 diabetes. However, no change was seen in either FMD or NID in patients with long-term diabetes or those with macrovascular diseases.

We investigated the effect of pioglitazone on vascular reactivity in patients with insulin treated type 2 diabetes. This particular cohort was chosen since patients with type 2 diabetes treated with insulin are more likely to have a longer duration of disease. Very little data is available on the effects of thiazolidinediones on endothelial function in patients with a long duration of insulin resistance and hyperglycemia.

METHODS

Subjects

Twenty patients, age 18–75 years, with insulin treated type 2 diabetes (with or without oral hypoglycemic agents) and poor glycemic control (HbA1c > 7.5%), were recruited for the study. All patients were on stable lipid lowering (with statins) and antihypertensive therapy (including ACE inhibitors in all) which was not changed during the study.

The exclusion criteria were active liver disease, pregnant or breast-feeding women, history or recent myocardial infarction within the last 6 months or recent major surgery within the last 6 months.

Procedure

Patients were recruited from the various diabetes clinics affiliated with Tulane University School of Medicine, New Orleans. All patients came for the visits following an overnight fast. If they were on a sulfonylurea or rapid acting insulin, they were asked to omit their morning dose on that day. Physical examination was performed on each visit.

Addition of other treatments known to affect endothelial function—such as estrogen, arginine, statins, and ACE inhibitors—was not permitted during the course of the study. Patients on such drugs were included only if they had been on stable doses for at least 2 months prior to being randomized. No change in such treatments was made during the study.

After screening, patients continued their medication, including insulin, and were randomized to either pioglitazone 30 mg or placebo to be taken once daily with breakfast. Randomization was double blind and was carried out by a research pharmacist using a predetermined randomization code. Patients had brachial artery reactivity assessed at baseline and on study completion. Baseline and 4 months laboratory assessments included hs-CRP, plasma homocysteine, HbA1c, lipid profile, free fatty acids, and liver function tests.

Brachial artery studies

Endothelial function was assessed after an overnight fast by brachial artery reactivity. Two dimensional images were obtained using a linear array 12.5-MHz ultrasound transducer by HDI 5000 colorflow Doppler imaging system (ATL, Bothell, WA). The artery was visualized 6 cm above the ante cubital fossa. Color flow was used to identify the artery. The probe was initially angled at 90° isonation, with a 20° beam steering giving a 70° doppler angle to measure flow velocity. Patients were rested in the supine position for at least 15 minutes before initiation of the procedure. A simultaneous continuous EKG tracing was recorded.

Brachial artery diameter was measured at baseline. A straight segmental tourniquet cuff (Hokanson SC-5) was used to occlude the artery by increasing pressure of 40 mm of Hg over the systolic pressure for 5 min. This was confirmed by monitoring for blood flow while the cuff was inflated. The cuff was then de-
flated and the post-ischemic diameter was recorded at 45–60 sec post-deflation. Vessel diameter was measured as an average of reading taken during four cardiac cycles at end diastole, incident with the R wave on the continuous EKG tracing. All scans were recorded on videotape. FMD was then calculated as a percentage increase over baseline diameter.

After a 15-min rest, another scan was done to assure return to baseline of the arterial diameter. Sublingual nitroglycerin 400 mcg was then administered and a scan was done 3 min later. NID was calculated as a percentage increase over the baseline diameter.

**Statistical analysis**

The Sigma stat 2.03 software package was used for statistical analysis. Analysis of variance (ANOVA) was used to compare the baseline characteristics between those patients receiving active treatment and those receiving placebo in all groups.

Comparisons were made within groups for all parameters comparing baseline with post-treatment values. The study was powered to show a significant ($p < 0.05$) improvement in FMD based on data from other studies at our center demonstrating improvements in FMD with appropriate interventions.8

**RESULTS**

Twenty patients were enrolled in the study in a randomized, double-blind fashion, 10 each to the placebo arm and the active treatment (pioglitazone) arm. Sixteen patients (eight in each group) completed the study. Three were unable to follow up due to scheduling difficulties, and one withdrew due to a coincidental illness (not an adverse event). The baseline demographics for these patients are included in Table 1. There were no significant differences in age/sex of the two groups (Table 2).

At the end of the study, HbA1c decreased from 10.0 ($\pm$ 2.3) to 8.4 ($\pm$ 2.0) in the pioglitzone group, a statistically significant improvement in glycemic control ($p = 0.018$), and was unchanged in the placebo group ($p = 0.477$).

Endothelial function as assessed by FMD significantly improved from 10.1 ($\pm$ 4.0)% to 14.6 ($\pm$ 6.2)% in the pioglitazone group ($p = 0.036$) as compared to the placebo group ($p = 0.705$) (Fig. 1). There was a trend towards improvement in the NID (from 13.3 $\pm$ 8.0% to 18.9 $\pm$ 5.4%; $p = 0.056$).

**DISCUSSION**

This study demonstrates that pioglitazone improves endothelial function as assessed by flow-mediated dilatation while improving glycemic control in insulin treated type 2 diabetes patients. There was a trend towards improvement in nitroglycerin dependent dilatation in pioglitazone-treated patients. Although we studied a small number of patients the study was adequately powered to show a significant treatment effect on FMD.

