CALL FOR PAPERS | Chronic Kidney Disease and Fibrosis

Proximal tubule PPARα attenuates renal fibrosis and inflammation caused by unilateral ureteral obstruction

Shenyang Li,1,3 Nithya Mariappan,1,3 Judit Megyesi,1,3 Brian Shank,1,3 Krishnaswamy Kannan,2 Sue Theus,3 Peter M. Price,1,3 Jeremy S. Duffield,4 and Didier Portilla1,3

1Division of Nephrology, University of Arkansas for Medical Sciences, Little Rock, Arkansas; 2Division of Rheumatology, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas; 3Central Arkansas Veterans Healthcare System, Little Rock, Arkansas; and 4Division of Nephrology, Departments of Internal Medicine and Pathology, Center for Lung Biology, Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, Washington

Submitted 31 May 2013; accepted in final form 18 June 2013

Li S, Mariappan N, Megyesi J, Shank B, Kannan K, Theus S, Price PM, Duffield JS, Portilla D. Proximal tubule PPARα attenuates renal fibrosis and inflammation caused by unilateral ureteral obstruction. Am J Physiol Renal Physiol 305: F618–F627, 2013. First published June 26, 2013; doi:10.1152/ajprenal.00309.2013.—We examined the effects of increased expression of proximal tubule peroxisome proliferator-activated receptor (PPARα) in a mouse model of renal fibrosis. After 5 days of unilateral ureteral obstruction (UUO), PPARα expression was significantly reduced in kidney tissue of wild-type mice but this downregulation was attenuated in proximal tubules of PPARα transgenic (Tg) mice. When compared with wild-type mice subjected to UUO, PPARα Tg mice had reduced mRNA and protein expression of proximal tubule transforming growth factor (TGF)-β1, with reduced production of extracellular matrix proteins including collagen 1, fibronectin, α-smooth muscle actin, and reduced tubulointerstitial fibrosis. UUO-mediated increased expression of microRNA 21 in kidney tissue was also reduced in PPARα Tg mice. Overexpression of PPARα in cultured proximal tubular cells by adenoviral transduction reduced aristolic acid-mediated increased production of TGF-β, demonstrating PPARα signaling reduces epithelial TGF-β production. Flow cytometry studies of dissociated whole kidneys demonstrated reduced macrophage infiltration to kidney tissue in PPARα Tg mice after UUO. Increased expression of proinflammatory cytokines including IL-1β, IL-6, and TNF-α in wild-type mice was also significantly reduced in kidney tissue of PPARα Tg mice. In contrast, the expression of anti-inflammatory cytokines IL-10 and arginase-1 was significantly increased in kidney tissue of PPARα Tg mice when compared with wild-type mice subjected to UUO. Our studies demonstrate several mechanisms by which preserved expression of proximal tubule PPARα reduces tubulointerstitial fibrosis and inflammation associated with obstructive uropathy.

Peroxisome proliferator-activated receptor; transforming growth factor-β; interleukin-10

RECENT STUDIES SUPPORT the notion that patients who develop acute kidney injury (AKI) are at higher risk of developing chronic kidney disease (CKD) (15, 26). The mechanisms that lead to progression from AKI to CKD in humans are not entirely clear, but available studies using numerous animal models suggest that the development of progressive interstitial fibrosis, diminished capillary density, and increased levels of angiotensin II, among other factors, represent mechanistic pathways by which AKI can lead to CKD (6). There is increased evidence suggesting that a sub-family of nuclear receptor transcription factors, known as peroxisome proliferator-activated receptors (PPARs), reduces inflammation and ameliorates tissue fibrosis, but the mechanisms involved in this cytoprotective response have not been elucidated (16, 39, 42, 44). PPARs are metabolic regulators with anti-inflammatory properties in various organs including the kidney (41). Our previous work using PPARα ligands and PPARα transgenic (Tg) mice demonstrated that preserving intact function of proximal tubule epithelial PPARα is cytoprotective during AKI (22–24, 32). PPARα is a nuclear receptor transcription factor expressed predominantly in the proximal tubule and the thick ascending limb of Henle that plays an important role in the modulation of energy utilization through the regulation of fatty acid oxidation (FAO) and oxidant production in mitochondria and peroxisomes (17, 27, 36). The importance of PPARα in modulating FAO by peroxisomes and mitochondria in kidney tissue is underscored by the response of renal PPARα to dietary lipids and by the cytoprotective effects of increasing its expression in the proximal tubule during AKI (23, 24, 35).

