Protective effects of PPARγ agonist in acute nephrotic syndrome

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Abstract

Background. Peroxisome proliferator-activated receptor gamma (PPARγ) agonists have beneficial effects on renal structure and function in models of diabetes and chronic kidney diseases. However, the increased incidence of weight gain and edema potentially limits their usefulness. We studied an acute minimal-change disease-like nephrotic syndrome model to assess effects of PPARγ agonist on acute podocyte injury and effects on fluid homeostasis.

Methods. Acute podocyte injury and nephrotic syndrome were induced by puromycin aminonucleoside (PAN) injection in rats.

Results. PPARγ agonist, given at the time or after, but not before PAN, reduced proteinuria, restored synaptopodin, decreased desmin and trended to improve foot process effacement. There was no significant difference in glomerular filtration, effective circulating volume, blood pressure or fractional sodium excretion. PAN-injured podocytes had decreased PPARγ, less nephrin and α-actinin-4, more apoptosis and reduced phosphorylated Akt. In PAN-injured cultured podocytes, PPARγ agonist also reversed abnormalities only when given simultaneously or after injury.

Conclusions. These results show that PPARγ agonist has protective effects on podocytes in acute nephrotic syndrome without deleterious effects on fluid homeostasis. PPARγ agonist-induced decrease in proteinuria in acute nephrotic syndrome is dependent at least partially on regulation of peroxisome proliferator-response element-sensitive gene expression such as α-actinin-4 and nephrin and the restoration of podocyte structure.

Keywords: nephrotic syndrome; podocytes; PPARγ; puromycin aminonucleoside

Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ) agonists, also known as thiazolidinediones (TZDs), improve insulin resistance and other metabolic parameters and are widely used in type 2 diabetic patients [1, 2]. PPARγ agonists also have pleiotropic nonmetabolic effects, including regulating cell cycle and differentiation, controlling inflammation and cytokine production [3, 4]. PPARγ agonists have beneficial effects on the kidney [5–13], but fluid retention and edema, observed in up to 5% of TZD-treated diabetic patients, could limit their use [14]. The underlying mechanisms of these adverse effects remain obscure. Vascular permeability, vasodilation and increased activity of the epithelial sodium channel (ENaC) in the collecting duct have been suggested to cause TZD-induced fluid retention [15–20].

Previously, our studies in the chronic focal segmental glomerulosclerosis (FSGS) model induced by uninephrectomy combined with multiple small doses of puromycin aminonucleoside (PAN) injection found that pioglitazone, a potent PPARγ agonist, ameliorates progression of this podocyte injury-associated glomerulosclerosis and the associated low-grade proteinuria if given after injury was established [7]. However, those previous studies left unanswered effects of PPARγ agonist on edema in nephrotic syndrome. We therefore have now studied a model of acute nephrotic syndrome. This single PAN dose model is characterized by disruption of fluid homeostasis, marked proteinuria, edema and foot process effacement and mimicks the functional and morphological aspects of minimal-change disease (MCD) or early stage FSGS [21–24]. We used this model to investigate whether PPARγ agonist can protect podocytes from injury and reduce proteinuria in acute nephrotic syndrome and also assessed effects on fluid homeostasis in this setting. Our results demonstrate that PPARγ agonist decreases proteinuria in this acute MCD-like nephrotic syndrome by podocyte-specific mechanisms, without exacerbation of fluid homeostasis.

Materials and methods

In vivo studies

Study design. Adult male Sprague–Dawley rats (250–300 g; Harlan Laboratories, Indianapolis, IN) were studied in accordance with National Institutes of Health and Vanderbilt University animal care facility guidelines. Rats were housed under normal conditions and received standard rat powdered diet (Purina Rodent ‘5001’ meal; Tusculum Feed Center, Nashville, TN) and tap water ad libitum. Rats received PAN (100 mg/kg body weight (Bwt), intraperitoneally, Sigma, St. Louis,
MO or saline and were divided into six groups: control saline (n = 15), PAN without treatment (PAN, n = 19), pioglitazone (Pio, 10 mg/kg/day, mixed with powdered chow) starting 7 days before PAN (prePio, n = 20). Pio starting the same day as PAN (Pio0, n = 20). Pio starting 4 days after PAN (PostPio, n = 20) and pioglitazone starting 7 days before saline injection (PioControl, n = 5). The dose of Pio was based on our previous experience [7]. In this single dose PAN injection model, proteinuria emerges around 4-5 days, reaches a peak at 10 days and is maintained until 21 days [25, 26]. Thus, animals were sacrificed at day 4, 10 or 21.

