Pioglitazone Improves Myocardial Blood Flow and Glucose Utilization in Nondiabetic Patients With Combined Hyperlipidemia

A Randomized, Double-Blind, Placebo-Controlled Study

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Objectives

This study’s aim was to examine whether treatment with pioglitazone, added to conventional lipid-lowering therapy, would improve myocardial glucose utilization (MGU) and blood flow (MBF) in nondiabetic patients with familial combined hyperlipidemia (FCHL).

Background

Thiazolidinediones were found to improve insulin sensitivity and MGU in type 2 diabetes and MBF in Mexican Americans with insulin resistance. Familial combined hyperlipidemia is a complex genetic disorder conferring a high risk of premature coronary artery disease, characterized by high serum cholesterol and/or triglyceride, low high-density lipoprotein (HDL) cholesterol, and insulin resistance.

Methods

We undertook a randomized, double-blind, placebo-controlled study in 26 patients with FCHL, treated with pioglitazone or matching placebo 30 mg daily for 4 weeks, followed by 45 mg daily for 12 weeks. Positron emission tomography was used to measure MBF at rest and during adenosine-induced hyperemia and MGU during euglycemic hyperinsulinemic clamp at baseline and after treatment.

Results

Whereas no change was observed in the placebo group after treatment, patients receiving pioglitazone showed a significant increase in whole body glucose disposal (3.93 ± 1.59 mg/kg/min to 5.24 ± 1.65 mg/kg/min; p = 0.004) and MGU (0.62 ± 0.26 μmol/g/min to 0.81 ± 0.14 μmol/g/min; p = 0.0007), accompanied by a significant improvement in resting MBF (1.11 ± 0.20 ml/min/g to 1.25 ± 0.21 ml/min/g; p = 0.008). Furthermore, in the pioglitazone group HDL cholesterol (+28%; p = 0.003) and adiponectin (+156.2%; p = 0.0001) were increased and plasma insulin (−35%; p = 0.017) was reduced.

Conclusions

In patients with FCHL treated with conventional lipid-lowering therapy, the addition of pioglitazone led to significant improvements in MGU and MBF, with a favorable effect on blood lipid and metabolic parameters. (A study to investigate the effect of pioglitazone on whole body and myocardial glucose uptake and myocardial blood flow/coronary vasodilator reserve in patients with familial combined hyperlipidemia: http://www.controlled-trials.com/mrct/trial/230761/ISRCTN78563659; ISRCTN78563659) © 2007 by the American College of Cardiology Foundation.
Activated receptor-lidinediones, which activate the peroxisome proliferator-improvement of the lipid parameters with thiazolidinediones, have demonstrated that subjects at an increased risk of developing CAD, such as smokers (8) and those with hyperlipidemia (9), often have abnormal myocardial blood flow (MBF) and coronary flow reserve (CFR) despite normal coronary angiograms. The latter has been interpreted as evidence of coronary microvascular dysfunction which might precede development of more typical CAD. The FCHL phenotype, however, is associated with insulin resistance, and the lipid abnormalities in this condition are therefore similar to those seen in patients with metabolic syndrome and type 2 diabetes (3).

The complex nature of FCHL, involving genetic and environmental factors, and the multiple lipid abnormalities that characterize FCHL make this condition difficult to treat, frequently necessitating combination drug therapy with different classes of lipid-lowering agents. Given the association of FCHL with insulin resistance, there is a potential for combination drug therapy with different classes of lipid-lowering agents. Given the association of metabolic syndrome and type 2 diabetes (3) with metabolic syndrome and type 2 diabetes (3), FCHL patients frequently have hypertension (14). Thiazolidinediones have been reported to improve endothelial function (15) and myocardial glucose utilization (MGU) (16,17) in patients with type 2 diabetes. However, treatment with pioglitazone had no effect on MBF in patients with insulin-treated type 2 diabetes (17), although thiazolidinediones did improve coronary vasomotor abnormalities in Mexican Americans with insulin resistance (18).