In the present study, we extended previous observations made in our laboratory and compared wild-type with PPARα Tg mice using the model of unilateral ureteral obstruction (UUO)-mediated renal fibrosis to define potential cellular mechanisms by which increased expression of proximal tubule PPARα reduces tubulointerstitial fibrosis. Our studies suggest that activation of proximal tubule PPARα may represent a novel therapeutic target to reduce renal fibrosis and ameliorate progression to CKD.

METHODS

Generation of proximal tubule PPARα Tg mice. Proximal tubule-specific expression of PPARα under the control of the androgen-sensitive KAP (kidney androgen-regulated protein) promoter was achieved with KAP2-PPARα Tg mice, which were generated and characterized as previously described (23). Eight- to ten-week-old, age- and weight-matched PPARα Tg and wild-type (C57 BL/6) female mice were used for the studies. To investigate the effects of
increased expression of proximal tubule PPARα, wild-type and PPARα Tg mice received a 5-mg subcutaneous testosterone pellet (Innovative Research of America, Sarasota, FL) and after 9 days were subjected to surgery (see scheme of experimental time course in Fig. 1A). The animals were housed at the Veterinary Medical Unit at the Central Arkansas Veterans Healthcare System (Little Rock, AR). When appropriate, animals were painlessly euthanized according to methods of euthanasia approved by the Panel on Euthanasia of the American Veterinary Medical Association. Our animal study protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Central Arkansas Veterans Healthcare System.

Renal fibrosis model of UUO. KAP2-PPARα Tg and wild-type mice, 8–10 wk old, were assigned to four treatment groups. Either UUO or sham surgeries were performed on both wild-type and PPARα Tg mice. All four groups of animals received 5-mg (21-day release) subcutaneous testosterone pellets 9 days before surgery. The left kidney was exposed through a midline incision under sterile conditions; the ureter was dissected and securely tied at two places with 6–0 silk sutures. Volume depletion was prevented by administration of −0.1 ml saline into the peritoneal cavity. The midline incision was closed; the mice were returned to their cages and allowed free access to food and water for 5 days. As control, sham surgery was performed the same way as UUO without tying the ureter. After 5 days, the mice were euthanized and the left kidneys from UUO and sham mice were collected for protein, RNA isolation, and histological evaluation.

