Peroxisome proliferator-activated receptor-δ activation ameliorates albuminuria by preventing nephrin loss and restoring podocyte integrity in Type 2 diabetes

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Abstract

Background. Peroxisome proliferator-activated receptor (PPAR)-δ is a ligand-activated transcription factor in regulating gene expression and is believed to play an important role in various kidney diseases including diabetic nephropathy. This study investigated the efficacy of GW610742, a highly specific agonist for PPAR-δ, for the treatment of diabetic nephropathy.

Methods. Type 2 diabetic Otsuka Long-Evans Tokushima Fatty rats were randomized into an untreated diabetic group (n = 9) and a GW610742-treated diabetic group (n = 9). The GW610742 was administered (10 mg/kg/day) orally for 11 weeks. Long-Evans Tokushima Otsuka rats (n = 9) were used as a non-diabetic control.

Results. Albuminuria was markedly increased and renal PPAR-δ expression was decreased in diabetes. Diabetic albuminuria and renal injury markers, such as glomerular basement membrane thickening, decreased number of slit pores between podocyte foot processes, decreased nephrin expression, increased desmin expression and increased CCL2 expression, were significantly reversed through the treatment with GW610742. PPAR-δ agonist GW610742 markedly increased nephrin expression in cultured podocytes. Nephrin mRNA expression was markedly decreased in response to high glucose in cultured podocytes and effectively prevented by GW610742.

Conclusions. PPAR-δ activation by GW610742 ameliorates albuminuria by preventing diabetes-induced nephrin loss and restoring podocyte integrity, implying that GW610742 may be a potential therapeutic agent for diabetic nephropathy.

Keywords: albuminuria; diabetic nephropathy; nephrin; podocyte; PPAR-δ

Introduction

Peroxisome proliferator-activated receptor (PPAR)-δ is a nuclear receptor protein that acts as a ligand-activated transcription factor in regulating gene expression and is implicated in cellular metabolism, differentiation, proliferation, survival and inflammation [1]. PPAR-δ is also believed to play an important role in many kidney diseases [2]. PPAR-δ+/− and PPAR-δ−/− mutant mice exhibit much greater kidney injury and dysfunction than wild-type mice after renal ischemia/reperfusion injury. Genetic deletion of PPAR-δ-developed proteinuria suggests that PPAR-δ activity could influence renal damage and dysfunction [3]. Conversely, PPAR-δ activation has been reported to decrease renal injury and the markers of glomerular dysfunction in the models of renal ischemia/reperfusion [4, 5], and the overexpression of PPAR-δ provides the protection against hypertonicity-induced cell death in cultured medullary interstitial cells, which suggests that PPAR-δ is an important survival factor in the kidney [6].

With regard to diabetic nephropathy, however, the role of PPAR-δ in the pathogenesis of kidney injury has barely been studied. Renal PPAR-δ mRNA expression is known to be markedly decreased in proteinuric Type 1 diabetic mice [7], and PPAR-δ protein expression is markedly decreased in response to insulin-like growth factor-1 in cultured mesangial cells [8]. These findings suggest that reduced renal PPAR-δ expression possibly represents an underlying mechanism involved in diabetic kidney injury, and that PPAR-δ agonists may be considered a novel means of conferring renal protection in diabetic nephropathy. Newly developed synthetic PPAR-δ ligands have been under recent evaluation for their efficacy in animal models of insulin resistance and in Type 2 diabetes.
mellitus patients; however, the potential therapeutic role of PPAR-δ in diabetic kidney disease has not yet been evaluated [9, 10].

In this study, we examined whether diabetes influences renal PPAR-δ expression, and investigated the effects of a highly specific synthetic ligand for PPAR-δ, GW610742, on functional and structural renal changes in Type 2 diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats. We also attempted to elucidate the mechanism of renoprotective effects of PPAR-δ activation in cultured podocytes.