We hypothesized that treatment with thiazolidinediones, added to conventional management, might improve both whole body and myocardial insulin sensitivity and MBF in FCHL patients. Therefore, we undertook a randomized, double-blind, placebo-controlled study in FCHL patients using PET to measure MGU, MBF, and CFR at baseline and after 4 months of treatment with the thiazolidinedione pioglitazone.

Methods

Patients. Index patients with FCHL from our well characterized cohort were invited to participate in the study. A description of the FCHL cohort and recruitment and diagnostic criteria have been previously published in detail (3,19). Patients were eligible to participate in the present study if despite lipid-lowering medication with a statin (with or without other conventional lipid-lowering agents) 1 or more of their serum lipid parameters remained above target levels (total serum cholesterol >5 mmol/l, triglycerides >1.7 mmol/l, HDL cholesterol <1.0 mmol/l, or total cholesterol to HDL cholesterol ratio >5.0). Patients were deemed to have CAD based on clinical evidence (i.e., coronary angiography in symptomatic patients, history of previous myocardial infarction, and coronary revascularization). The patients who were designated CAD negative were asymptomatic, with no chest pain history and normal resting electrocardiograms, and they did not have coronary angiography.

Patients were excluded from the study for any of the following: type 1 or type 2 diabetes, obesity, abnormal liver function tests (defined as alanine aminotransferase >2.5 times the upper limit of the reference range), and significant renal impairment (defined as creatinine >135 μmol/l).

A total of 32 British Caucasians with FCHL were enrolled in the study, and 26 completed the study according to the protocol. Four patients did not meet the recruitment criteria, and 2 patients refused to have the PET scan repeated at the end of the study period and discontinued taking the medication. All 26 patients who completed the study were included in the analysis.

All of the patients attended the same lipid clinic for at least 2 years and had been on stable medical therapy and diet for at least 3 months before inclusion in the study. Therapy and dietary habits remained the same during the trial period.

All patients gave written informed consent before participation, and the study protocol was approved by the Research Ethics Committees of the 2 participating hospitals (Hammersmith Hospital, Imperial College, and University College Hospitals, University College London) and by the U.K. Administration of Radioactive Substances Advisory Committee. The study was conducted according to the guidelines of the Declaration of Helsinki.

The study was registered in a public access database for controlled clinical trials (register number ISRCTN78563659).

Study design and protocol. The study protocol is shown on Figure 1. The study was designed as a 2-center double-blind randomized placebo-controlled trial; patients who met the inclusion and exclusion criteria at visit 1 were randomly allocated to receive treatment with pioglitazone or placebo from visit 2 and treated for a period of 16 weeks. For the first 4 weeks patients received 30 mg pioglitazone or placebo daily (both manufactured by Takeda, Osaka, Japan), and the dosage was increased to 45 mg daily for the rest of the study.
If adverse events were observed, patients remained on 30 mg. A PET scan was performed twice: before and after treatment.

All patients refrained from caffeine-containing beverages for the 12 h preceding the PET studies. The 4 smokers continued to smoke throughout the study although they did not smoke in the 2 to 3 h preceding the PET studies when they were attended by the investigators. Compliance with study medication was calculated based on the dispensing and return of study medication tablets and showed that the mean dose was 28.3 ± 3.58 mg during the first 4 weeks and 41.5 ± 6.5 mg daily for the rest of the study, which represents 94% and 92% compliance, respectively.

