Pioglitazone in addition to metformin improves erythrocyte deformability in patients with Type 2 diabetes mellitus

Thomas FORST∗†, Matthias M. WEBER†, Mirjam LÖBIG∗, Ute LEHMANN‡, Jürgen MÜLLER§, Cloth HOHBERG∗, Christiane FRIEDRICH∗, Winfried FUCHS‡ and Andreas PFÜTZNER∗

∗Institute for Clinical Research and Development, University of Mainz, D-55116 Mainz, Germany, †Department of Endocrinology, Johannes Gutenberg University, D-55131 Mainz Germany, ‡Takeda Pharma GmbH, D-52066 Aachen, Germany, and §acromion GmbH, D-50266 Frechen, Germany

A B S T R A C T

The aim of the present study was to compare the effect of PIO (pioglitazone) or GLIM (glimepiride) on erythrocyte deformability in T2DM (Type 2 diabetes mellitus). The study covered 23 metformin-treated T2DM patients with an HbA1c (glycated haemoglobin) >6.5%. Patients were randomized to receive either PIO (15 mg, twice a day) or GLIM (1 mg, twice a day) in combination with metformin (850 mg, twice a day) for 6 months. Blood samples were taken for the measurement of fasting glucose, HbA1c, fasting insulin, intact proinsulin, adiponectin and Hct (haematocrit). In addition, the erythrocyte EI (elongation index) was measured using laser diffractoscopy. Both treatments significantly improved HbA1c levels (PIO, −0.9 ± 1.1%; GLIM, −0.6 ± 0.4%; both P < 0.05) and resulted in comparable HbA1c levels after 6 months (PIO, 6.5 ± 1.2%; GLIM, 6.2 ± 0.4%) Treatment with PIO reduced fasting insulin levels (−8.7 ± 15.8 milli-units/l; P = 0.098), intact proinsulin levels (−11.8 ± 9.5 pmol/l; P < 0.05) and Hct (−1.3 ± 2.3%; P = 0.09), whereas adiponectin levels increased (8.2 ± 4.9 μg/ml; P < 0.05). No significant change in these parameters was observed during GLIM treatment. PIO improved the EI, resulting in a significant increase in EI at all physiological shear stress ranges (0.6–6.0 Pa; P < 0.05). The improvement in EI correlated with the increase in adiponectin levels (r = 0.74; P = 0.001), and inversely with intact proinsulin levels (r = −0.47; P < 0.05). This is the first study showing an improvement in EI during treatment with PIO, which was associated with an increase in adiponectin and a decrease in intact proinsulin levels, but independent of glycaemic control.

INTRODUCTION

T2DM (Type 2 diabetes mellitus) patients suffer from an increased incidence of micro- and macro-vascular complications. It is widely accepted that rheological properties of erythrocytes are of considerable significance in many circulatory disorders, such as atherosclerosis, essential hypertension and diabetes mellitus [1–3]. Several clinical studies have documented decreased erythrocyte deformability in diabetic patients [4–7], and, impaired erythrocyte deformability was observed even in children with a short duration of diabetes [8]. Impaired tissue perfusion, as observed in diabetes mellitus, could be attributed to a reduction in erythrocyte deformability.

Key words: adiponectin, erythrocyte deformability, insulin resistance, metformin, sulfonylurea, thiazolidinedione, Type 2 diabetes mellitus.

Abbreviations: EI, elongation index; EImax, maximal EI; GLIM, glimepiride; HbA1c, glycated haemoglobin; Hct, haematocrit; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PIO, pioglitazone; SS1/2, shear stress required for half of EImax; T2DM, Type 2 diabetes mellitus.

Correspondence: Professor Thomas Forst (email ThomasF@ikfe.de).
and an increase in blood viscosity [9,10]. It is crucial for the erythrocytes to pass through the capillaries in order to supply oxygen to the surrounding tissues, and decreased erythrocyte deformability contributes to an impaired oxygen and nutrition supply in diabetic microangiopathy [11,12].

