Beneficial effect of pioglitazone in proteinuric IgA nephropathy with concomitant ACEI/ARB treatment

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Background: Proteinuria is a major predictor for renal progression in IgA nephropathy (IgAN). Thiazolidinediones were demonstrated to reduce proteinuria in patients with diabetic nephropathy.

Objective: To investigate the effect of pioglitazone (PGZ) in proteinuric IgAN using randomized, double-blinded approach.

Methods: Forty-one biopsy-proven IgAN patients with proteinuria (≥0.5 g/day) who were currently treated with renin angiotensin system inhibitors and had at least two out of four risk factors for progressive disease (male gender, blood pressure >150/90 mmHg, creatinine clearance of 20-80 mL/min/1.73m², and chronicity index >1) were randomly assigned to receive either PGZ 30 mg/day (PGZ group; n=21) or placebo (control group, n=20) for 16 weeks.

Results: Baseline characteristics of patients in both groups were comparable. Following 16-week treatment, proteinuria in the PGZ group was significantly lower than the control group, [1.2 vs. 2.1 g/day (p<0.05)]. Patients in the PGZ group also showed a significant reduction in urinary excretion of TGF-β (from 361.4 to 234.4 ng/gCr) and VEGF (from 1353.1 to 765.1 ng/gCr) after 16-week treatment (p<0.05, both).

Conclusion: PGZ significantly reduced proteinuria, urinary TGF-β, and urinary VEGF in IgAN patients. These findings suggest that PGZ could have a role in the treatment of proteinuric IgAN. Further studies with larger cases and longer follow-up time are warranted.

Keywords: IgA nephropathy, PPAR-γ, proteinuria, TGF-β, VEGF.

IgA nephropathy (IgAN) is well recognized as the most prevalent glomerular disease worldwide [1, 2]. It is characterized by the accumulation of IgA deposit, predominantly in the glomerular mesangium accompanied by mesangial proliferation [3]. A significant proportion of patients follows a progressive course and eventually reaches end-stage renal failure [4]. A large body of evidence shows that proteinuria is associated with disease progression and reduction of proteinuria becomes one of therapeutic goals for IgAN [5, 6]. Recently, urinary biomarkers such as urinary transforming growth factor-β (TGF-β), and urinary vascular endothelial growth factor (VEGF) have been suggested to have prognostic role in various kidney diseases including IgAN [7-9].

Although IgAN has long been characterized since 1969 by Berger, no effective therapy for IgAN has been established [10, 11]. Blood pressure (BP) control and treatment with angiotensin converting enzyme inhibitors (ACEI) and/or angiotensin type II receptor blocker (ARB) are currently the mainstay therapy for IgAN. Other treatments, such as fish oil or various immunosuppressive regimens have been advocated, but the roles of these treatment modalities remain controversial.

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Recent studies demonstrated that thiazolidinediones (TZD), a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist, could reduce the amount of proteinuria, lessen the synthesis of TGF-β, and attenuate pathological changes of the diabetic kidney [12-17]. Although there are scarce data regarding the effect of TZD in non-diabetic nephropathy [18], several lines of evidence suggested that TZD might be effective in IgAN. Mesangial cells, the principal cells in pathogenesis of IgAN, highly express PPAR-γ, which is the pharmacological target of TZD [19]. Moreover, activation of PPAR-γ has been shown to prevent mesangial proliferation, extracellular matrix synthesis, and mesenchymal transdifferentiation in various mesangial cell injury models [12, 20]. In this study, we investigated the potential effects of a PPAR-γ agonist, pioglitazone (PGZ), on proteinuria in IgAN patients.

**Patients and methods**

**Participants**

Participants were recruited from Nephrology Clinic, King Chulalongkorn Memorial Hospital between January 2007 and December 2008. Patients with biopsy-proven IgAN who had macroalbuminuria (24-hour urine protein >0.5 g) despite receiving ACEI or ARB therapy and had at least two risk factors for progressive disease (male gender, BP over 150/90 mmHg, creatinine clearance (CrCl) of 20-80 mL/min/1.73 m² and chronicity index greater than one point) were eligible to participate in the present study. Patients who were receiving immunosuppressive agents or steroid (>10 mg/day of prednisolone) were excluded. This randomized, double-blinded study was approved by the Ethics Committee of Research, Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from each participating patient.