Functional assessments

Body weight, systolic blood pressure (SBP) and 24-h urine protein, sodium and potassium excretions were assessed at baseline, day 1, 3, 5, 10, 14 and 21 after PAN or saline injection. Glomerular filtration rate (GFR) (creatinine clearance, CCr) was assessed at baseline, day 1, 4, 10 and 21. Plasma volume was assessed at day 10. SBP was measured using tail-cuff plethysmography in unanesthetized prewarmed trained rats at an ambient temperature of 29°C. Twenty-hour urine protein was measured by the Pierce Protein Assay Kit (Pierce Laboratories, Hercules, CA). Urine creatinine, sodium and potassium were measured by the creatinine ELISA kit (Exocell, Philadelphia, PA) and flame photometer analyzer (Instrumentation Laboratory, Lexington, MA), respectively. Urine and serum osmolality was determined with an advanced micro-osmometer (The Advanced Instruments, Inc., Pomona, CA). Values of CCr, fractional excretion of sodium (FENa) and potassium (FEK) and solute-free water clearance (FEH2O) were calculated using standard formulas. Plasma volume was measured by the Evans blue dye dilution method in anesthetized rats (pentobarbital sodium, 40 mg/kg Bwt) [19, 27]. Epididymal fat was re-measured by the Evans blue dye dilution method in anesthetized rats at sacrifice and its water content was calculated, after a 7-day incubation at 70°C [28, 29]. Glomeruli were isolated and cells were confirmed as podocytes by immunostaining before the first passage [31–33]. These cells were then passaged three to four times and cultured with complete growth medium for primary podocytes to obtain final cells for experiments. Western blot analysis

Western blot of cultured podocytes was done for α-actinin-4 (1:400; ZYMED Laboratories, S. San Francisco, CA), phosphorylated Akt (1:1000; Cell Signaling Technology, Inc., Danvers, MA) and total Akt (1:1000; Cell Signaling Technology, Inc., Danvers, MA). Densitometric quantitation was performed using NIH image (version 1.63).

Primary podocyte cell culture

Glomeruli were isolated and cells were confirmed as podocytes by immunostaining before the first passage [31–33]. These cells were then passaged three to four times and cultured with complete growth medium for primary podocytes to obtain final cells for experiments. Western blot analysis was done using PI3K inhibitor and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to normalize the protein content. All data are expressed as mean ± SEM. Statistical difference was assessed by single-factor variance (analysis of variance) followed by least significant difference correction for multiple comparisons. Nonparametric

### Table 2. Body weight at baseline, at sacrifice and % increase from baseline

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th></th>
<th></th>
<th>Day 10</th>
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<th>Day 21</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>SAC</td>
<td>Baseline</td>
<td>SAC</td>
<td>Baseline</td>
<td>SAC</td>
<td>Baseline</td>
<td>SAC</td>
</tr>
<tr>
<td>N</td>
<td>288 ± 3</td>
<td>329 ± 3</td>
<td>(14.0 ± 0.4%)</td>
<td>290 ± 13</td>
<td>337 ± 11</td>
<td>(16.5 ± 3.4%)</td>
<td>289 ± 6</td>
<td>363 ± 2</td>
</tr>
<tr>
<td>PAN</td>
<td>294 ± 16</td>
<td>327 ± 10</td>
<td>(11.5 ± 2.5%)</td>
<td>286 ± 13</td>
<td>308 ± 18</td>
<td>(7.6 ± 3.1%)</td>
<td>281 ± 4</td>
<td>308 ± 11*</td>
</tr>
<tr>
<td>prePio</td>
<td>285 ± 9</td>
<td>312 ± 2</td>
<td>(9.5 ± 2.9%)</td>
<td>276 ± 2</td>
<td>303 ± 11</td>
<td>(10.0 ± 3.9%)</td>
<td>275 ± 5</td>
<td>286 ± 18*</td>
</tr>
<tr>
<td>Pio0</td>
<td>293 ± 12</td>
<td>326 ± 14</td>
<td>(11.8 ± 6.6%)</td>
<td>299 ± 15</td>
<td>320 ± 16</td>
<td>(7.3 ± 3.5%)</td>
<td>278 ± 4</td>
<td>304 ± 25*</td>
</tr>
<tr>
<td>PostPio</td>
<td>ND</td>
<td></td>
<td></td>
<td>274 ± 3</td>
<td>309 ± 11</td>
<td>(12.6 ± 4.2%)</td>
<td>278 ± 9</td>
<td>318 ± 17*</td>
</tr>
</tbody>
</table>