Materials and methods

Experimental animals

All protocols using rats were approved by the Institutional Animal Care and Use Committee. All rats were kindly provided at 5 weeks of age by GlaxoSmithKline Pharmaceuticals, Stevenage, UK. The GW610742 (GlaxoSmithKline Pharmaceuticals, Stevenage, UK) [11], a specific and high-affinity agonist for PPAR-δ, was administered (10 mg/kg/day) via oral gavage once a day for 11 weeks. Male Long-Evans Tokushima Otsuka rats (n = 9) were used as a non-diabetic control. Body weights and plasma glucose levels were checked every other week, the latter using glucose oxidase methods (Surestep; Lifescan, Evans, WA), in the diabetic group (n = 9) in antibody diluent solution (Golden Bridge International, Mukilteo, WA), nephri (1:100, Abcam), desmin (1:100, Abcam) and ED-1 anti-bodies (1:100, Millipore, Billerica, MA) in a humidified chamber at 4°C. Polymer HRP-linked anti-mouse or anti-rabbit IgG (Golden Bridge International) were used as secondary antibodies. Sections were covered with a 3,3-diaminobenzidine substrate solution (Sigma-Aldrich, St. Louis, MO), then dehydrated in ethanol, cleared in xylene and mounted without counterstaining. All of these sections were examined in a masked manner using light microscopy (Olympus BX-50; Olympus Optical) equipped with a digital camera (Olympus DP2; Olympus Optical). Glomerular desmin staining was graded as follows: signal area in the glomerular capillary tuft of 0 = 0%; 1+ = 1–25%; 2+ = 26–50%; 3+ = 51–75% and 4+ = 76–100% [16]. ED-1 positive macrophage numbers were assessed on paraffin-embedded sections. A total of 50 glomeruli were analyzed per animal by a blinded observer.

A human kidney specimen for PPAR-δ immunostaining was obtained from a diagnostic renal biopsy performed at the Soon Chun Hyang University Cheonan Hospital (Cheonan, Korea). As a normal control, non-tumor kidney tissue from a patient who had renal cell carcinoma and underwent a nephrectomy was used. Studies using human tissues were approved by the institutional review board.

Western immunoblotting

The kidney cortex was homogenized in PRO-PREP™ Protein Extraction Solution (Introne Biotechnology, Korea). The supernatant protein concentrations were measured using the Lowry assay (Bio-Rad, Hercules, CA) and equalized with the addition of Laemmli buffer prior to sodium dodecyl sulfate-based electrophoresis through a sodium dodecyl sulfate-PAGE gel. Following electrical wet transfer of the proteins to a nitrocellulose membrane, the membrane was incubated with anti-PPAR-δ (Progen Biotechnik, Heidelberg, Germany) or anti-β-actin (Sigma). After the incubation with horseshadish peroxidase-conjugated secondary antibody (anti-guinea pig IgG (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-mouse IgG (Sigma)), the chemiluminescent reaction was developed using SuperSignal West Pico (Pierce, Rockford, IL). Computer-assisted densitometry (Image J) was used to quantify the bands captured on radiographic film, and after normalization for the quantity of β-actin, the mean results for each protein in the three groups of rats were graphed as a percentage of the control, which was assigned a value of 100%.

Cell culture

Conditionally, immortalized mouse podocytes (gift of Dr Mundel, University of Miami, Miami, FL) were cultured as previously described [17, 18]. The cells were allowed to differentiate at 37.5°C without interferon in DMEM containing 5.5 mM glucose. Differentiated podocytes were synchronized into quiescence by growing cells in a serum-free medium for 24 h prior to the experiment. To examine the effects of PPAR-δ activation on PPAR-δ target gene transcription, podocytes were treated with PPAR-δ agonist GW610742 and/or PPAR-δ antagonist GW9662.
Real-time quantitative RT-PCR

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instruction. cDNA was synthesized from 1 μg of total RNA using an iScript™cDNA Synthesis Kit (Bio-Rad) and a PCR reaction was performed in 20 μL reaction mixtures containing 10 ng of cDNA template, SYBR® Green PCR Master Mix-Plus (Toyobo, Osaka, Japan), and primers. The sequence of each primer was as follows: mouse nephrin, forward: 5′ GAG GAG GAT CGA ATC AGG AA 3′ and reverse: 5′ GGT CCA CTT CTG CTG TA 3′; rat nephrin, forward: 5′ CGA GGC ACT TCG TGA AAC 3′ and reverse: 5′ CGA CTT GCT CTC CCA GGA CT 3′; rat CCL2, forward: 5′ CTG GAT CGG AACCAA ATG AG 3′ and reverse: 5′ CGG GTG AAC TAC ATC ACATCC AA 3′; mouse β-actin, forward: 5′ GGA CTC CTA TGT GGG TGA CG 3′ and reverse: 5′ CTT CCT CTC CAT GTC CTG CCA GT 3′; rat β-actin, forward: 5′ CCA TGA AGA TCA AGA TCA TTG CTC C 3′ and reverse: 5′ TGC TTG CTG ATC GAC ATC GAC G 3′. For all real-time RT-PCR analysis, β-actin mRNA was used to normalize RNA inputs. Fold change expression with respect to the control was calculated for all samples.