Gene expression studies. PPARα, transforming growth factor–β1 (TGF-β1), α-smooth muscle actin (α-SMA), collagen type I, α1 (Col1A1), fibronectin (Fn), collagen type IV α1 (Col4A1), laminin β, interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), arginine 1, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), CD86 mRNA levels, and miRNA21 (miR21) mRNA levels were determined by quantitative RT-PCR. Total RNA was extracted from cells or mouse kidney tissue and treated with RNase-free DNase before RT reaction. Real-time PCR was carried out using the StepOnePlus real-time PCR system (Invitrogen, Foster City, CA) with iTaqSYBR Green Supermix with Rox (Bio-Rad, Hercules, CA). In each experiment, triplicates of 50-ng cDNA (total RNA equivalent) samples were amplified in a 20-μl reaction. Specificity of the amplified product was confirmed by melting curve analysis and agarose gel electrophoresis. For relative quantification, a standard curve was generated from a six-step cDNA dilution series. Samples were amplified with primers for PPARα, TGF-β1, α-SMA, Col1A1, Fn, Col4A1, laminin β, IL-1β, IL-6, IL-10, TNF-α, arginine 1, MCP-1, MIP-1α, CD86, miR21, and 18S RNA. The relative expression of genes was calculated from the standard curve. Relative quantity was calculated by the ratio of the gene-specific and the appropriate 18S rRNA expression. The primer sequences in the RT-PCR were the following: for PPARα, 5′-AAA GAG GCA GAG GTC CGA TT-3′ (forward), 5′-AGC AAG GTG ACT TGG TCG TT-3′ (reverse); for TGF-β1, 5′-CGA GGC GGT GCT GGT TTG-3′ (forward), 5′-CAT AGA TGG CGT TGT GTC GGT CCA-3′ (reverse); for α-SMA, 5′-TCTG ACG TCA GGA ACC ATG CTA CG-3′ (forward), 5′-ATC TTC ACC ACC AGC AGA GCC GGC G-3′ (reverse); for fibronectin 1, 5′-TCC ACA GAC ATT CCT GCC CGC-3′ (forward), 5′-GTT CAC CAC CCG GTA GC-3′ (reverse); for Col1A1, 5′-GCC CCA AGG AGC TCT CCG GTG CTT-3′ (forward), 5′-AGC AGG GCC GGT ACC AGG-3′ (reverse); for Col4A1, 5′-GTT ATT CAG GGA GAC CGT GG-3′ (forward), 5′-ACC TTG GTG CAC CCC TAG AT-3′ (reverse); for laminin β, 5′-CTA CTG TAA GGC CCT GTG GA-3′ (forward), 5′-GCT CGG CTG GAG TAT TGT GAG TCA-3′ (reverse); for IL-1β, 5′-GAC CCC AAA AAG TGA AGG GCT-3′ (forward), 5′-GCT GGT GCT GCG AGA TTA GGT GA-3′ (reverse); for IL-6, 5′-TCC GGA GAG GAC ACT TCA CA-3′ (forward), 5′-TTG...
CCA TTG CAC AAC TCT TTT CT-3' (reverse); for IL-10, 5'-GCA TGG CCC AGA AAT CAA GG-3' (forward), 5'-AGG GGA AAA ATC GAT GAC AGC-3' (reverse); for TNF-α, 5'-ATG GCC TCC CTC TCA TCA GT-3' (forward), 5'-CTT GGT GTT TGT TCA CTA CG-3' (reverse); for arginase 1, 5'-GGG GAC TAT CCT CAG TTC CAG GTC AGT-3' (reverse); for MCP-1, 5'-AGG TGT TTG AGT TAT TTC CAG GTC AGT-3' (reverse), 5'-GAC TTT ACT GTA GGG CTT GAC AGT-3' (forward); for IL-10, 5'-GGG AGG TGT CAT TTT CCT GAC TA-3' (forward), 5'-AGG TTC GTT GTT TCA CTT ACT GCC-3' (reverse); for IL-10, 5'-AGG AGT GGG CTT GGT TCA AGT-3' (forward), 5'-AAC GCC CAT GCA CCA CCA CC-3' (reverse). Quantitative PCR for miR21 or the housekeeping small nuclear RNA Smo234 was performed using the reverse universal primer specific to the annealed poly(A) tail and the specific forward primer to the miR21 (5'-CTT GGT GGT TTG CTA CGA CTT-3').

Protein expression by Western blotting. Control and experimental (UUO) kidneys were lysed in 1× lysis buffer (Cell Signaling) containing 20 mM Tris HCl (pH 7.5), 150 mM NaCl, 1 mM Na2 EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1× protease inhibitor cocktail (Calbiochem, San Diego, CA), and 1 mM PMSF. Protein concentration was measured with the BioRad Protein Assay Kit (Pierce, Rockford, IL). After protein concentration was determined, 70 μg protein per lane were loaded with 4× SDS loading buffer, resolved on 15% polyacrylamide (PAGE) gel (Bio-Rad), and transferred onto a PVDF membrane using a Bio-Rad mini-blot apparatus. The membranes were blocked in 5% nonfat milk solution for 1 h and then incubated with primary antibodies against TGF-β1 (dilution 1:1,000; cat. no. SC-146, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Membranes were washed extensively, incubated with anti-rabbit secondary antibodies (1:5,000 dilution; Abcam, Cambridge, MA), and then washed again. Detection was performed with the Amersham ECL Western blotting analysis kit (Thermo Scientific, West Palm Beach, FL). The membranes were stripped and then probed with GAPDH as loading control. Signals on the blots were visualized by autoradiography and quantified by densitometry using the ImageQuant image analysis system (Agfa Duoscan HID Scanner; Hamrick Software, Phoenix, AZ).

