PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease

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1Education and Research Center of Animal Models for Human Diseases, 2Division of Molecular Genetics, Institute for Comprehensive Medical Science, 3School of Health Sciences, Fujita Health University, Toyoake, Aichi, Japan; 4Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; and 5The Kidney Institute, University of Kansas Medical Center, Kansas City, Kansas

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Yoshihara D, Kurahashi H, Morita M, Kugita M, Hiki Y, Aukema HM, Yamaguchi T, Calvet JP, Wallace DP, Nagao S. PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. Am J Physiol Renal Physiol 300: F465–F474, 2011. First published December 8, 2010; doi:10.1152/ajprenal.00460.2010.—In autosomal recessive polycystic kidney disease (ARPKD), progressive enlargement of fluid-filled cysts is due to aberrant proliferation of tubule epithelial cells and transcapillary fluid secretion leading to extensive nephron loss and interstitial fibrosis. Congenital hepatic fibrosis associated with biliary cysts/dilatations is the most common extrarenal manifestation in ARPKD and can lead to massive liver enlargement. Peroxisome proliferator-activated receptor γ (PPAR-γ), a member of the ligand-dependent nuclear receptor superfamily, is expressed in a variety of tissues, including the kidneys and liver, and plays important roles in cell proliferation, fibrosis, and inflammation. In the current study, we determined that pioglitazone (PIO), a PPAR-γ agonist, decreases polycystic kidney and liver disease progression in the polycystic kidney rat, an orthologous model of human ARPKD. Daily treatment with 10 mg/kg PIO for 16 wk decreased kidney weight (%) of body weight), renal cystic area, serum urea nitrogen, and the number of Ki67-, pERK1/2-, and pS6-positive cells in the kidney. There was also a decrease in liver weight (% of body weight), liver cystic area, fibrotic index, and the number of Ki67-, pERK1/2-, pERK5-, and TGF-β-positive cells in the liver. Taken together, these data suggest that PIO inhibits the progression of polycystic kidney and liver disease in a model of human ARPKD by inhibiting cell proliferation and fibrosis. These findings suggest that PPAR-γ agonists may have therapeutic value in the treatment of the renal and hepatic manifestations of ARPKD.

congenital hepatic fibrosis; peroxisome proliferator-activated receptor; pioglitazone

POLYCYSTIC KIDNEY DISEASES (PKD) are characterized by progressive enlargement of countless fluid-filled cysts in the kidneys, often leading to end-stage renal disease. Autosomal dominant PKD (ADPKD) has an incidence of 1:1,000 and is the most common inherited human renal disease. Progressive enlargement of the kidneys is due to aberrant proliferation of the cyst epithelial cells, together with an accumulation of fluid within the cyst cavities due to transcapillary fluid secretion (10). Hepatic cysts, which are the most common extrarenal manifestation in ADPKD, can originate from biliary microhe- matomas or focal proliferation of biliary ducts and peribiliary glands (2, 18, 25). In ADPKD, the liver may become grossly enlarged by the expansion of cysts and have interstitial fibrosis; however, functional impairment is rare.

Autosomal recessive PKD (ARPKD) is a juvenile form of cystic disease with an incidence of 1:20,000 (10, 11, 39). ARPKD kidneys are characterized by cystic fusiform dilations of the collecting ducts accompanied by increased cell proliferation and fluid secretion, leading to massive kidney enlargement and renal failure within the first few years of life (45, 46). Aberrant cell proliferation, increased fluid secretion, and interstitial fibrosis are also common to cystic liver disease in ARPKD (40). Congenital hepatic fibrosis is common in ARPKD and can lead to significant clinical liver complications (40).

Two key signaling pathways, cyclic AMP (cAMP)-activated B-Raf/MEK/ERK (20, 24, 32, 50, 51) and AKT/mTOR/S6K/S6 (8, 9, 26, 33, 34, 41, 42), have been implicated in renal cyst growth and are associated with PKD progression in humans and animal models. In the polycystic kidney (PKC) rat, an orthologous model of human ARPKD, inhibition of renal cAMP production by treating with a vasopressin V2 receptor antagonist or by increasing water intake to reduce plasma vasopressin decreased cell proliferation and ameliorated cystogenesis with an associated reduction of B-Raf/MEK/ERK activity and led to improved renal function (21, 47). Inhibition of the Akt/mTOR/S6K/S6 pathway by rapamycin treatment reduced renal cyst growth in pcy mice, a model of nephronophthisis; in Han:SPRD Cy rats, a model of ADPKD; and in orthologous conditionally targeted Pkd1 mice (9, 34, 37, 42); however, the results of rapamycin treatment were not as definitive in PCK rats and in human clinical trials (28, 31, 44, 49).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor family. PPAR-γ, one of PPARs that is activated by naturally occurring fatty acids or fatty acid derivatives, is widely expressed in a number of tissues, including the kidneys and liver (7). Several reports showed that PPAR-γ activation has a crucial role in growth inhibition, cell cycle arrest, and induction of apoptosis in cancer cells (1). PPAR-γ agonists have been shown to affect renal fibrosis and inflammation and hepatic regeneration (13, 52). In previous reports, treatment with pioglitazone (PIO), a PPAR-γ agonist, improved survival and ameliorated cardiac defects and the degree of renal cystogenesis in embryos of Pkd1−/− mice (19). Also, long-term treatment with PIO improved endothelial function by increasing production of nitric oxide in adult heterozygous Pkd1+/− mice (19). Rosiglitazone,
another PPAR-γ agonist, was shown to attenuate PKD progression and to prolong survival in Han:SPRD Cy rats (3).