Statistical analysis

Data except albuminuria are expressed as the mean ± standard error of the mean (SEM) and differences between groups were examined for statistical significance using ANOVA with the Bonferroni correction (SPSS 11.5; SPSS, Chicago, IL) for multiple comparisons when appropriate. Albuminuria data are expressed as median and interquartile range as data are not normally distributed. We used non-parametric Kruskal–Wallis test to compare albuminuria in the three groups (control, diabetic and treated) and follow-up Mann–Whitney tests were conducted to evaluate pairwise differences among the three groups, controlling for Type I error across tests by using the Bonferroni approach. P value of <0.05 was considered as a statistically significant difference.

Results

The effects of PPAR-δ agonist on the clinical characteristics of the diabetic rats

As expected, the diabetic OLETF rats were more obese and hyperglycemic than the non-diabetic control rats. Body weight and fasting glucose concentrations were not affected by GW610742 (Table 1). Renal function assessed by blood urea nitrogen and serum creatinine was not different among the three groups. The absolute kidney weight (data not shown) and the kidney weight expressed as a function of body weight of the diabetic rats, perhaps indicative of renal hypertrophy, were also significantly higher than that of the control rats. Treatment with GW610742 did not affect kidney weight (Table 1). Blood levels of total cholesterol and triglycerides were increased in the OLETF diabetic rats compared with those of the non-diabetic rats; however, renal contents of total cholesterol, triglycerides and free fatty acids were not different between the diabetic and non-diabetic groups. GW610742 did not affect blood and renal lipid concentrations, either (Table 1).

Table 1. Effects of GW610742 on body and kidney weights, fasting blood glucose levels, renal lipid concentrations and renal functional parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 9)</th>
<th>DM (n = 9)</th>
<th>DM + GW610742 (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>518 ± 12</td>
<td>623 ± 19*</td>
<td>627 ± 9*</td>
<td>0.000</td>
</tr>
<tr>
<td>Kidney weight (g/100 g bw)</td>
<td>0.64 ± 0.01</td>
<td>0.70 ± 0.02*</td>
<td>0.71 ± 0.02*</td>
<td>0.028</td>
</tr>
<tr>
<td>Fasting blood glucose (mM)</td>
<td>5.4 ± 0.1</td>
<td>8.0 ± 0.4*</td>
<td>7.1 ± 0.2*</td>
<td>0.000</td>
</tr>
<tr>
<td>Blood urea nitrogen (mM)</td>
<td>3.31 ± 0.35</td>
<td>3.42 ± 0.25</td>
<td>2.73 ± 0.25</td>
<td>0.257</td>
</tr>
<tr>
<td>Serum creatinine (mM)</td>
<td>19.6 ± 1.3</td>
<td>16.7 ± 1.0</td>
<td>13.3 ± 1.9</td>
<td>0.066</td>
</tr>
<tr>
<td>Blood total cholesterol (mM)</td>
<td>4.6 ± 0.1</td>
<td>5.7 ± 0.4*</td>
<td>6.0 ± 0.4*</td>
<td>0.012</td>
</tr>
<tr>
<td>Blood triglycerides (mM)</td>
<td>0.34 ± 0.04</td>
<td>2.31 ± 0.36*</td>
<td>3.12 ± 0.56*</td>
<td>0.000</td>
</tr>
<tr>
<td>Renal total cholesterol (μM)</td>
<td>145 ± 8</td>
<td>163 ± 9</td>
<td>149 ± 6</td>
<td>0.240</td>
</tr>
<tr>
<td>Renal triglycerides (mM)</td>
<td>1.12 ± 0.09</td>
<td>1.37 ± 0.14</td>
<td>1.20 ± 0.03</td>
<td>0.216</td>
</tr>
<tr>
<td>Renal free fatty acid (μM)</td>
<td>154 ± 8</td>
<td>148 ± 13</td>
<td>147 ± 7</td>
<td>0.852</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM. DM, diabetes mellitus, bw, body weight. P: overall P-value for the ANOVA. *P < 0.05 from post-hoc analysis versus non-diabetic control rats.