In situ determination and quantitative analysis of fibrotic markers in kidney tissues. Formalin-fixed, paraffin-embedded 5-μm kidney sections from sham and UUO kidneys were stained with picro-sirius red for 1 h. Positive collagen staining in the interstitium was detected by light microscopy using circularly polarized light. Photographs from the entire cross-sections of kidneys were analyzed by Image J software (http://rsbweb.nih.gov/ij/download.html) to quantify the collagen accumulation. Five-micrometer sections from the same kidneys were stained with periodic acid Schiff reagent and used to evaluate the basement membrane could also be seen. PPARα expression ameliorates UUO-induced morphological damage. Sham-operated kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes. Five days after UUO, PPARα expression was reduced by ~75% in wild-type mice. Although it was also reduced in PPARα Tg mice subjected to UUO, the level of expression was similar to that observed in sham-operated wild-type mice. The effect of PPARα expression on UUO-induced tubular damage was further examined by measuring tubular lumen diameter and number of tubules per field. As shown in Figure 1B, PPARα expression significantly increased the number of tubular lumen diameters, indicating a protective effect against UUO-induced tubular damage.

Results

PPARα expression downregulated after UUO is attenuated in PPARα Tg mice. PPARα expression was increased in the Tg mice by 2.3-fold (Fig. 1A) in sham-operated mice when compared with wild-type mice. Five days after UUO, PPARα expression was reduced by ~75% in wild-type mice. Although it was also reduced in PPARα Tg mice subjected to UUO, the level of expression was similar to that observed in sham-operated wild-type mice. Increased proximal tubule PPARα expression ameliorates UUO-induced morphological damage. Sham-operated kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes. Five days after UUO, kidneys from wild-type mice showed significant dilation with no tubular dilation or interstitial changes. Five days after UUO, kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes. Five days after UUO, kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes. Five days after UUO, kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes.
Increased proximal tubule PPARα expression reduces UUO-mediated increased expression of fibrogenic genes. We found that Tgfβ1, Coll1a1, Acta2 (α-SMA), and Fn1 transcripts were upregulated by UUO in wild-type mice. There was a 4.1-, 10.4-, 12.6-, and 10.1-fold increased level of mRNA expression for Tgfβ1, Coll1a1, Acta2, and Fn1, respectively (P < 0.001), after 5 days of UUO compared with sham-operated mice, as shown in Fig. 2A. Prior overexpression of PPARα in the Tg mice significantly attenuated the UUO-induced upregulation of Tgfβ1, Coll1a1, Acta2, and Fn1 transcripts when compared with wild-type mice (P < 0.05).

TGF-β1 protein expression increased sevenfold in wild-type mice 5 days after UUO. This increased expression was significantly reduced in PPARα Tg mice when compared with wild-type mice (Fig. 2B). Formalin-fixed, paraffin-embedded kidney sections were also used for the in situ localization of TGF-β1. Indistinguishable positive immune reaction could be detected in medullary and cortical thick ascending limbs in both wild-type and Tg kidneys. Five days after UUO showed a strong immunostaining for TGF-β in cortical thick ascending limbs, but also a positive staining could be seen in the basolateral surfaces of convoluted proximal tubules (S1-S2 segments) of the wild-type kidneys. The positive TGF-β1 immunoreaction was almost completely diminished from the PPARα Tg mouse kidneys 5 days after UUO. Only occasional weak staining could be found in some cortical thick ascending limbs. There was no obvious staining in proximal tubule segments in the Tg UUO group (Fig. 2C).