The thiazolidinediones reduce plasma insulin concentrations while simultaneously lowering blood glucose by increasing insulin-mediated glucose uptake in muscle and adipocytes.9 Interestingly, perhaps by addressing insulin resistance, these drugs have effects on several nontraditional cardiovascular risk factors. These effects have been reviewed extensively elsewhere.3,5

The importance of endothelial dysfunction in the pathogenesis of cardiovascular diseases in diabetes has been recognized,3 and, in fact, it may be a prognostic/risk marker.10–12 Functional assessment of endothelial function is dependent on the ability of blood vessels to dilate in response to a stimulus, and includes

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of Insulin-Treated Type 2 Diabetes Patients that Completed the Study</th>
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<tbody>
<tr>
<td>Pioglitazone</td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
</tr>
<tr>
<td>A1c</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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</tbody>
</table>

None of the parameters reported were statistically significantly different between groups.
Hyperglycemia may exacerbate endothelial dysfunction. Recent data suggest that the metabolic and vascular actions of insulin are probably linked to its action on the endothelium. Thus, insulin sensitizers may improve endothelial function either by enhancing the effect of insulin or by decreasing free fatty acids. The effect of glycemic control on endothelial function is controversial, although one study has demonstrated a significant improvement in function with insulin treatment. Pistrosch et al. showed that insulin resistance per se is related to endothelial dysfunction, independent of glycemic control. Most importantly, they showed that the insulin resistance and endothelial dysfunction are amenable to treatment by rosiglitazone. Improvement of vascular reactivity in obese, nondiabetic patients after treatment with rosiglitazone has also been reported. Our study demonstrated a significant improvement in FMD in insulin-treated type 2 diabetes patients with pioglitazone treatment, while improving glycemic control (even though it was blood glucose was not normalized). The FMD at the end of the study in the pioglitazone group is near normal for our laboratory.

A limitation of our study is that the two groups were not perfectly matched at baseline and by chance the HbA1c was higher at baseline in the pioglitazone group and fell significantly lower in the pioglitazone group compared to placebo. The HbA1c at the end of the study in the pioglitazone group is near normal for our laboratory.

### Table 2. Effect of Pioglitazone Treatment for 4 Months Compared to Placebo (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pioglitazone</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>A1c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10.0</td>
<td>2.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Post</td>
<td>8.4*</td>
<td>2.0</td>
<td>0.018</td>
</tr>
<tr>
<td>FMD (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10.1</td>
<td>4.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Post</td>
<td>14.6*</td>
<td>6.2</td>
<td>0.036</td>
</tr>
<tr>
<td>NID (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>13.3</td>
<td>8.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Post</td>
<td>18.9</td>
<td>5.4</td>
<td>0.056</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to pre-treatment.

FMD, flow-mediated dilatation; NID, nitroglycerine-induced dilatation; tHcy, plasma homocysteine concentration; FFA, free fatty acid concentration.

![Effect of pioglitazone compared to placebo on flow-mediated dilatation. *p value = 0.04.](image)

**FIG. 1.** Effect of pioglitazone compared to placebo on flow-mediated dilatation. *p value = 0.04.
cantly with treatment. While it could be argued that the improvement seen in endothelial function related to improved glycemic control, we do not believe that this is likely. Firstly, endothelial dysfunction in diabetes appears to be related more to insulin resistance than hyperglycemia. Secondly we have not observed and there are no reports in the literature of improvements in FMD related to this degree of improved glycemic control using agents that have their effects predominantly on glycemia such as the sulfonylureas. At the end of the study, the pioglitazone treatment resulted in an average A1c that was very similar to the placebo treated group. Since these values are still well above goal, it suggests that the beneficial effect of pioglitazone on FMD was related to improvement in insulin resistance or a direct vascular effect.

The FMD test is considered to be a marker of endothelial nitric oxide production, whereas the NID is an index of the ability of vascular smooth muscles to relax when exposed to nitric oxide. Since insulin resistance has been associated with impaired nitric oxide production, our results are compatible with the concept that pioglitazone, an insulin sensitizer, can improve nitric oxide production even at a relatively late stage of the disease.

Earlier studies have demonstrated the efficacy of troglitazone to improve endothelial function. Our study is the first double blind randomized study to demonstrate that pioglitazone can improve FMD in insulin-treated type 2 diabetes patients. Our data are also compatible with the findings of Satoh et al., who demonstrated that pioglitazone improves pulse wave velocity, independent of changes in glucose metabolism.

Our data provide support and possible mechanistic insight into the results of the PROACTIVE study. PROACTIVE was a prospective, randomized, controlled trial in 5,238 patients with type 2 diabetes, who had evidence of cardiovascular disease. These patients were randomized to pioglitazone or placebo. Importantly, the study drug was taken in addition to the patient's usual glucose-lowering medications and therefore this study was designed to assess the pure effect of pioglitazone, independent from any of its effects on lowering blood glucose.

The results of the study show that pioglitazone had a modest, and not statistically significant, 10% reduction in the risk of the primary composite endpoint, which consisted of all-cause mortality, nonfatal myocardial infarction, stroke, acute coronary syndrome, and revascularization or amputation. However, the “main secondary endpoint,” consisting only of certain of the primary outcome measures (namely all-cause mortality, myocardial infarction, and stroke), was significantly reduced by 16%. A large proportion of patients in the study were on insulin and these patients had outcomes that were no different from those on oral agents. Thus, they received benefit from a combination of insulin and pioglitazone such as used in our study. The PROACTIVE study results did not provide mechanistic insights into the mechanism underlying the benefit and studies such as ours are therefore important in this context.

In conclusion, pioglitazone improves endothelial function in patients with type 2 diabetes treated with insulin. These findings may have implications for preventing cardiovascular events in high-risk patients with diabetes.

ACKNOWLEDGMENTS

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