PPAR-δ agonist ameliorates diabetic albuminuria

Median levels of the albumin excretion rate, measured with ELISA on the 24-h urine samples, was markedly increased in the Type 2 diabetic OLETF rats as compared with the non-diabetic control rats, whether the albuminuria was corrected for urinary creatinine (Figure 1A) or expressed per day (Figure 1B). In the diabetic rats, GW610742 treatment resulted in the prevention of albuminuria (Figure 1).

PPAR-δ agonist recovers GBM thickening in diabetes

The GBM thickness measured across its full thickness was markedly increased in the diabetic OLETF rats as compared with the non-diabetic control rats. However, in the diabetic rats that were treated with GW610742, diabetic GBM thickening was effectively prevented (Figure 2). Representative electron photomicrographs of the GBM in the non-diabetic control, diabetic and GW610742-treated diabetic rats are shown in Figure 2A.

PPAR-δ agonist improves podocyte injury in diabetes

Podocyte foot process effacement was observed in the diabetic OLETF rats, as expected. Slit pore density, the number of slit pores between podocyte foot processes per unit length of GBM that can be measured in a representative electron photomicrograph (Figure 3A), was also significantly reduced in the diabetic OLETF rats. GW610742 treatment effectively restored the effacement and fusion of the podocyte foot processes and recovered slit pore density along the GBM in the diabetic rats (Figure 3B). The intensity of desmin, a marker of glomerular podocyte injury, was markedly increased in the diabetic rats as compared with the non-diabetic control rats.
GW610742 treatment effectively improved podocyte injury (Figure 3C and D). However, the calculated glomerular volume and mesangial expansion assessed by the glomerular matrix index, which were markedly increased in the untreated diabetic rats compared with the control rats, were not changed by treatment with GW610742 (data not shown).

**PPAR-δ agonist recovers nephrin expression in diabetic kidney tissue**

Nephrin mRNA expression was markedly decreased in the OLETF diabetic rats as compared with the non-diabetic control rats. In the diabetic rats that were treated with GW610742, nephrin expression was effectively restored (Figure 4B). The intensity of nephrin was decreased in the diabetic rats as compared with the non-diabetic control rats, as assessed by immunohistochemistry. The administration of GW610742 also significantly restored glomerular nephrin levels in the diabetic rats (Figure 4C).

**The effects of PPAR-δ agonist on CCL2 and macrophage accumulation in diabetic kidney**

CCL2 mRNA expression by real-time RT-PCR was markedly increased in the kidneys in the OLETF diabetic rats as compared with the non-diabetic control rats (Figure 5A). Renal CCL2 protein expression measured with ELISA was also markedly increased in the OLETF diabetic rats as compared with the non-diabetic control rats (Figure 5B). GW610742 treatment effectively inhibited renal CCL2 mRNA expression, but renal CCL2 protein expression is only partially decreased in the diabetic kidneys (Figure 5A and B). The number of ED-1 positive macrophages was negligible in the kidneys of non-diabetic normal control rats. Although the number of renal macrophages looks slightly increased, there was no statistical difference among three groups by ANOVA test (Figure 5C and D).

**Renal expression of PPAR-δ in diabetes**

In the non-diabetic normal control kidney tissue, PPAR-δ was constitutively expressed in both humans (Figure 6A) and rats (Figure 6B). PPAR-δ was also expressed in the podocytes (Figure 6B). Glomerular PPAR-δ expression was markedly decreased in the proteinuric diabetic human and rats as compared with the non-diabetic human and rat tissue, as assessed by immunohistochemistry (Figure 6A and C). However, PPAR-δ expression was not modified by GW610742 in the diabetic rats (Figure 6C and D).