Proximal tubule PPARα reduces UUO-mediated interstitial fibrosis. To quantify the amount of collagen produced in the interstitium in wild-type and PPARα Tg mice subjected to UUO, kidney sections were stained with picro-sirius red staining. Positive red stain in the interstitium was increased 5 days after UUO in wild-type kidneys compared with sham ones (P < 0.05), and it was significantly reduced (P < 0.05) in

Fig. 2. A: effect of UUO on renal fibrogenic gene transcripts transforming growth factor (TGF)-β1, collagen type I, α1 (Col1A1), α-smooth muscle actin (SMA), and fibronectin. Bars represent means ± SE mRNA levels for at least 4 mice in each group. *P < 0.001 when comparing WT sham vs. WT-UUO mice. ‡P < 0.05 when comparing WT UUO vs. PPARα Tg UUO mice. B: TGF-β1 protein expression in kidney tissue of WT and PPARα Tg mice subjected to sham and 5 days UUO. B: densitometry and quantification of TGF-β1 signals, normalized to GAPDH levels from Western blot analysis; GAPDH was used as a loading control. Data are expressed as means ± SE. *P < 0.005 when comparing WT sham vs. WT UUO mice. ‡P < 0.005 when comparing WT UUO vs. PPARα Tg UUO mice by unpaired Student’s t-test. C: representative photomicrographs of TGF-β1 immunostaining in WT and Tg mice 5 days after UUO. Positive staining is obvious in the cortical thick ascending limbs and proximal convoluted tubules of WT animals. The positive staining is reduced from Tg UUO kidney. *, thick ascending limb of loop of Henle; PT, proximal convoluted tubules. Magnification: ×244.
PPARα Tg mice as shown in Fig. 3, A and B, indicating that PPARα upregulation ameliorated UUO-induced kidney fibrosis.

**Increased proximal tubule PPARα reduces UUO-mediated increased miR21 expression.** miR21 directly suppresses expression of PPARα and silences other genes in the PPARα downstream signaling pathway in mitochondria and peroxisomes (5). As expected, miR21 expression determined by qPCR was significantly upregulated after UUO (3.5-fold) in wild-type mice ($P < 0.05$). Overexpression of PPARα in the kidney tubule after UUO, however, reduced miR21 levels close to those in normal kidneys (Fig. 4A). This observation was recapitulated in vitro in TKPTS cells injured by the tubular cell toxin AA (see Fig. 4B).

![Figure 3](https://example.com/figure3.png)

Fig. 3. A: representative photomicrographs of picro-sirius red-stained sham and 5 days UUO kidney sections from WT and Tg animals. Collagen accumulation can be seen, indicated by the red staining in the WT UUO section. No significant positive staining was seen in shams and Tg UUO kidneys. Magnification: x122. B: quantitative analysis of picro-sirius red staining in kidney sections from sham and 5 days UUO WT and Tg animals. Collagen accumulation was significantly increased in WT UUO kidneys, and it was unchanged in the Tg UUO samples compared with sham-operated ones. *$P < 0.05$ when comparing WT sham vs. WT UUO mice. ‡$P < 0.05$ when comparing WT UUO vs. Tg UUO mice in unpaired Student’s $t$-test.

![Figure 4](https://example.com/figure4.png)

Fig. 4. PPARα repressed UUO-mediated upregulation of miRNA21 (miR21) in kidney tissue and also reduces aristolochic acid (AA)-stimulated miR21 expression in TKPTS cells. Level of miR21 mRNA was determined by qPCR. A: quantitative analysis of miR21 expression of WT and PPARα Tg mice subjected to sham and UUO surgery. *$P < 0.05$ when comparing changes in miR21 expression between sham sham and WT UUO mice. ‡$P < 0.05$ when comparing changes in miR21 expression between WT UUO and Tg UUO mice in unpaired Student’s $t$-test. B: TKPTS cells were incubated with PPARα adenovirus for 18 h before being treated with AA and grown for an additional 24 h. Bars represent means ± SE mRNA levels for at least 4 independent experiments in each group. *$P < 0.05$ when comparing untreated cells (control) vs. AA-treated cells. ‡$P < 0.05$ when comparing AA-treated cells vs. AA in the presence of PPARα (AA + PPARα) in unpaired Student’s $t$-test.
injured epithelial cells are believed to secrete increased tubule basement membrane proteins including collagen IV and laminin. To evaluate the effect of PPARα on cultured epithelial cells, we overexpressed PPARα in TKPTS cells by adenoviral transduction and injured epithelial cells with AA. Whereas AA stimulated epithelial TGF-β production, as well as degradation of collagen I, collagen IV, and laminin, PPARα overexpression blunted these effects. These results suggest that PPARα reduces tubular epithelial cell injury by preventing AA-mediated degradation of tubule basement membrane (Fig. 5).