In the current study, we tested the effects of PIO on polycystic kidney and liver disease in PCK rats, an orthologous model of human ARPKD. The results demonstrate for the first time that PIO significantly reduces kidney disease progression in association with inhibition of ERK and mTOR signaling pathways, and significantly reduces liver disease progression in association with inhibition of the ERK and TGF-β signaling pathways in a model of human ARPKD.

METHODS

PCK rat and study design. PCK rats were originally derived from a strain of Sprague-Dawley rats in Japan. PKD in these rats is caused by a splicing mutation with subsequent skipping of exon 36 and a frameshift in the orthologous Pkhd1 gene (48). PCK rats are characterized by renal cysts derived from collecting ducts and congenital hepatic fibrosis associated with biliary cysts (17, 21). The main cause of death in PCK rats is renal insufficiency. The life span is ~1 yr in males and 1.5 yr in females. Descendants of this colony have been maintained at the Education and Research Center of Animal Models for Human Diseases, Fujita Health University. PCK rats and normal Sprague-Dawley rats (+/+) were randomly assigned to one of two groups: treatment with 10 mg/kg PIO (Takeda Pharmaceutical Limited, Osaka, Japan) or vehicle control (0.5% DMSO) by gavage every day from 4 to 20 wk of age. The protocol for the use of these animals was approved by the Animal Care and Use Committee at Fujita Health University.

Food and water intake were measured in 19-wk-old rats placed in metabolic cages for 24 h. At 20 wk of age, rats were anesthetized with pentobarbital sodium (Schering-Plough, Kenilworth, NJ), and the kidneys and liver were removed rapidly, causing lethal exsanguination. Body weight, total kidney weight, and liver weight were measured. Blood samples were collected for measurements of serum urea nitrogen (SUN), a marker of kidney function, and serum glucose, aspartate amino transferase (AST), and total bilirubin, markers of liver function. Half of the left kidney and 0.5 g of liver were homogenized in TLB lysis buffer to extract proteins.

![Fig. 1. Effect of pioglitazone (PIO) in the kidney. Male and female polycystic kidney (PCK) and +/+ rats (n = 5 per group) received 10 mg/kg PIO or control vehicle (0.5% DMSO) [CONT (vehicle)] by gavage every day from 4 to 20 wk of age. Kidney weights (A) were represented as a percent of total body weight (F). Serum urea nitrogen (SUN) was measured as a renal function marker (B). Representative kidney sections from PCK male rats were stained with hematoxylin eosin (C: control vehicle-treated kidney; D: PIO-treated kidney). Cystic area (E) and fibrotic index (G) were determined from representative kidney sections by morphometric analysis. Comparison between vehicle-treated and PIO-treated PCK and +/+ rats (female or male), *P < 0.05, **P < 0.01.](http://ajprenal.physiology.org/Downloadedfrom/AJP-Renal-Physiol-VOL300-FEBRUARY2011-www.ajprenal.org)
Half of the right kidney and a part of the right medial liver lobe were embedded in 4% carboxy methyl cellulose (FINETEC, Tokyo, Japan) after fixation in 4% paraformaldehyde (4% PFA) for 60 min at 4°C and sequential incubations in 10, 20, and 30% sucrose for 30, 60, and 120 min, respectively, at 4°C and were sectioned for immunofluorescence staining. The other half of the right kidney and a part of the right medial liver lobe were immersed in 4% PFA, embedded in paraffin, and sectioned for immunohistochemistry.

Measurement of SUN, blood sugar, AST, and total bilirubin levels. SUN measurements were performed using a colorimetric assay using a urease-indophenol method (Wako Pure Chemicals, Osaka, Japan). Blood sugar level determinations were performed using the mutarotase-glucose oxidase method from a commercial kit (Glucose CII-B-test; Wako). Total bilirubin levels were determined by an enzymatic method using bilirubin oxidase (Mitsubishi Chemical Medience, Tokyo, Japan). Blood sugar level, SUN, AST, and total bilirubin levels were expressed as means ± SE (n = 5 rats per treatment group per gender).