Insulin resistance as observed in patients with T2DM is associated with endothelial dysfunction [13,14] and an increase in blood viscosity [15,16]. PIO (pioglitazone) has been shown to improve insulin sensitivity [17], to improve endothelial function [18–21] and to improve the overall cardiovascular risk profile in diabetic and in non-diabetic subjects [22–25]. In contrast, there is increasing evidence that anti-diabetic treatment with sulfonylurea, especially in combination with metformin, might have a negative impact on the cardiovascular outcome in patients with T2DM [26–28]. The aim of the present investigation was to compare the effect on erythrocyte deformability of adding PIO or GLIM (glimepiride) to patients with T2DM already treated with metformin.

**MATERIALS AND METHODS**

**Patients**

This is a sub-analysis of the patients from the PIOfix study, treated at the Institute for Clinical Research and Development study site in Mainz, in whom erythrocyte deformability was measured immediately after venous blood draw. Patients had to be on a stable treatment with metformin monotherapy at a maximal individually tolerated dosage (850–2000 mg) within the last 12 weeks and with an HbA1c (glycated haemoglobin) ≥6.5%. Subjects were excluded if they had been treated with insulin or other oral anti-diabetic drugs, with the exception of metformin, within the last 12 weeks. All other concomitant treatments were kept stable during the study. Patients were randomized to receive PIO (15 mg, twice a day) or GLIM (1 mg, twice a day) in combination with 850 mg of metformin, twice a day.

Fasting blood samples were taken at baseline and after 24 weeks of treatment for the measurement of plasma glucose, HbA1c, insulin, intact proinsulin, adiponectin, Hct (haematocrit) and the assessment of erythrocyte deformability.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethical committee. All subjects gave written informed consent. The study was registered at ClinicalTrials.gov with the clinical trial registry number NCT 00770653.

**Measurement of erythrocyte deformability**

The measurement of erythrocyte deformability was performed using laser diffractoscopy from freshly obtained venous blood samples. Laser diffractoscopy was performed using a Rheodyn SSD shear stress diffractometer and corresponding Dextran FP-60 24 mPas (Myrenne). The method of laser diffractoscopy has been described in detail previously [29]. In summary, a He/Ne laser detects deformation of erythrocytes between two parallel glass discs, one of them rotating and executing defined shear stresses as per the following formula:

$$\tau (\text{Pa}) = 2\pi r \times \eta/t \times b$$

where \(\tau\) is the shear stress, \(r\) is the laser beam distance from rotation centre, \(\eta\) is the viscosity, \(b\) is the height of the gap between discs and \(t\) is time. Adjusting for equipment-specific values (\(r = 25\ \text{mm}, b = 0.5\ \text{mm and } \eta = 24\ \text{mPas}\)), the formula is condensed to

$$\tau (\text{Pa}) = 7536\ \text{Pa} \times \text{rev./min}$$

The applied shear stress range is electronically regulated and consists of eight levels (0.3, 0.6, 1.2, 3, 6, 12, 30 and 60 Pa).

The erythrocyte deformability measurement detects scattered light intensities along orthogonal axes \((A, B)\) of erythrocytes within the laser diffusion light cone. The erythrocyte EI (elongation index) is calculated by the following equation:

$$\text{EI} (%) = \left(\frac{(A - B)}{(A + B)}\right) \times 100$$

The erythrocyte EImax (maximal EI) and SS1/2 (shear stress required for half of EImax) were calculated as described by Baskurt et al. [30].

**Additional laboratory measurements**

Blood samples for all other laboratory measurements were centrifuged and kept at ~20°C until analysis. Plasma glucose concentrations were determined using the glucose dehydrogenase method (Super GL; RLT). Insulin and intact proinsulin were measured using a chemiluminescence assay (Invitron), and adiponectin was measured using an ELISA (TECOmedical). HbA1c was determined by HPLC (Menarini Diagnostics).