Increased proximal tubule PPARα reduces infiltration of kidney tissue macrophages. Single cell preparations from whole kidney were initially analyzed by flow cytometry for CD45+, 7-AAD-negative cells to identify the total live leukocyte population. A significant increase in the total CD45+ population indicated a major influx of leukocytes into kidneys was seen as early as 48 h but persistent accumulation was seen after 5 days of UUO in wild-type mice (Fig. 6, A–B), whereas the sham-operated mice did not show similar perturbation. These results were confirmed by Image Stream X flow cytometry (data not included). After ascertaining that the mononuclear phagocytes were one of the major infiltrating cells in the UUO kidney, we investigated the role of PPARα-mediated protection by flow cytometry analysis of inflammatory mononuclear cells. CD45+/CD11b+/F4/80+ cells were considered proinflammatory monocyte/macrophages based on previous studies (11). Tg overexpression of PPARα in proximal tubules markedly blunted this response such that leukocyte recruitment was reduced by more than 50% as shown in Fig. 6, A and B.

PPARα increases IL-10 expression and suppresses renal UUO-induced proinflammatory cytokines/chemokines IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α. To investigate the mechanisms by which increased expression of proximal tubule PPARα ameliorated UUO-mediated renal fibrosis, mRNA expression levels of pro- and anti-inflammation cytokines/chemokines were investigated. As shown in Fig. 7, there were 5.2-, 9.7-, 46.9-, 2.1-, 10.6-, and 8.2-fold increases of mRNA expression for IL-1β, TNF-α, IL-6, IL-10, MCP-1, and MIP-1α, respectively (P < 0.001), 5 days after UUO surgery in wild-type mice when compared with sham-operated mice. Overexpression of PPARα in Tg mice significantly attenuated the UUO-induced upregulation of proinflammation cytokines/chemokines IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α mRNA expression when compared with wild-type mice (P < 0.05). Anti-inflammatory cytokine IL-10 was further increased (7-fold) in Tg mice 5 days after UUO surgery compared with wild-type sham mice and was also significantly elevated (P < 0.05) when compared with wild-type UUO mice (Fig. 7B).

PPARα increases expression of arginase-1 and repressed expression of macrophage marker CD86. To study the roles of PPARα on macrophages in UUO-induced renal fibrosis, we examined the mRNA expression of surface markers/receptors of M1/M2 macrophages. As shown in Fig. 7, B and C, there were 9.1- and 3.4-fold increases of mRNA expression for the macrophage markers arginase-1 and CD86, respectively (P < 0.001), 5 days after UUO surgery in wild-type mice when compared with sham mice. Overexpression of PPARα in Tg mice significantly suppressed the UUO-induced upregulation of CD86 mRNA expression when compared with wild-type mice (P < 0.05). In contrast, arginase-1 was further increased (22.5-fold) in Tg mice 5 days after UUO surgery when compared with wild-type sham mice, but arginase-1 levels were further increased when compared with wild-type UUO mice (P < 0.05).