Abbreviations: N, normal; PAN, puromycin aminonucleoside alone without further treatment; prePio, pioglitazone treatment given before PAN; Pio0, simultaneous pioglitazone and PAN treatment; PostPio, pioglitazone treatment given after PAN; ND, not done. Data are given as gram and percent increase from baseline, mean ± SEM.

*p<0.05 vs N.

For baseline body weight, all pNS vs N. At sacrifice, body weights were not significantly different for PAN-injected groups receiving pioglitazone treatment vs PAN alone.

### Table 1. Real-time RT–PCR primer sequences forward reverse

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
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<tbody>
<tr>
<td>Nephrin</td>
<td>tggactctgagcgaacctca</td>
</tr>
<tr>
<td>PPARγ</td>
<td>gaggagcaccactgcatt</td>
</tr>
<tr>
<td>GAPDH</td>
<td>gagaggccotactcactc</td>
</tr>
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</table>
data were compared by Mann–Whitney U-test. A P-value <0.05 was considered significant.

Results

Systemic and renal functional changes

SBP was normal and similar over time in all PAN-injected groups versus saline control. Baseline body weights were similar in all PAN-injected rats and saline control. At day 4 and day 10, neither body weight nor percent increase from baseline was different among PAN-injected rats versus saline control. At day 21, body weight decreased similarly in all PAN-injected groups compared to saline control (Table 2). At day 10, compared to normal control, epididymal fat water content was increased in groups treated with PPARγ agonist before or at the same time as PAN (Table 3). Proteinuria increased similarly in all PAN-injected rats versus control by day 4, continued to increase by day 10 and was maintained at day 21. Treatment with PPARγ agonist at the time of injury or 4 days later significantly reduced subsequent proteinuria (Figure 1). By contrast, pretreatment with PIO failed to reduce subsequent proteinuria (Figure 1). At day 21, serum albumin decreased similarly in all PAN-injected groups compared to control, while blood urea nitrogen did not differ among all groups (data not shown).

CCr was similar in all groups at Day 1 but was markedly and similarly decreased in all PAN-injected groups by day 10. Plasma volume was not affected by PPARγ agonist (Table 4). At day 21, CCr was similar in all groups (N 0.72 ± 0.02, PAN 0.71 ± 0.13, prePIO 0.72 ± 0.06, PIO0 0.62 ± 0.03, PostPIO 0.77 ± 0.04 mL/min/100 g Bwt, all pNS), reflecting previously documented resolution of injury without progressive sclerosis in this acute PAN model.

There was a trend in a pioglitazone rats to have decreased urine volume compared with PAN alone (data not shown). Pioglitazone rats also had a trend towards a decrease in FENa and FEK compared with PAN alone at day 4 and day 10 (data not shown). At day 21, all PIO rats had decreased FENa compared to PAN alone (Table 4). FEK also decreased in PIO0 and PostPIO rats but not in prePIO compared to PAN alone (Table 4). FEH2O did not differ among all PAN-injected groups at day 10 (Table 4).

There was no significant difference between saline control and the additional pioglitazone control (pioglitazone starting 7 days before saline injection) in the following functional parameters assessed at day 10: GFR (Table 4), proteinuria (Figure 1), urine volume (data not shown), FENa (data not shown), FEK (data not shown) and FEH2O (Table 4). These data indicate that pioglitazone by itself had no effects on these functional parameters under normal conditions.