**Subjects sample size considerations and statistical analysis**

No clinical information was available about the effect of PIO or GLIM on erythrocyte deformability in patients with T2DM. Therefore no confirmatory study size calculation was possible and the results obtained were interpreted in an exploratory sense.

For the erythrocyte deformability, two-sided \(P\) values for within- and between-group treatment differences were calculated for the absolute change from baseline, considering an ANCOVA (analysis of co-variance) model with the fixed effect factors for the treatment group and centre and with the baseline value as the covariate. For all other within- and between-group comparisons, two-sided \(P\) values resulting from the
Table 1  Clinical characteristics of the two groups investigated

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PIO (n = 11)</th>
<th>GLIM (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 9</td>
<td>58 ± 12</td>
</tr>
<tr>
<td>Gender (♂/♀)</td>
<td>9/2</td>
<td>7/5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>33.5 ± 5.1</td>
<td>32.0 ± 3.6</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>6.1 ± 3.9</td>
<td>6.4 ± 4.8</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5 ± 0.9</td>
<td>6.8 ± 0.3*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128.6 ± 11.8</td>
<td>123.5 ± 16.9</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.0 ± 8.2</td>
<td>81.5 ± 9.7</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>43.4 ± 4.9</td>
<td>39.3 ± 5.6</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>105.9 ± 34.3</td>
<td>68.8 ± 30.5*</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>200.1 ± 31.3</td>
<td>149.3 ± 50.3*</td>
</tr>
</tbody>
</table>

Values are means ± S.D. *P < 0.05 compared with PIO-treated group. BP, blood pressure.

Table 2  Change in erythrocyte EI from baseline

<table>
<thead>
<tr>
<th>Shear stress (Pa)</th>
<th>PIO</th>
<th>GLIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>1.3 ± 2.1</td>
<td>−0.4 ± 1.7</td>
</tr>
<tr>
<td>0.6</td>
<td>2.4 ± 1.3*†</td>
<td>−0.5 ± 1.1</td>
</tr>
<tr>
<td>1.2</td>
<td>3.2 ± 2.2*†</td>
<td>−1.1 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>3.3 ± 2.8*†</td>
<td>−1.5 ± 3.1</td>
</tr>
<tr>
<td>6</td>
<td>3.1 ± 2.9*</td>
<td>−1.4 ± 2.9</td>
</tr>
<tr>
<td>12</td>
<td>2.7 ± 2.8</td>
<td>−1.3 ± 2.9</td>
</tr>
<tr>
<td>30</td>
<td>2.5 ± 2.6</td>
<td>−1.3 ± 3.5</td>
</tr>
<tr>
<td>60</td>
<td>2.7 ± 2.6</td>
<td>−1.3 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± S.D. *P < 0.05 compared with baseline; †P < 0.05 compared with the GLIM-treated group.

RESULTS

A total of 23 patients with T2DM were included in the analysis. Of these, 11 were randomized to the PIO group and 12 patients were randomized to the GLIM group. The clinical characteristics of the patients are shown in Table 1. At baseline, HbA1c, LDL (low-density lipoprotein) and triacylglycerol (triglyceride) were lower in the GLIM group compared with the PIO group. No other relevant differences were observed between the two groups.

After 24 weeks of study treatment, both groups had improved metabolic control and, at the end of the study, HbA1c and HDL (high-density lipoprotein) levels were comparable between both treatment groups (HbA1c, 6.5 ± 1.2 and 6.2 ± 0.4% in the PIO and GLIM groups respectively; HDL, 46.2 ± 5.6 and 39.9 ± 6.8 mg/dl in the PIO and GLIM groups respectively; P values were not significant in either case). Triacylglycerol levels remained significantly higher in the PIO group compared with the GLIM group (203.3 ± 36.5 and 158.1 ± 58.0 mg/dl in the PIO and GLIM groups respectively; P < 0.05).