**PPAR-δ ligand increases nephrin expression**

To determine whether PPAR-δ activation directly regulates nephrin gene expression in the kidneys, differentiated mouse podocytes were treated with incremental doses of PPAR-δ agonist GW610742. Podocytes constitutively express moderate levels of nephrin mRNA, and this level increased following the activation of PPAR-δ. Compared with the control, GW610742 markedly increased nephrin mRNA expression in a concentration-dependent manner, as measured using quantitative real-time RT-PCR (Figure 7A). To examine the role of PPAR-δ in nephrin gene regulation in diabetic nephropathy, we investigated the effect of high glucose on nephrin expression in vitro. Nephrin mRNA expression was significantly decreased in response to high glucose in cultured podocytes. PPAR-δ agonist GW610742 markedly prevented high glucose-induced decrease in nephrin expression. GW9662, which is an irreversible inhibitor of PPAR-δ, significantly abrogated GW610742-induced changes of nephrin expression (Figure 7B). These data indicate clearly that nephrin
expression is regulated in the diabetic milieu in a PPAR-δ-dependent manner.

Discussion

We have identified PPAR-δ as a newly recognized participant in the pathogenesis of diabetic kidney disease. Renal PPAR-δ expression appears to be decreased in Type 2 diabetes, and the diabetic rats treated with GW610742, a highly specific agonist for PPAR-δ, were functionally and morphologically protected against diabetic renal injury. This is the first report showing the therapeutic benefit of PPAR-δ agonist GW610742 in albuminuric Type 2 diabetic kidney disease.

We determined the role of PPAR-δ in the development of diabetic renal injury. We firstly confirmed PPAR-δ expression in normal kidney tissue and cultured podocytes and then found that renal PPAR-δ expression was markedly decreased in diabetic rat and human tissues. Although a decrease in the expression of PPAR-δ has been described in the diabetic heart [19], reports of the same condition occurring in kidney tissues are lacking. Until now, only two groups have shown the renal PPAR-δ expression in Type 1 diabetes; however, the results were discordant [7, 20]. Ours is the first to show decreased renal PPAR-δ expression in proteinuric Type 2 diabetic rats and human kidney tissue, which raises the possibility of an important role of PPAR-δ in the protection of kidney injury in Type 2 diabetes. And the fact that the altered PPAR-δ expression in diabetes is associated with albuminuria and well-known diabetes-induced changes, including GBM thickening, podocytopathy and loss of slit diaphragm integrity, support the essential role of PPAR-δ in maintaining urinary filtration barrier integrity.

We also evaluated the therapeutic benefit of PPAR-δ activation on functional and structural diabetic renal injury. GW610742 treatment effectively inhibited albuminuria and diabetic renal injury markers. It also effectively restored diabetes-induced renal morphological changes, including foot process effacement, GBM thickening and distorted slit diaphragms. This is the first study to investigate the effect of this PPAR-δ selective agent on the ultrastructural changes of the diabetic kidney.

Finally, we examined the mechanism underlying the anti-albuminuric effect of the PPAR-δ agonist in diabetes. Nephrin, a major component of the slit diaphragm and in glomerular urinary filtration barrier integrity, was evaluated. Untreated diabetic OLETF rats showed lower nephrin protein expression than non-diabetic controls, as we expected [21, 22]. Interestingly, we found that PPAR-δ activation restores nephrin expression in diabetic kidneys. Recently, Miglio et al. [5] showed that a PPAR-δ agonist increases nephrin expression in podocytes after oxygen/glucose deprivation-reoxygenation in vitro. The
Fig. 3. Podocyte injury. Representative electron photomicrographs show where podocyte slit pore (arrowhead) density is decreased in diabetes, and its prevention in the GW610742-treated diabetic rats is evident by visual inspection (A). As assessed by electron microscopy, the number of slit pores between the podocyte foot processes is expressed per millimeter length of GBM to arrive at the mean numerical density for each of the three groups of rats. The diabetic rats had decreased slit pore density, but these changes were significantly prevented by treatment with GW610742. The data are displayed as the slit pore density mean ± SEM as evaluated in at least 10 glomeruli from each rat (B). The intensity of desmin, a marker of glomerular podocyte injury, was markedly increased in the diabetic rats as compared with the non-diabetic control rats, and GW610742 effectively improved podocyte injury (C and D). The overall P value from ANOVA is shown in the graph. *P < 0.001 versus non-diabetic control rats, †P < 0.005 versus untreated diabetic rats.
relationship between PPAR-δ and nephrin has never been previously evaluated in vivo, although it has been published in other isoforms of PPAR, such as PPARα or PPARγ [23, 24]. One important and interesting finding in this study is that PPAR-δ regulates podocyte function and morphology. The data presented here, that PPAR-δ is expressed constitutively in the podocytes and PPAR-δ agonist increases nephrin as PPAR-δ target gene expression in cultured podocytes, suggests that PPAR-δ is functionally active in podocytes and regulates podocyte