AQP2 and ENaCγ expression

We next assessed key transporter molecules in water and sodium handling. In the normal rat kidney, AQP2 was present on the apical membrane and throughout the cytoplasm along the entire collecting duct as well as in the connecting tubeule. Neither the expression pattern nor the intensity was changed by PAN or PIO (Figure 2A). By western blot, both the nonglycosylated (29 kDa) and the glycosylated (35 kDa) bands of AQP2 were detected in the medulla, as previously reported [34], and AQP2 protein levels were similar in all groups (Figure 2B). All PAN-injected rats showed similarly increased ENaCγ expression in the medulla compared to normal at day 10 (Figure 2C).

Podocyte injury in vivo

In normal rat kidneys, PPARγ was present in small arteries, collecting ducts and transitional epithelium. PPARγ podocyte immunostaining was not different among groups at day 4 while it was increased similarly in all PAN-injected rats at day 10 and 21 compared with normal (Figure 3).

Table 3. Water content in epididymal fat

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 10</th>
<th>Day 21</th>
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<tbody>
<tr>
<td>N</td>
<td>47.7 ± 5.7%</td>
<td>50.0 ± 6.2%</td>
<td>43.9 ± 8.0%</td>
</tr>
<tr>
<td>PAN</td>
<td>50.1 ± 0.3%</td>
<td>64.2 ± 13.8%</td>
<td>52.6 ± 4.3%</td>
</tr>
<tr>
<td>prePIO</td>
<td>59.7 ± 5.7%</td>
<td>78.8 ± 10.8%</td>
<td>68.7 ± 10.4%</td>
</tr>
<tr>
<td>PIO0</td>
<td>58.4 ± 2.9%</td>
<td>75.8 ± 5.7%*</td>
<td>49.2 ± 3.9%</td>
</tr>
<tr>
<td>PostPIO</td>
<td>ND</td>
<td>70.6 ± 4.7%</td>
<td>46.9 ± 3.4%</td>
</tr>
</tbody>
</table>

N, normal; PAN, puromycin aminonucleoside alone without further treatment; prePIO, pioglitazone treatment given before PAN; PIO0, simultaneous pioglitazone and PAN treatment; PostPIO, pioglitazone treatment given after PAN; Data are mean ± SEM. *P < 0.05 versus N. Water content in epididymal fat in PAN-injected groups receiving pioglitazone treatment versus that in PAN alone, all pNS.

Fig. 1. Proteinuria time course. Proteinuria increased similarly in all PAN-injected rats versus control by day 4. At day 10 and day 21, the increased proteinuria was significantly reduced by simultaneous or delayed PIO treatment (PIO0, PostPIO) but not by pretreatment with PIO (prePIO). Pioglitazone by itself (PIOControl) had no effect on proteinuria (N, n = 15; PAN, n = 19; PIO0, n = 20; prePIO, n = 20; PostPIO, n = 20; PIOControl = 5; *P < 0.05 versus N, #P < 0.05 versus PAN).
Synaptopodin, a podocyte differentiation marker, was markedly and similarly decreased by immunostaining at day 4 and 10 in all PAN groups compared with normal. However, at day 21 after PAN, synaptopodin was restored in Pio0 and PostPio but not in prePio rats (Figure 4A). In contrast, desmin, a marker of early podocyte injury, was similarly increased in all PAN-injected groups at day 4, remained upregulated at day 10 and day 21 in PAN and prePio but was less in Pio0 and PostPio (Figure 4B).

PAN rats showed prominent podocyte degeneration with complete foot process effacement (FPE) by EM at day 4 and day 10, with vacuoles, microvillous transformation, and segmental podocyte detachment and glomerular basement membrane denudation. PostPio and Pio0 had a trend towards less podocyte injury with subtotal FPE and lesser podocyte detachments (Figure 4C).

FPW was increased 3.6-fold in response to PAN compared to normal (1551 ± 475 versus 334 ± 21 nm, P < 0.05). FPW was numerically highest in prePio rats, even higher than in PAN alone and numerically lowest in PostPio (prePio 1715 ± 223, Pio0 1543 ± 370, PostPio 1278 ± 43 nm versus PAN).