In PIO-treated patients, fasting insulin levels tended to decrease from 18.0 ± 14.9 to 9.3 ± 2.7 pmol/l (P = 0.098), and intact proinsulin levels declined from 21.4 ± 10.4 to 9.7 ± 4.0 pmol/l (P < 0.05). Adiponectin levels increased from 4.1 ± 1.7 to 12.3 ± 5.7 μg/ml (P < 0.05). No significant changes in these laboratory parameters were observed during GLIM treatment (fasting insulin increased from 17.2 ± 8.4 to 18.5 ± 8.0 pmol/l; intact proinsulin decreased from 16.2 ± 16.3 to 15.9 ± 8.9 pmol/l; adiponectin increased from 4.3 ± 2.5 to 4.7 ± 2.6 μg/ml; P values were not significant in each case).

Treatment with PIO increased erythrocyte deformability at all of the shear stress rates tested in our present study protocol (Figure 1A). In the physiological shear stress range of 0.6 to 6.0 Pa, a significant improvement in erythrocyte deformability compared with baseline was observed during treatment with PIO. In contrast, GLIM treatment produced a slight, albeit non-significant, deterioration in erythrocyte deformability (Figure 1B). In the physiological shear stress range between 0.6 and 6.0 Pa, the change from baseline in erythrocyte EI was significantly different between the two treatment groups. During PIO treatment, EImax decreased from 82.1 ± 8.5 to 76.9 ± 17.8% (P < 0.01) and the SS1/2 decreased from 6.3 ± 1.4 to 5.2 ± 3.7 Pa (P < 0.001). No significant effect of GLIM treatment on EImax (from 87.9 ± 19.9 to 86.3 ± 15.1%) or SS1/2 (from 6.4 ± 3.2 to 6.7 ± 2.5) was observed in our present study.

During PIO treatment, Hct slightly decreased from 42.5 ± 2.8 to 41.1 ± 3.8% (P = 0.09), whereas a slight increase from 41.0 ± 2.4 to 41.2 ± 2.6% (P value was not significant) was observed during treatment with GLIM.

A significant correlation was observed between the increase in adiponectin plasma levels and the increase in the EI (r = 0.74; P < 0.001; Figure 2), whereas an inverse relationship was observed between EI and intact proinsulin plasma levels (r = −0.47; P < 0.05; Figure 3). No association between erythrocyte deformability and fasting glucose, insulin and HbA1c levels, Hct or lipids was found in our present study.

DISCUSSION

T2DM is linked to numerous functional and morphological disturbances in micro- and macro-vascular blood flow, merging in malperfusion and tissue damage. As erythrocytes usually have a size of 8 μm and the capillary lumen is in the range of 4–9 μm, the property of the erythrocyte to assimilate while passing the nutritive capillaries becomes a crucial factor for sufficient...
Figure 1  Erythrocyte EI at baseline and 6 months after treatment with PIO (A) and GLIM (B)

Values are means ± S.D. In (A), baseline values (□) and those after 6 months of treatment with PIO (■) are shown; *P < 0.05 compared with baseline. In (B), baseline values (○) and those after 6 months of treatment with GLIM (●) are shown.

Figure 2  Linear correlation between the increase in adiponectin levels and the increase in erythrocyte EI at a physiological shear stress rate of 1.2 Pa

Figure 3  Linear correlation between the decrease in intact proinsulin level and the increase in erythrocyte EI at a physiological shear stress rate of 1.2 Pa

tissue perfusion. In patients with diabetes mellitus, increased capillary recruitment could only incompletely compensate for rigid diabetic erythrocytes [31,32]. It has been suggested that reduced erythrocyte deformability could, at least in part, account for the impaired tissue perfusion observed in several tissues in patients with diabetes mellitus [9,10].