**Intervention**

During one month before allocation, the BP in each patient was controlled by ACEI or ARB the dose of which could be titrated up to the maximally recommended dose for hypertension. The target of BP was below 140/90 mmHg. Calcium channel blocker could be added to achieve the target BP.

The enrolled patients were randomly allocated to receive either 30 mg of oral pioglitazone daily (PGZ group), or placebo (control group) for 16 weeks. PGZ and placebo were provided in a package of similar capsules labeled with the study number. Participants, investigators that followed the patients, and technicians who measured laboratory parameters, were all blinded to the group assignment.

During the study period, alpha-adrenergic blockers was added, if necessary, to maintain the BP at the level below 140/90 mmHg. The compliance of the patients was monitored by counting the remaining drug.

**Outcomes**

Primary outcome was proteinuria measured by 24-hour protein excretion after 16-week treatment. Secondary outcomes were CrCl, urinary TGF-β1 excretion, and urinary VEGF excretion.

Fasting blood sugar, blood urea nitrogen (BUN), serum creatinine (SCr), CrCl, cholesterol, triglyceride, high-density lipoprotein (HDL), 24-hour urinary protein excretion, urinary TGF-β1, and urinary VEGF were determined at baseline and after 16-week treatment. Urinary protein excretion was additionally determined after four- and eight-week treatment.

CrCl was calculated from SCr, and 24-hour urinary creatinine excretion. Urinary TGF-β1 and VEGF were analyzed using a human TGF-β1 ELISA kit and a human VEGF ELISA kit (R&D Systems, Minneapolis, USA). The sensitivities of these assays were 4.6 and 5.0 pg/mL, respectively. The intra-assay and inter-assay coefficients of variation were less than 10%. The results of these urinary biomarker concentrations were normalized by urine creatinine concentration and expressed as nanogram per gram of creatinine (ng/gCr).

**Statistical analysis**

The distribution of all parameters was assessed using Shapiro-Wilk analysis. Data with normal distribution were expressed as means ± standard deviation (SD). Data with skewed distribution was expressed as geometric mean (95% confidence interval, 95%CI) and was logarithmically transformed before subjected to statistical analysis. Statistical analysis was performed using STATA/SE version 9.2. Paired t-tests were used for comparison between the values of all parameters at baseline and after 16-week treatment. Unpaired t-tests were used for comparison between PGZ and control groups.
Results

Patient characteristics

Forty-one patients were included in this study. Twenty patients were assigned to the control group and twenty-one patients were assigned to the PGZ group. As demonstrated in Table 1, both control and PGZ groups were comparable in all baseline parameters.

Urinary protein excretion

At baseline, there was no statistical significance in the values of 24-hour urinary protein excretion between groups [2.0 (95%CI, 0.9-3.1) vs. 2.1 (1.6-2.6) g/day] (Fig. 1A). In the control group, urinary protein excretion was unchanged throughout the study period [2.0 (0.9-3.1) vs. 2.1 (1.3-2.9) g/day, baseline vs. 16 weeks, NS]. In contrast, urinary protein excretion of the PGZ group was progressively decreased [2.1 (1.6-2.6) vs. 1.2 (0.7-1.7) g/day, baseline vs. 16 weeks, p<0.05] and was significantly lower than that of the control group [1.2 (0.7-1.7) vs. 2.1 (1.3-2.9) g/day, PGZ versus control, p<0.05] after 16-week treatment.

Creatinine clearance

There were no significant differences in CrCl measured by 24-hour urine collection between PGZ and control groups, at baseline as well as after 16-week treatment (Fig. 1B). However, there was a non-significant trend of increasing CrCl after 16 weeks of PGZ treatment while CrCl had a tendency to decrease in the control group.

Urinary TGF-β1 and VEGF excretions

Pooled baseline data of all patients (n=41) showed no significant correlation between urinary TGF-β1 excretion and age, sex, hypertension, Scr, or CrCl (data not shown). However, there was a significant correlation between urinary TGF-β1 excretion and 24-hour urinary protein excretion (r² = 0.75, p < 0.01). In the control group, urinary TGF-β1 excretion was slightly, but not significantly, elevated from 353.5 (188.0-519.0) to 430.4 (220.7-460.2) ng/gCr in the control group (Fig. 2A). On the other hand, after 16 week treatment with PGZ, urinary TGF-β1 excretion was significantly decreased, from 361.4 (95%CI, 259.4-463.4) to 234.4 (170.9-298.0) ng/gCr (p<0.05). Urinary TGF-β1 excretion of the PGZ group was not significantly different from the control group, at baseline and after 16-week treatment (Fig. 2A). The magnitude of urinary TGF-β1 excretion change was significantly different between the two groups (Fig. 2A; 25.8% decrease in the PGZ vs. 37.2% increase in the control, p = 0.02).