**Podocyte injury and PPARγ agonist in vitro**

We quantitated podocyte apoptotic changes by assessing Annexin V, an early marker of apoptotic events. PAN induced increased apoptosis (% cells apoptotic: DMSO 4.0 ± 1.1 versus PAN 11.5 ± 1.5, P < 0.05). Both simultaneous and delayed treatment with Pio, but not

### Table 4. Renal function and fluid/electrolyte balance

<table>
<thead>
<tr>
<th></th>
<th>Ccr (mL/min/100 g Bwt)</th>
<th>Plasma volume (mL/100 g Bwt)</th>
<th>FeH2O (mL/h)</th>
<th>FEna (%)</th>
<th>FeK (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.90 ± 0.08</td>
<td>2.30 ± 0.11</td>
<td>−2.32 ± 0.23</td>
<td>0.51 ± 0.07</td>
<td>26.1 ± 1.8</td>
</tr>
<tr>
<td>PAN</td>
<td>0.45 ± 0.10*</td>
<td>3.03 ± 0.20*</td>
<td>−1.36 ± 0.15*</td>
<td>1.03 ± 0.23*</td>
<td>44.5 ± 6.2*</td>
</tr>
<tr>
<td>PrePio</td>
<td>0.40 ± 0.08*</td>
<td>2.72 ± 0.18*</td>
<td>−1.38 ± 0.23*</td>
<td>0.52 ± 0.02†</td>
<td>44.9 ± 6.0*</td>
</tr>
<tr>
<td>Pio0</td>
<td>0.40 ± 0.10*</td>
<td>3.11 ± 0.20*</td>
<td>−1.03 ± 0.15*</td>
<td>0.34 ± 0.01†</td>
<td>26.6 ± 3.7†</td>
</tr>
<tr>
<td>PostPio</td>
<td>0.53 ± 0.17*</td>
<td>2.89 ± 0.21*</td>
<td>−0.94 ± 0.23*</td>
<td>0.39 ± 0.05†</td>
<td>29.7 ± 4.6†</td>
</tr>
<tr>
<td>PioControl</td>
<td>0.86 ± 0.14</td>
<td>ND</td>
<td>−2.77 ± 0.46</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*P < 0.05 versus N, †P < 0.05 versus PAN.

### Footnotes for Table 4:

*N*, normal; PAN, puromycin aminonucleoside alone without further treatment; prePio, pioglitazone treatment given before PAN; Pio0, simultaneous pioglitazone and PAN treatment; PostPio, pioglitazone treatment given after PAN; PioControl, pioglitazone pretreatment before saline injection; ND, not done; Ccr, creatinine clearance, plasma volume and FeH2O, solute-free water clearance data are from day 10; FEna, fractional excretion of sodium; FeK, fractional excretion of potassium data are from day 21. Data are mean ± SEM.
pretreatment, significantly attenuated PAN-induced apoptosis (% cells apoptotic: Pio0 6.2 ± 1.1, PostPio 6.8 ± 1.4, both P < 0.05 versus PAN; prePio 10.0 ± 3.1, NS versus PAN). We next explored the expression of Akt, a key signaling protein involved in the regulation of podocyte survival. Decreased phosphorylated Akt/total Akt induced by PAN was significantly restored when Pio was given with PAN or after, but not by pretreatment (DMSO 1.10 ± 0.10, PAN 0.75 ± 0.11, Pio0 0.99 ± 0.08 and PostPio 1.06 ± 0.07, all P < 0.05 versus PAN; prePio 0.85 ± 0.03, NS versus PAN) (Figure 5A).