Fig. 5. PPARα inhibited AA-stimulated TGF-β1, Col1A1, Col4A1, and laminin-β mRNA expression in TKPTS cells. Levels of mRNA were determined by qPCR. TKPTS cells were incubated with PPARα adenovirus for 18 h before being treated with AA and grown for an additional 24 h. *P < 0.05 when comparing untreated cells (control) vs. AA-treated cells. †P < 0.05 when comparing AA-treated cells vs. cells treated with AA in the presence of PPARα (AA+PPARα) in unpaired Student’s t-test.
DISCUSSION

Our study is the first to evaluate the role of and mechanisms by which increased expression of proximal tubule PPARα attenuates renal fibrosis. Several important findings are supported by our data. Our studies confirm previous observations demonstrating increased expression of TGF-β in cortical proximal tubules (PCT and PST) of UUO-treated wild-type mice, although there was some level of expression of TGF-β in the thick ascending limb, collecting duct, and also in the interstitial compartment of the obstructed kidney (10). The observed peritubular fibrosis present after 5 days of UUO in wild-type mice was almost absent in the kidney tissue of PPARα Tg mice. Importantly, PPARα Tg mice also produced less proximal tubule TGF-β, which was demonstrated by reduced mRNA, protein, and reduced immunostaining in the basolateral domain of proximal tubules. TGF-β is a pleiotropic cytokine that plays a major role in stimulating extracellular matrix production after UUO, and blocking TGF-β has been shown to reduce tubular apoptosis in several models of chronic kidney injury (25, 30). It contributes to renal fibrosis by several mechanisms including induced loss of phosphatase tensin homolog that contributes to the failure of regenerating epithelial cells to redifferentiate, thereby causing the retention of proliferative signaling and giving rise to profibrotic peptides (19). TGF-β also increases renal fibrosis not only by a direct effect on myofibroblasts but by inducing the production of Notch (1), CTGF (34), and PDGFβ (40). TGF-β also mediates upregulation of osteopontin in injured epithelial cells and triggers a focal inflammatory process with migration of monocytes and macrophages (28). We find that the UUO-mediated increased expression of fibrogenic gene transcripts including Col1A1, α-SMA, and fibronectin in wild-type mice was significantly reduced in PPAR-α Tg mice subjected to UUO. These results indicate that PPARα overexpression in the proximal tubule alone effectively reduces interstitial fibrosis in the model of UUO.

Similar to the in vivo model of UUO, in the in vitro model of fibrosis using AA to injure proximal tubule epithelial cells in
culture, increased expression of PPARα directly reduces production of TGF-β and prevented AA-mediated degradation of collagen 4 and laminin, the major components of tubular basement membrane. In addition to injured tubule epithelium, TGF-β can be produced also by interstitial kidney fibroblasts, macrophages, and endothelium (12). Our findings as well as recently published data (3, 43) support the concept that, during injury, the induction of tubulointerstitial fibrosis occurs as a result of altered cross talk mechanisms between tubular epithelial cells and interstitial fibroblasts. We demonstrated in AKI models (23) that proximal tubule PPARα could increase mitochondrial FAO and reduce oxidant production, and we speculate that these cellular mechanisms could affect the production of TGF-β or its effect on transcription of fibrotic genes. Our in vivo studies do not allow us to examine the cellular mechanisms by which PPARα reduces TGF-β expression; however, a recent study suggests that mitochondrial reactive oxygen species derived from complex III of the mitochondrial electron transport chain are required for TGF-β-mediated transcription of profibrotic genes (18).

Another important observation in the present study is the significant inhibition of UUO-induced miR21 expression in kidney tissue of proximal tubule PPARα Tg mice that is also accompanied by reduced fibrosis. miRNAs are a class of ~20-nucleotide single-strand endogenous RNAs that control the translation of mRNAs by promoting the degradation of target mRNAs or preventing their translation (4, 33). MiRNAs have recently been demonstrated to regulate cell proliferation, differentiation, and apoptosis (29). In a recent study, we described that PPARα expression and activity measured as FAO in kidney tissue were significantly reduced in models of renal fibrosis. In addition, miR21 expression was significantly elevated in mice subjected to an ischemic fibrosing model or UUO (5). Reducing miR21 expression in kidney tissue by using anti-miR21 oligonucleotides or miR21 knockout mice resulted in epithelial cell protection and less interstitial fibrosis in response to kidney injury (5). Moreover, using an array assay we demonstrated that PPARα and many genes regulating fatty acid metabolism are seed-matched targets for miR21 in the kidney indicating that miR21 directly silences PPARα and the downstream signaling pathways mediated by PPARα. The findings here of reduced expression of miR21 when proximal tubule PPARα was increased in Tg mice lend support to the notion of a feedback circuit that modulates miR21 expression in kidney tissue at the transcriptional level via the PPARα/AP1 signaling cascade (45). The reduced expression of miR21...
during UUO also could be explained by reduced expression of TGF-β in PPARα Tg mice. TGF-β receptor-mediated signaling has been shown to directly regulate miRNA biogenesis by several mechanisms including direct gene transcription of miRNA genes, direct binding of TGF-β receptor-induced SMAD proteins to specific miRNAs, and by stabilization of miRNA processing machinery (7, 14). The net effect of TGF-β signaling is to upregulate miRNA biogenesis.