**Laboratory analyses.** All blood samples were obtained after a 12-h fast at week 0 and again at week 16. Total cholesterol, triglyceride, and HDL cholesterol serum levels, plasma glucose, and hemoglobin A1c were determined by automated methods using commercial kits and interassay controls. The LDL cholesterol levels were calculated from the standard formula: LDL cholesterol = total cholesterol − (HDL cholesterol + [triglycerides/2.2]). Serum lipoprotein (a) was measured using an automated immunoturbidimetric assay (Beckman Instruments, Galway, Ireland). Plasma insulin was analyzed using an established radioimmunoassay (20). All samples were frozen and subsequently assayed in duplicate on a single occasion. The detection limit of the assay was 6 pmol/l, and the intra-assay coefficient of variation was 5.4%. As a proxy for small dense LDL particles, we used the triglycerides to HDL cholesterol molar ratio, which is known as the atherogenic index of plasma (21,22). Nonesterified fatty acids (NEFA) were measured in serum using the NEFA C, ACS-ACOD Method (Wako Chemicals, Neuss, Germany). Plasma plasminogen activator inhibitor (PAI)-1 was determined by enzyme-linked immunosorbent assay (ELISA) (Hyphen BioMed, Neuville sur Oise, France). Plasma levels of oxidized LDL were measured in an mAB-4E6–based competition ELISA (Mercodia, Uppsala, Sweden) and adiponectin plasma levels in the Quantikine ELISA (R&D Systems, Abingdon, United Kingdom).

**PET, REGIONAL MYOCARDIAL PERFUSION.** The PET studies were performed in a 3-dimensional imaging mode for oxygen-15–labeled water (H215O) and a 2-dimensional imaging mode for 2-[18F]fluoro-2-deoxy-D-glucose (FDG) using a 962 (HR+) scanner (Siemens, Knoxville, Tennessee) after an overnight fast and abstinence from caffeine-containing beverages for at least 24 h. A 20-min transmission scan was performed and used for subsequent attenuation correction of all emission scans. The blood pool was then imaged using oxygen-15–labeled carbon monoxide (C15O) (3 MBq/ml at 500 ml/min for 4 min). A 9-min single-frame emission scan was initiated 1 min after the end of the C15O inhalation to allow for equilibration (23). After allowing 10 min for the decay of 15O radioactivity, resting and hyperemic (intravenous adenosine, 140 μg/kg/min over 7 min) MBF were measured using H15O (185 MBq), injected intravenously over 20 s at a rate of 10 ml/min with flushing for a further 2 min, as previously described (23). The following scanning protocol was used: 1 × 30 s (background), 1 × 20 s, 14 × 5 s, 3 × 10 s, 4 × 20 s, and 4 × 30 s, for a total scanning time of 350 s.

**REGIONAL MGU.** To measure whole body glucose disposal (M) and provide a standardized metabolic milieu for the measurement of MGU, a hyperinsulinemic-euglycemic clamp was performed as previously described (23–25). After at least 90 min of hyperinsulinemia, 185 MBq FDG was injected over a 2-min period and a 37-frame dynamic PET scan acquired over 50 min (1 × 30 s [background], 12 × 10 s, 3 × 20 s, 4 × 30 s, 5 × 60 s, 8 × 150 s, 4 × 300 s) (25).

**PET DATA ANALYSIS.** The sinograms were corrected for attenuation and reconstructed using standard algorithms. Subsequent images were analyzed with Matlab (Mathworks, Natick, Massachusetts) software. Myocardial images, for the definition of regions of interest (ROIs), were generated directly from the dynamic H15O, as previously reported (26). Sixteen ROIs were drawn within the left ventricular myocardium (27) and projected onto the dynamic H215O images to obtain tissue activity curves. A separate set of
ROIs was defined for the right ventricular cavity and the left atrium. Myocardial and blood time-activity curves were then generated from the dynamic image and fitted to a single-tissue compartment tracer kinetic model to give values of MBF (ml/min/g) (28). The CFR was calculated as the ratio of MBF during hyperemia to MBF at rest (29). Coronary resistance was calculated as mean blood pressure divided by MBF (whole left ventricle).

Tissue FDG time-activity curves were analyzed by a linearized approach using the same 16 myocardial ROIs defined for $H_2^{15}$O (30,31). The MGU data were corrected for partial volume effect using extravascular volume measurement, obtained from the $C^{15}$O and transmission scans, as previously described (25). The final MGU values are expressed as $\mu$mol/g/min.

**Statistical methods.** Statistical analysis was performed in the 26 patients who completed the study. All data are given as least-squares mean ± SEM. Change from baseline data was tested by analysis of covariance with factor treatment (placebo or pioglitazone) taken into account. The baseline value of the variable was included as a covariate to control for differences at baseline between study groups. A p value of <0.05 was considered to be statistically significant.