PIO is used for the treatment of insulin resistance in T2DM patients. Beyond improving blood glucose control, treatment with PIO has been shown to effect micro- and macro-vascular blood flow, and to reduce the risk for cardiovascular complications in T2DM patients [10,19,23,33–35]. PIO has been shown to reduce inflammation [35], to improve endothelial function [21], to reduce the intima media layer of the carotid artery [24,36] and to decrease atherosclerotic plaques in the cardiovascular system [25]. In contrast with GLIM, in the present study, PIO improved erythrocyte deformability at all physiological shear stress ranges. The improvement in erythrocyte deformability was found to be associated with an increase in plasma adiponectin concentrations and a decrease in intact proinsulin concentrations.

Adiponectin, an adipokine released from visceral adipose tissue, has been found to increase insulin sensitivity, to improve endothelial function and to exhibit several anti-inflammatory and anti-oxidative effects.
[37,38]. Decreased adiponectin plasma levels contribute to the development of insulin resistance and T2DM, and a causal relationship between low adiponectin levels and the development of cardiovascular disease has been suggested [39,40]. Treatment with PIO has been shown to reduce intima media thickness of the carotid artery, which was found to be associated with an increase in adiponectin levels [41]. Erythrocyte deformability is highly dependent on the fluidity of the erythrocyte membrane [42,43]. In hypertensive patients, it has been shown that the membrane fluidity of erythrocytes is inversely related to plasma adiponectin levels, indicating that hypoadiponectinaemia might be associated with decreased membrane fluidity and decreased erythrocyte deformability [44]. It has been demonstrated that adiponectin stimulates the production of NO in endothelial cells [45]. Many studies have demonstrated an inter-relationship between NO and erythrocyte deformability [46,47]. In our present study, treatment with PIO in T2DM patients increased plasma adiponectin levels and improved erythrocyte deformability over a wide range of physiological shear stress rates. Therefore it appears conceivable that treatment with PIO in T2DM patients improves erythrocyte deformability by increasing plasma adiponectin levels and subsequent stimulation of endothelial NO release.

In T2DM patients, an increased Hct effects endothelial function [48] and is assumed to contribute to an increase in the risk of coronary vascular disease [49]. In accordance with previous studies [50,51], our present study confirmed a slight decrease in Hct during treatment with PIO. In addition, no association was observed between the improvement in erythrocyte flexibility and the reduction in Hct; however, interference between changes in Hct and erythrocyte deformability cannot be excluded, due the limited size of the present study.

In conclusion, the present study is the first to show an improvement in erythrocyte deformability during treatment with PIO in T2DM patients. In consideration with findings from the literature, it could be assumed that the increase in adiponectin levels caused by PIO treatment might affect erythrocyte membrane fluidity and thereby improve erythrocyte deformability. Our results further confirm the important effects of PIO in the modification of the cardiovascular risk profile which can be observed beyond glucose control.

**Study limitation**

A potential limitation of our present findings is that the results were obtained from a sub-group treated at one site within a large multi-centre study. Because erythrocyte deformability was a secondary end point and no confirmatory study size estimation for this parameter was deemed possible, the findings should be interpreted in an exploratory sense. Even if no association was found between erythrocyte deformability and HbA1c levels or lipid parameters in the present study, insufficient statistical power does not allow such a relationship to be ruled out.

**AUTHOR CONTRIBUTION**

Thomas Forst designed the study, developed the protocols, conducted the clinical study, analysed the data and prepared the manuscript; Matthias Weber developed the study and prepared the manuscript; Mirjam Löhig performed the laboratory analysis; Ute Lehmann designed the study, developed the protocols, analysed the data and prepared the manuscript; Jürgen Müller analysed the data; Cloth Höhberg conducted the study and prepared the manuscript; Christiane Friedrich conducted the clinical study; Winfried Fuchs designed the study, developed the protocols, analysed the data and prepared the manuscript; and Andreas Pfützner designed the study, developed the protocols and prepared the manuscript.

**FUNDING**

This study was supported by an unrestricted fund from Takeda Pharma.

**REFERENCES**


© The Authors Journal compilation © 2010 Biochemical Society
Pioglitazone and erythrocyte deformability