We next assessed PPARγ activity and gene expression in podocytes. PPARγ activity was increased in all Pio-treated podocyte groups (prePio 6.4 ± 0.5, Pio0 11.0 ± 0.9 and PostPio 8.5 ± 0.3, versus PAN 3.8 ± 1.3 and DMSO 4.4 ± 0.2, P < 0.05). PAN suppressed PPARγ gene expression compared with control. Treatment with Pio at the same time or 24 h later significantly restored gene expression (DMSO 1.00 ± 0.00, PAN 0.37 ± 0.03, Pio0 0.54 ± 0.03 and PostPio 0.56 ± 0.02, P < 0.05 versus PAN, Figure 5B). By contrast, pretreatment with Pio failed to increase PPARγ levels (0.30 ± 0.04, NS versus PAN). Thus, although in vivo, glomerular PPARγ protein was increased in expression intensity in response to PAN, protein expression and mRNA levels of PPARγ were not changed in parallel in vitro in podocytes. These differences likely reflect in vivo effects of glomerular cell cross talk, effects of feedback with endogenous PPARγ ligands or effects of other systemic and/or hemodynamic parameters not captured in the in vitro setting.

α-Actinin-4, a key cytoskeletal protein, was significantly decreased by PAN. Treatment with PPARγ agonist at the time of injury or 24 h later significantly restored α-actinin-4 protein. In contrast, pretreatment with Pio failed to increase α-actinin-4 (Figure 5C). Decreased nephrin gene expression induced by PAN was significantly restored when Pio was given with PAN or after, but not by pretreatment (Figure 5D).

Discussion

In the current study, we studied an acute MCD-like nephrotic syndrome model, which is characterized by fluid retention, proteinuria and podocyte FPE, without progressive sclerosis. We first demonstrated that PPARγ agonist ameliorated podocyte damage and reduced proteinuria. Importantly, the administration of PPARγ agonist did not potentiate or worsen fluid and ion retention in these nephrotic rats, and PPARγ agonist by itself in normal rats had no effect on GFR, proteinuria, urine volume or fractional excretion of Na, K or water. In addition, PPARγ agonist in PAN rats showed time-dependent effects, similar to those observed in our previous chronic PAN-associated glomerulosclerosis model [7]. Thus, PPARγ agonist, when given at the time of, or after, but not before injury, reduced proteinuria in the acute PAN model. Both in vivo and in vitro data showed that these differential effects were dependent, at least partially, on restoring differentiation markers and structure in podocytes.

Proteinuria may be modulated by hemodynamic abnormalities (including increased systemic or glomerular pressure, increased circulating volume or GFR, etc.) and/or structural alteration of the glomerular filtration barrier. Troglitazone, a PPARγ agonist, dilated both afferent and efferent arterioles in an in vitro study, suggesting that these vasodilator actions may play a role in its renoprotective effects [18]. However, we found no difference in blood pressure or GFR with PPARγ agonist, suggesting that proteinuria effects were not hemodynamically mediated. Treatment with PPARγ agonist also did not change body weight or total plasma volume in acute PAN nephrotic syndrome.

PPARγ agonists have ameliorated injury in models of progressive sclerosis and immune complex disease [5–10]. However, in patients, these compounds have been associated with fluid retention and edema [35–37]. The mechanisms for these side effects remain obscure but have been linked to arterial vasodilation, endothelial permeability and renal sodium reabsorption [15–20]. Regulation of aquaporin-2, different subunits of ENaC and other transporters may be associated with PPARγ agonist-induced fluid retention [29, 34, 38] and also are implicated in nephrotic syndrome [39]. We therefore examined PPARγ agonist effects on these mechanisms in nephrotic syndrome. We observed reduced CCR in the acute nephrotic syndrome with increased sodium and fluid retention and increased expression of ENaCγ. Our data also show that PPARγ agonist before or with PAN increased epididymal fat water content versus normal control. However, there

Figure 3. PPARγ expression. PPARγ podocyte immunostaining was similar in all groups at day 4 but increased similarly in all PAN-injected rats at day 10 and 21 compared with normal (×400) (N, n = 10; PAN, n = 14; Pio, n = 15; prePio, n = 15; PostPio, n = 15; *P < 0.05 versus N).
was no significant change in body weight or epididymal fat water content in PAN rats receiving PPARγ agonist versus PAN alone. Administration of PPARγ agonists in normal rats caused early alterations in banding patterns for AQP2 and ENaC subunits with later reduction in these molecules, perhaps representing an attempt to reestablish sodium and fluid balance [34]. However, treatment with PPARγ agonist in PAN rats did not significantly change these functional indices or key transporter proteins, such as aquaporin-2 and ENaCγ. Taken together, these results demonstrate that PPARγ agonist treatment did not worsen existing fluid and sodium retention in the acute nephrotic syndrome setting.