**Results**

**Baseline characteristics.** The clinical characteristics of the patients in the placebo and pioglitazone groups and their medication are presented in Table 1. The mean age of the pioglitazone group was lower compared with the placebo group (p < 0.05) and there were more current smokers in the active treatment group, whereas the 2 groups were similar regarding concomitant medication. There was also difference with regard to body mass index (BMI) between the placebo and pioglitazone group (26.47 ± 2.19 kg/m$^2$ vs. 28.92 ± 1.79 kg/m$^2$, respectively; p < 0.05). All of the study patients were on treatment with statins. The patients from the pioglitazone and placebo groups were on treatment mainly with atorvastatin (64% and 67% of the patients from the 2 groups, respectively; p = NS). The median dose of atorvastatin was 20 mg daily for both groups. Single patients from both groups were treated with pravastatin, simvastatin, and rosuvastatin on similar doses. Combination of statin and fibrate was used in 43% of the patients from the pioglitazone group and 42% of the patients from the placebo group. The median dose of fibrate for both treatment groups was 267 mg daily. The lipid-lowering medication for all patients remained unchanged during the study.

**Effect of pioglitazone treatment on clinical and laboratory parameters.** The effect of 16 weeks’ treatment on laboratory parameters is summarized in Table 2. Pioglitazone therapy significantly increased BMI (from 28.92 ± 1.79 kg/m$^2$ to 29.38 ± 1.71 kg/m$^2$; p < 0.05), whereas no change occurred in the placebo group (from 26.47 ± 2.19 kg/m$^2$ to 26.60 ± 2.37 kg/m$^2$). A significant increase in HDL cholesterol, LDL cholesterol, and adiponectin and a reduction in plasma insulin were observed in pioglitazone group compared with the placebo group with no change in total cholesterol, triglycerides, atherogenic index of plasma, oxidized LDL, NEFA, lipoprotein (a), plasma glucose, or hemoglobin A1c. In addition, a significant reduction in total cholesterol to HDL cholesterol ratio (p = 0.04) and PAI-1 (p = 0.01) was observed in the pioglitazone group after treatment (within-treatment effect).

**Effect of the treatment on PET findings.** A significant increase in M and MGU was observed in the pioglitazone group compared with the placebo group (Table 3, Fig. 2). In addition, patients treated with pioglitazone had a significant improvement in resting MBF and a reduction in resting coronary resistance (Table 3) compared with the placebo group. And hyperemic MBF increased significantly (p = 0.01) in the pioglitazone group after treatment (within-treatment effect) (Fig. 3).

**Discussion**

The major novel findings in this study are that the addition of pioglitazone to conventional lipid-lowering therapy in nondiabetic FCHL patients leads to: 1) additional beneficial effects on serum lipid and metabolic parameters beyond those achieved with standard lipid-lowering therapy; and 2) significant improvement in M, MGU, and MBF.

**Effects of pioglitazone on blood parameters.** The insulin-sensitizing thiazolidinediones have been tested as experimental therapies with variable success (10) in other groups of patients with nondiabetic insulin resistance, such as nonalcoholic fatty liver disease (32), polycystic ovary syndrome (33), hypertension (14), and lipodystrophies (34,35). Patients with FCHL have numerous metabolic abnormalities similar to those in the metabolic syndrome and require complex management. The addition of pioglitazone to the
therapeutic regimen of the present FCHL patients, all of whom were on statins and nearly one-half of them on a combination with a fibrate, led to further changes in HDL cholesterol, total cholesterol to HDL cholesterol ratio, adiponectin, plasma insulin, and PAI-1. The lack of effect from the present study may be due to the smaller dose of pioglitazone used by Abbink et al. (38) and the lack of suppression (39). In a subsequent double-blind placebo-controlled randomized study from the same group, 54 patients with type 2 diabetes and CAD were treated for 16 weeks with rosiglitazone or placebo. Rosiglitazone treatment led to improved MGU in both normal myocardium and regions with evidence of exercise-inducible ischemia (16). In a smaller study, 16 patients with insulin-treated type 2 diabetes were randomly assigned to 12 weeks’