**Fig. 4.** Nephrin mRNA and protein expression. (A) Nephrin mRNA expression as measured by real-time RT-PCR was markedly decreased in the OLETF diabetic rats as compared with the non-diabetic control rats. In the diabetic rats that were treated with GW610742, the nephrin mRNA expression was recovered. (B) The nephrin protein by western immunoblotting. The densitometric ratio of nephrin to β-actin was decreased in the diabetic rats as compared with the non-diabetic control rats. The nephrin protein was recovered successfully by GW610742 treatment in diabetic rats. Representative western blots of nephrin and β-actin are also shown. The overall P value from ANOVA is shown in the graph. *P < 0.05 versus non-diabetic control rats, †P < 0.05 versus untreated diabetic rats. (C) The nephrin protein was analyzed by immunohistochemistry. Immunodetectable nephrin was markedly decreased in the OLETF diabetic rats as compared with the non-diabetic control rats. However, in the diabetic rats that were treated with GW610742, the nephrin expression was effectively restored.
function and morphology. And the production of the nephrin gene was decreased in response to high glucose in cultured podocytes. Treatment with PPAR-δ agonist was markedly prevented high glucose-induced decrease in nephrin expression in vitro. Also PPAR-δ antagonist abolished the effect of PPAR-δ activation on nephrin. These findings suggest that PPAR-δ is an endogenous regulator of nephrin gene expression.

After ligand binding, PPAR-δ acts by modifying their shape and binding with the specific DNA consensus sequence known as peroxisome proliferator-responsive element. As peroxisome proliferator-responsive element occurs in the promoter region of a nephrin gene [23], in the present study, PPAR-δ agonist GW610742 increased nephrin gene transcription via the peroxisome proliferator-responsive element in the promoter region of the nephrin gene, which discloses a novel mechanism of renoprotection for the PPAR-δ agonist.

PPAR-δ might also exert direct and indirect anti-inflammatory activities. This study shows that the renal

Fig. 5. Renal CCL2 expression and macrophage accumulation. (A) CCL2 mRNA expression by real-time RT-PCR was markedly increased in the kidneys of the OLETF diabetic rats as compared with the non-diabetic control rats. GW610742 treatment restored renal CCL2 levels in the diabetic kidney. (B) Renal CCL2 protein measured using ELISA was markedly increased in the OLETF diabetic rats as compared with the non-diabetic control rats. GW610742 treatment decreased renal CCL2 levels in the diabetic kidney, although the change was not statistically significant. (C and D) The number of ED-1 positive macrophages was counted in the kidneys. The accumulation of glomerular macrophages was reduced in the GW610742-treated diabetic rats as compared with the untreated diabetic rats, although the change was not statistically significant. The overall P value from ANOVA is shown in the graph. *P < 0.05 versus non-diabetic control rats, †P < 0.05 versus untreated diabetic rats.
Fig. 6. Glomerular expression of PPAR-δ. (A) In non-diabetic normal human kidney tissue, PPAR-δ is expressed in glomerular cells, including podocytes. Renal PPAR-δ expression was markedly decreased in diabetic human kidney tissue as compared with non-diabetic human kidney tissue, as assessed by immunohistochemistry. (B) PPAR-δ was constitutionally expressed in the normal rat kidney tissues and cultured mouse podocytes. (C) PPAR-δ expression by immunohistochemistry was markedly decreased in the diabetic renal glomeruli as compared with the non-diabetic rats, and PPAR-δ expression was not modified by GW610742 treatment. (D) PPAR-δ expression was markedly decreased in the diabetic renal cortex as compared with the non-diabetic rats, as assessed by immunoblotting, and PPAR-δ expression was not modified by GW610742 treatment. The overall P value from ANOVA is shown in the graph. *P < 0.05 versus control.
CCL2 mRNA and protein expression is markedly increased in diabetic rats as compared with the non-diabetic control rats. And GW610742 treatment effectively inhibited renal CCL2 mRNA expression in diabetes, although GW610742 did not affect ED1 immunostaining. This finding is also consistent with our in vitro podocyte culture study [18], which showed that CCL2 causes podocytes to increase permeability to albumin, migrate and change cytoskeleton. We did not measure blood pressure during the experiment, although blood pressure is important in the development of diabetic kidney injury. However, we anticipate the effect of PPAR-δ on BP might be negligible as Takata et al. [25] showed that the administration of the PPAR agonist GW0742 (1 or 10 mg/kg) did not alter blood pressure.