Our studies also show that increased expression of proximal tubule PPARα during UUO is accompanied by reduced infiltration of macrophages. In addition, kidney tissues of PPARα Tg mice had reduced expression of inflammatory cytokines including MCP-1 and MIP-1α, which help recruit macrophages to kidney tissue during UUO. Macrophages and dendritic cells derived from monocytes are abundant in kidney injury and play an important role in inflammation, tissue repair, and fibrosis. Ablative studies in several models of kidney interstitial disease indicate that macrophages significantly contribute to the development of fibrosis (8, 13, 31, 38). Several mechanisms have been proposed to explain monocyte/macroage function. One is that the way monocytes are activated upon entering the diseased kidney dictates their differentiation and function. Another is that monocytes exist as functionally discrete subsets. We also find that increased expression of proximal tubule PPARα markedly reduced production of proinflammatory cytokines (TNFα, IL-6, and IL-1β) and increased the expression of anti-inflammatory cytokine IL-10. Thus, the increased expression of proximal tubule PPARα seems to affect gene expression of CD11b+/F4/80+ cell subpopulations suggesting that renal inflammation involves at least two phenotypically different monocyte/macrophage subpopulations: CD11b+/F4/80+ monocytes showing M1-type activation and CD11b+/F4/80+ IL-10 and arginase-1 producing M2-type macrophages. In a previous study, we described that the anti-inflammatory effect of PPARα ligand in the cisplatin model of AKI was mediated by inhibition of kidney tissue NF-κB and this could be an additional mechanism for the anti-inflammatory effect of PPARα on renal fibrosis (22). Our results are different from the ones published by a group of investigators that showed that using a PPARα ligand was cytoprotective, but they did not find any effects of the PPARα ligand on renal inflammatory markers using a similar model of UUO (2). We speculate that increased expression of proximal tubule PPARα protein in our PPARα Tg mice (23) as opposed to a nonspecific saturating effect of the PPARα ligand could account for the observed differences between the two studies.

In summary, we describe several mechanisms in this study by which increased expression of proximal tubule PPARα reduces inflammation and interstitial fibrosis including reduced production of TGF-β, reduced expression of miR21, and reduced influx of inflammatory macrophages. Together, these mechanisms contribute to reduced extracellular matrix production. Additional studies are needed to further examine the mechanisms by which PPARα affects production of TGF-β in renal epithelium, inflammatory cells, and/or pericytes, as well as the effects of increased expression of PPARα on UUO-mediated macrophage activation, production of IL-10, and the activation of pericytes to myofibroblasts.

GRANTS

D. Portilla’s laboratory is funded by National Institutes of Health Grant DK75976, a Veterans Affairs Merit Award, and a Research Enhancement Award Program award. N. Mariapan was a T32 fellow supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant T32 DK061921.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.L., N.M., J.M., B.S., K.K., P.M.P., and D.P. performed experiments; S.L., N.M., J.M., K.K., P.M.P., and D.P. analyzed data; S.L., N.M., J.M., B.S., K.K., T.P., J.S.D., and D.P. edited and revised manuscript; S.L., N.M., J.M., B.S., K.K., T.P., M.P.F., J.S.D., and D.P. approved final version of manuscript; D.P. conceived and designed research; D.P. drafted manuscript.

REFERENCES