Urinary VEGF excretion was modestly correlated with 24-hour urine protein (r² = 0.41, p < 0.01) and with urinary TGF-β1 at pooled baseline data (r² = 0.42, p < 0.01). No correlations were found between urinary VEGF excretion and demographic, clinical characteristics, or pathologic findings. Urinary VEGF levels were comparable in both groups at baseline. However, the level was significantly decreased, from 1,353.1 to 765.1 ng/gCr (p <0.05) after 16-week treatment with PGZ. No significant change was detected in the control group (Fig. 2B).

Table 1. Baseline characteristics of the patients. Data are expressed as geometric mean (95%CI) or mean±SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=20)</th>
<th>PGZ (n=21)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>41.4±11.4</td>
<td>42.1±13.6</td>
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<tr>
<td>Gender (male/female)</td>
<td>10/10</td>
<td>7/17</td>
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<tr>
<td>BUN (mg/dL)</td>
<td>20.7(16.3-25.2)</td>
<td>21.4(16.6-26.3)</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>1.5(1.1-1.8)</td>
<td>1.62(1.3-1.9)</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>2.0(0.9-3.1)</td>
<td>2.1(1.6-2.6)</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>61.1(48.6-73.7)</td>
<td>57.4(47.5-67.4)</td>
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<tr>
<td>Pathological findings</td>
<td></td>
<td></td>
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<tr>
<td>Crescent formation (%)</td>
<td>0.7(0.0-6.7)</td>
<td>2.3(0.0-10.5)</td>
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<td>Glomerular sclerosis index</td>
<td>1.7±0.6</td>
<td>1.8±1.1</td>
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<tr>
<td>Tubulointerstitial score</td>
<td>1.6±1.0</td>
<td>1.6±0.8</td>
</tr>
<tr>
<td>Chronicity score</td>
<td>3.7±1.9</td>
<td>3.8±1.9</td>
</tr>
<tr>
<td>Haas Classification</td>
<td>2.8±1.5</td>
<td>3.5±1.4</td>
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Fig. 1. Urinary protein excretion (A) and creatinine clearance (B) during 16 weeks of treatment. The 24-hour proteinuria in the pioglitazone group (indicated by black square) was gradually reduced during 16 weeks of treatment while the level remained stable in the control group (indicated by white circle). Creatinine clearance in both groups did not change during 16 weeks of treatment. \( a = p<0.05 \) vs. baseline, \( b = p<0.05 \) vs. control.
Fig. 2. Urinary TGF-β1 excretion (A) and urinary VEGF excretion (B) during 16 weeks of treatment. Urinary TGF-β1 excretion and urinary VEGF excretion in the pioglitazone group (indicated by black square) were significantly reduced after 16 weeks of treatment while the levels did not change in the control group (indicated by white circle). a = p < 0.05 vs. baseline.
Adverse effects

None of the PGZ-treated patients developed congestive heart failure, hepatitis, or hypoglycemic symptoms. Fasting plasma glucose was not changed after PGZ treatment. Patients in the PGZ group had slight, but not significant weight gain of 2.0 kg after 16 weeks of treatment. There was also a non-significant decrease in hematocrit (1.1%) after PGZ treatment (Table 2).

Discussion

Although IgAN has long been recognized, and the prognostic factors have been widely explored, no treatment up to now can cure the disease [11, 21]. Only renin-angiotensin aldosterone system blockages had been advocated to reduce proteinuria and slow progression of the disease. However, most studies reported partial reduction of proteinuria [8, 22]. In the present study, PGZ effectively reduced urinary protein excretion in high risk IgAN who had been treated with ACEI/ARB therapy. To our knowledge, this is the first randomized, double-blinded study that demonstrated the effectiveness of PPAR-γ agonists in patients with IgAN. Since proteinuria is associated with disease progression and reduction of proteinuria is one of the main therapeutic goals for IgAN [5, 6], our findings suggest potential use of PGZ in IgAN.