In conclusion, the deficiency of renal PPAR-δ in a model of Type 2 diabetic nephropathy likely contributes to the aggravation of albuminuria and the pathologic changes observed in this model. PPAR-δ activation by GW610742 ameliorates diabetic albuminuria by preventing diabetes-induced nephrin loss and restoring podocyte integrity, implying that GW610742 may be a potential therapeutic agent for diabetic nephropathy.

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Conflict of interest statement. None declared.

References
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Fig. 7. (A) PPAR-δ ligand increases nephrin expression in cultured podocytes. Differentiated mouse podocytes were treated with incremental doses of PPAR-δ ligand GW610742 for 24h. Compared with the control, GW610742 markedly increased nephrin mRNA expression, as assessed by real-time RT-PCR (n=3). *P<0.05 versus control. †P<0.05 versus 5μM of GW6107842. (B) Podocytes were treated with 30 mM D-glucose (5.6 mM of D-glucose as a normal glucose control) for 24h in the presence or absence of the PPAR-δ ligand GW610742 and/or GW6662, which is an irreversible inhibitor of PPAR-δ. PPAR-δ inhibition attenuated PPAR-δ agonist-induced upregulation of nephrin (n=3). *P<0.05, †P<0.001.
Urine podocin:nephrin mRNA ratio (PNR) as a podocyte stress biomarker

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Abstract
Background. Proteinuria and/or albuminuria are widely used for noninvasive assessment of kidney diseases. However, proteinuria is a nonspecific marker of diverse forms of kidney injury, physiologic processes and filtration of small proteins of monoclonal and other pathologic processes. The opportunity to develop new glomerular disease biomarkers follows the realization that the degree of podocyte depletion determines the degree of glomerulosclerosis, and if persistent, determines the progression to end-stage kidney disease (ESKD). Podocyte cell lineage-specific mRNAs can be recovered in urine pellets of model systems and in humans. In model systems, progressive glomerular disease is associated with decreased nephrin mRNA steady-state levels compared with podocin mRNA. Thus, the urine podocin:nephrin mRNA ratio (PNR) could serve as a useful progression biomarker. The use of podocyte-specific transcript ratios also circumvents many problems inherent to urine assays.

Methods. To test this hypothesis, the human diphtheria toxin receptor (hDTR) rat model of progression was used to evaluate potentially useful urine mRNA biomarkers. We compared histologic progression parameters (glomerulosclerosis score, interstitial fibrosis score and percent of podocyte depletion) with clinical biomarkers [serum creatinine, systolic blood pressure (BP), 24-h urine volume, 24-h urine protein excretion and the urine protein:creatinine ratio (PCR)] and with the novel urine mRNA biomarkers.

Results. The PNR correlated with histologic outcome as well or better than routine clinical biomarkers and other urine mRNA biomarkers in the model system with high specificity and sensitivity, and a low coefficient of assay variation.

Conclusions. We concluded that the PNR, used in combination with proteinuria, will be worth testing for its clinical diagnostic and decision-making utility.

Keywords: glomerular disease; podocyte; proteinuria; urine biomarker; urine podocin:nephrin mRNA ratio

Introduction

An ideal biomarker for prevention of progression of glomerular diseases will predict who will progress to end-stage kidney disease (ESKD) (unless effective intervention is implemented) and who will not progress (and therefore does not need expensive monitoring or exposure to