We next assessed whether acute nephrotic syndrome altered PPARγ effects on podocytes. In order to support normal glomerular filtration barrier function, podocytes must maintain both their complex cytoskeletal architecture and differentiation [40–43]. We therefore examined effects of PPARγ agonist on podocytes in this acute nephrotic syndrome state induced by PAN and potential underlying mechanisms. We found that desmin, a marker of early podocyte injury, was upregulated by PAN and remained upregulated in PAN and pretreatment with PIO but was dramatically suppressed following simultaneous or delayed PIO treatment at day 10 and 21 (×400) (N, n = 4; PAN, n = 5; PIO, n = 5; PIO0, n = 5; PostPIO, n = 5; *P < 0.05 versus N, #P < 0.05 versus PAN). (B) Desmin, a marker of early podocyte injury, was similarly increased in all PAN-injected groups at day 4, remained upregulated in PAN and pretreatment with PIO but was dramatically suppressed following simultaneous or delayed PIO treatment at day 10 and 21 (×400) (N, n = 4; PAN, n = 5; PIO, n = 5; PIO0, n = 5; PostPIO, n = 5; *P < 0.05 versus N, #P < 0.05 versus PAN). (C) Ultrastructure of podocytes. Electron microscopic analysis at day 10 revealed prominent podocyte injury with complete FPE, cytoplasmic vacuoles and microvillous transformation in PAN rats, contrasting intact foot processes in normal animals (N). Podocyte injury was less severe in PAN rats receiving delayed treatment with PIO (PostPIO), with only subtotal FPE (transmission electron microscopy, ×22,000) (N, n = 3; PAN, n = 4; PIO, n = 4; PIO0, n = 4; PostPIO, n = 3).
that the beneficial effects of PPARγ agonist to decrease proteinuria in acute nephrotic syndrome are partially mediated through effects on podocytes. We next explored potential mechanisms of PPARγ effects on podocytes. PPARγ is a transcriptional factor. Heterodimers of PPARs and the retinoid X receptors recognize peroxisome proliferator-response elements (PPRE) that exhibit a consensus sequence in target genes. The nephrin gene contains PPRE in its promoter, and the group of Remuzzi linked pioglitazone’s renoprotection to its capacity to repair the injured slit diaphragm via nephrin reexpression [44]. We identified one putative consensus PPRE sequence in the promoter area of α-actinin-4 gene by TESS (Transcription ElementSearching System) analysis. Interestingly, in cultured podocytes, pretreatment with PPARγ agonist was associated with the lowest PPARγ gene expression among all groups and indeed failed to restore the suppressed nephrin, α-actinin-4 and phosphorylated Akt induced by PAN. Thus, we postulate that pretreatment with PPARγ agonist may downregulate its endogenous nuclear receptor, PPARγ, by negative feedback which may render podocytes insensitive to mount an appropriate response to injury. We speculate that the decreased PPARγ resulting from pretreatment with PPARγ agonist may downregulate the expression of PPRE-sensitive genes, such as nephrin and α-actinin-4, and eventually facilitate podocyte apoptosis and loss. Further studies will be needed to test this hypothetical mechanism.

In summary, our study shows that PPARγ agonist given at the time of or after injury provides protective effects against proteinuria without aggravating fluid and sodium retention in the PAN-induced nephrotic syndrome model. These complex time-dependent actions of PPARγ agonist on proteinuria are mediated at least partially through effects on podocytes by upregulation of PPRE-sensitive gene expression and restoration of podocyte structure.

Acknowledgements. Sources of support: This work was supported in part by NIH NIDDK P50 DK79341 and by Takeda Pharmaceutical Company Ltd. These studies were supported in part by a grant from Takeda Pharmaceutical Company Ltd. NIH NIDDK P50 DK79341 and Takeda Pharmaceutical Company Ltd.

Conflict of interest statement. None declared.

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Received for publication: 14.12.10. Accepted in revised form: 4.4.11