PGZ and other PPAR-γ agonists have been demonstrated to reduce proteinuria in diabetic kidney disease [12-15]. Therefore, antiproteinuric effect of PPAR-γ agonists is likely to be general in nature, and does not relate to the causes of glomerular injury. The antiproteinuric effect of PGZ is likely to be independent of insulin-sensitizing and lipid-lowering effects, since the levels of plasma glucose and lipids in patients who received PGZ were not significantly changed from the baseline levels, as seen in Table 2. PPAR-γ agonists may exert proteinuria attenuating effect, at least in part, via direct action on mesangial cells because activation of PPAR-γ has been shown to prevent mesangial proliferation, extracellular matrix synthesis, and mesenchymal transdifferentiation [12, 20]. Recent studies showed that progression of renal function in several glomerulonephritis including IgAN [23] depended mainly on tubulo-interstitial damage, which was mediated by increased renin angiotensin system in tubular epithelial cell (TEC) [24]. PPAR-γ agonists, in a PPAR-γ-dependent manner, could attenuate angiotensin II receptor type 1 expression in TEC [25] and potentiate the therapeutic effect of ARB in IgAN [26]. Therefore, it is likely that PPAR-γ agonists would slow progression of IgAN. However, significant difference in CrCl could not be demonstrated in the present study, probably due to the relatively short duration of the study.

Table 2. Blood pressure, hematocrit, and metabolic parameters at baseline and 16 weeks after treatment. Data are expressed as geometric means (95% CI).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>PGZ (n = 21)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>16 weeks</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>125.2 (118.8-131.7)</td>
<td>129.5 (123.6-135.3)</td>
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<tr>
<td>MAP (mmHg)</td>
<td>93.0 (89.0-97.1)</td>
<td>95.1 (90.7-99.4)</td>
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<tr>
<td>Hematocrit (%)</td>
<td>38.1 (35.8-40.5)</td>
<td>37.9 (35.4-40.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.3 (55.2-67.4)</td>
<td>61.3 (55.3-67.2)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>23.8 (21.5-26.2)</td>
<td>23.8 (21.5-26.1)</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>95.9 (90.2-101.7)</td>
<td>93.1 (84.7-101.6)</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
<td>228.5 (206.9-250.1)</td>
<td>234.5 (202.0-267.0)</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>141.3 (95.5-187.2)</td>
<td>141.3 (103-179.6)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>59.9 (52.9-66.9)</td>
<td>60.4 (52.2-68.5)</td>
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</table>
Enhanced ECM production with segmental sclerosis and tubulo-interstitial fibrosis are the two obvious morphological changes observed in IgAN [27]. These pathological changes lead to a declination of renal function and ultimately cause end stage renal disease (ESRD). TGF-β is well recognized as a profibrotic factor that augments ECM accumulation and fibrosis [28]. Local up-regulation of the TGF-β protein has been observed in the fibrotic and sclerotic areas of the kidney [29]. Therefore, urinary rather than blood levels of TGF-β are correlated with the renal disease progression. Previous studies demonstrated that urinary TGF-β excretion was significantly up-regulated in IgAN [7, 8]. The present study demonstrated that PGZ could significantly down-regulate urinary TGF-β excretion in patients with IgAN, as seen in Fig. 2A. These results suggest that PPAR-γ agonists may exert beneficial effects through inhibition of renal TGF-β. Noteworthy, a recent study using mouse model confirmed that PPAR-γ agonists could reduce renal TGF-β expression and attenuate renal interstitial fibrosis [30].

In the present study, PGZ also significantly reduced urinary VEGF excretion while the level was slightly increased in the control group. Previous studies documented increased VEGF expression in glomerular diseases including IgAN [9, 31]. Increased VEGF expression could lead to several pathological changes such as glomerular hypertrophy and collapsing glomerulopathy [32, 33]. Therefore, decreasing urinary VEGF after PGZ treatment is likely to be beneficial.

PPAR-γ agonists, which could effectively reduce proteinuria and urinary TGF-β as well as VEGF excretion in the present study, may have the therapeutic role in retarding the progression of IgAN. Data from the present study in high-risk IgAN patients with concomitant ACEI/ARB treatment would privilege this class of TZD agents, and TZD would be considered as one of the most preferable alternative drugs that could provide beneficial effect on the kidney.

In conclusion, activation of PPAR-γ using PGZ significantly reduced proteinuria in IgAN patients. These findings warrant further evaluation to determine the etiology of this benefit (possibly TGF-β & VEGF related) and to determine whether long-term benefit exists.

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References