Table 2
Effect of the Treatment (16 Weeks) on Clinical and Laboratory Parameters

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 12)</th>
<th>Pioglitazone (n = 14)</th>
<th>Difference</th>
<th>95% CI for Difference</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.77 ± 0.28</td>
<td>4.87 ± 0.25</td>
<td>1.09</td>
<td>0.42 to 1.33</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.63 ± 0.46</td>
<td>1.79 ± 0.14</td>
<td>0.24</td>
<td>0.09 to 0.38</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.21 ± 0.06</td>
<td>1.18 ± 0.05</td>
<td>0.17</td>
<td>0.02 to 0.38</td>
<td>0.047</td>
</tr>
<tr>
<td>AIP</td>
<td>1.41 ± 0.47</td>
<td>1.53 ± 0.15</td>
<td>0.13</td>
<td>0.04 to 0.30</td>
<td>0.097</td>
</tr>
<tr>
<td>Total cholesterol/HDL cholesterol</td>
<td>3.98 ± 0.28</td>
<td>4.25 ± 0.23</td>
<td>0.28</td>
<td>0.05 to 0.24</td>
<td>0.025</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.81 ± 0.23</td>
<td>2.82 ± 0.17</td>
<td>0.16</td>
<td>0.02 to 0.28</td>
<td>0.040</td>
</tr>
<tr>
<td>Oxidized LDL (mg/dl)</td>
<td>80.83 ± 5.97</td>
<td>77.23 ± 4.72</td>
<td>0.21</td>
<td>0.01 to 0.28</td>
<td>0.046</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>4.66 ± 0.98</td>
<td>4.13 ± 1.10</td>
<td>0.53</td>
<td>0.02 to 0.27</td>
<td>0.001</td>
</tr>
<tr>
<td>NEFA (mg/dl)</td>
<td>0.87 ± 0.10</td>
<td>0.73 ± 0.13</td>
<td>0.14</td>
<td>0.01 to 0.27</td>
<td>0.049</td>
</tr>
<tr>
<td>Lpa(α) (mg/dl)</td>
<td>50.17 ± 12.66</td>
<td>43.45 ± 4.04</td>
<td>0.77</td>
<td>0.08 to 0.15</td>
<td>0.012</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.58 ± 0.16</td>
<td>4.83 ± 0.13</td>
<td>0.74</td>
<td>0.06 to 0.20</td>
<td>0.030</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.6 ± 0.12</td>
<td>5.79 ± 0.06</td>
<td>0.59</td>
<td>0.05 to 0.20</td>
<td>0.029</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>93.77 ± 11.48</td>
<td>95.94 ± 11.02</td>
<td>0.17</td>
<td>0.02 to 0.29</td>
<td>0.017</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>18.45 ± 4.97</td>
<td>15.35 ± 3.97</td>
<td>0.36</td>
<td>0.05 to 0.26</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Data are presented as least-squares mean ± SEM.

AIP = atherogenic index of plasma; or triglycerides to HDL cholesterol molar ratio; CI = confidence interval; Hb = hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Lpa(α) = lipoprotein (a); NEFA = nonesterified fatty acids; PAI = plasminogen activator inhibitor.

Effect of pioglitazone on myocardial metabolism and blood flow. To our knowledge this is the first study where pioglitazone was used to investigate its effects on MGU and absolute resting and hyperemic MBF simultaneously. Three earlier studies using PET examined the effect of thiazolidinediones on MGU in patients with type 2 diabetes. In one study, patients (n = 44) were randomized to receive rosiglitazone, metformin, or placebo over a 26-week period. At the end of the study only rosiglitazone treatment produced a significant improvement in M (+36%) and MGU (+38%), which the authors ascribed to serum NEFAs suppression (39). In a subsequent double-blind placebo-controlled randomized study from the same group, 54 patients with type 2 diabetes and CAD were treated for 16 weeks with rosiglitazone or placebo. Rosiglitazone treatment led to improved MGU in both normal myocardium and regions with evidence of exercise-inducible ischemia (16). In a smaller study, 16 patients with insulin-treated type 2 diabetes were randomly assigned to 12 weeks’
treatment with pioglitazone or placebo. Despite favorable changes in glucose and lipid parameters in the pioglitazone group, the study did not demonstrate any significant change in resting and hyperemic MBF or CFR (17). In a nonrandomized study in 25 Mexican Americans with evidence of insulin resistance, 3 months’ treatment with thiazolidinediones did not lead to significant changes in resting and dipyridamole-stimulated MBF, although the MBF response to cold pressor test, which is a marker of endothelium dependent vasodilation, was significantly improved (18).

The present study is the first in which the effect of thiazolidinediones on MGU and MBF were tested in the same patients simultaneously. In addition to the discussed effects on blood parameters, which represent important cardiovascular risk factors, the results of the present study provide novel evidence of a combined effect of pioglitazone on MGU, MBF, and coronary resistance.

Based on the present results, it is not possible to prove beyond doubt that the improvement in MBF and coronary resistance is related to the effects of the drug on lipid and glucose metabolism, although some parameters which improved after treatment with pioglitazone (e.g., HDL cholesterol) are known to affect overall coronary function (9). Previous studies have demonstrated that patients at higher risk of developing CAD, such as smokers and those with hyperlipidemia, have abnormalities of coronary microvascular function which can improve after correction of the risk factors (40). Therefore, it is possible to speculate that the change in MBF and coronary resistance following pioglitazone is the result of improved coronary microvascular function related to the favorable effects of the drug on lipid and metabolic parameters.

In the present study, the beneficial effect of pioglitazone on MBF and coronary resistance was observed in the absence of changes in plasma glucose or hemoglobin A1c. This is contrary to the belief that hyperglycemia is a key modulator of vasodilator function, at least in patients with diabetes (17,41). The key could be the improved insulin sensitivity during treatment with pioglitazone, which might influence MBF directly. This is in agreement with a study showing that nondiabetic insulin-resistant patients manifest coronary vasomotor abnormalities that can be normalized by insulin-sensitizing thiazolidinedione therapy (18). The only predictor of improvement in endothelial function in this study was the change in fasting plasma insulin level. Furthermore, the recently published PROACTIVE (Prospective Pioglitazone Clinical Trial in Macrovascular Events) study demonstrated that pioglitazone reduced composite all-cause mortality in patients with type 2 diabetes when added to their standard medications. It might be speculated that, similarly to our study, the benefit observed in the PROACTIVE study were, at least in part, due to improvement in coronary microvascular function (42). In line with earlier reports, we observed a significant increase in adiponectin and a decrease in PAI-1 after treatment with thiazolidinediones (10,43). However, none of the parameters listed in Table 2 showed any significant association with the changes in MBF or coronary resistance.

Study limitations. The main limitation of the present study is the relatively small sample size, which, however, was sufficient to observe highly significant differences between the 2 groups. We believe that the limited number was responsible for some of the differences that were observed in
the baseline parameters, such as age and BMI. Another limitation was that only 2 women were enrolled out of 26 patients, although in our experience FCHL is more frequently observed in men. Finally, we did not repeat our measurements after cessation of treatment and therefore we cannot ascertain if the favorable effects, in particular those on the coronary circulation, would be long lasting. However, because PET involves administration of ionizing radiation, it would have been ethically difficult to justify a third scan.

Conclusions

The results of this pilot study have demonstrated that the addition of pioglitazone to conventional lipid-lowering therapy in FCHL patients leads to significant beneficial effects on metabolic and vascular parameters at both whole body and myocardial level beyond those seen with conventional lipid-lowering therapy.

Acknowledgment

The authors thank Dr. Seth Gbenado for the statistical analysis and his advice in the preparation of the manuscript.

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REFERENCES