Cyst index and fibrosis index. Cystic area was measured from five random fields (×100 magnification) of hematoxylin eosin (HE)-stained sections of kidneys and livers obtained from PCK and +/+ rats. Fibrosis area was measured from five random fields (×100 magnification) of picrosirius red-stained kidney and liver sections. Cystic and fibrotic indexes (% of total field) were measured by a naive observer using LUZEX FS software (Kideko, Tokyo, Japan) and were expressed as means ± SE.

Western blot analysis. Proteins (20 or 100 μg protein/lane) from kidney or liver lysates were separated by 10% SDS-PAGE and were transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in TBS-T for 1 h at room temperature and then incubated overnight at 4°C in primary antibody in 2% milk in TBS-T. Primary antibodies were ERK1/2 (K-23, SC-94; 1:100; Santa Cruz Biotechnology), Santa Cruz Biotechnology, Santa Cruz, CA) and pERK1/2 (SC-7383; 1:1,000; Santa Cruz Biotechnology). Membranes were then washed three times with TBS-T and incubated with secondary antibody conjugated to horseradish peroxidase in 2% milk in TBS-T. Specific antibody signals were detected using an enhanced chemiluminescence system (ECL or ECL Advance Western Blotting Detection System; Amer sham Life Sciences, Arlington Heights, IL). Images of the blots were captured, and the intensity of the protein bands was quantified using a CS Analyzer 2.0 with a CCD camera (ATTO, Tokyo, Japan). Relative band intensity was compared with +/+ kidneys (set to 100%).

Immunohistochemistry and immunofluorescence. Paraffin sections were heated to 100°C for 15 min in 10 mM sodium citrate buffer (pH 6.0) to unmask antigens. Endogenous peroxidase was destroyed by incubating sections in 0.3% H2O2/methanol. The sections were blocked with 1% bovine serum albumin and 0.05% NaN3 in phosphate-buffered saline buffer without Mg2+ and Ca2+ for 30 min at room temperature and were incubated with primary antibody overnight at 4°C. Primary antibodies for immunohistochemistry were ERK1/2 (K-23, SC-94; 1:100; Santa Cruz Biotechnology), pERK1/2 (M8159; 1:1,000; Sigma, St. Louis, MO), Ki-67 (ab16667; 1:100; Abcam, Cambridge, MA), ERK5 (C-20, SC-1284; 1:100; Santa Cruz Biotechnology), pERK5 (#3371, 1:50; Cell Signaling Technology, Danvers, MA), S6 (#2217, 1:100; Cell Signaling), and pS6 (#4858, 1:50; Cell Signaling). Rinsed sections were incubated with biotinylated anti-mouse and rabbit IgG/IgA/IgM secondary antibody (Histofine; Nichirei Biosciences, Tokyo, Japan) for immunohistochemistry. Immune reaction products were developed using 3,3'-diaminobenzidine. The 4% PFA-fixed thin sections were incubated with a primary antibody for TGF-β (3G1, 1:100; Santa Cruz Biotechnology) overnight at 4°C. Rinsed sections were incubated with anti-mouse IgG secondary antibody conjugated to Alexa568 (Invitrogen, Carlsbad, CA) for immunofluorescence detection. Positive-stained cells were counted in five random fields of kidney and liver sections.

A

B

Fig. 2. Effect of PIO on cell proliferation in PCK and +/+ kidneys. A: kidney sections from PCK and +/+ rats were stained with an antibody to Ki67, a proliferation marker. B: percentage of Ki67-positive cells was determined from the total number of cells in cystic and noncystic tubules in PCK kidney sections and from normal tubules in +/+ kidney sections. The total number of cells was counted in 5 random fields of one kidney section from each rat (n = 5 rats). Nuclei of Ki67-positive cells stained brown with 3,3'-diaminobenzidine, whereas nuclei of Ki67-negative cells appeared blue due to the counterstaining of hematoxylin. Comparison between vehicle-treated and PIO-treated PCK and +/+ rats (female or male), *P < 0.05, **P < 0.01.
obtained from five rats in each group by a naive observer using a ×40 objective.

**Statistics.** Results are expressed as means ± SE of the mean. Statistical comparisons were made using either Student’s t-test or two-way ANOVA, as appropriate, and differences were considered to be significant at \( P < 0.05 \).

**RESULTS**

**Effect of PIO on kidney weight, renal function, and cyst area in PCK rats.** Percent kidney weight relative to body weight (%KB) was 2.4- and 1.9-fold greater in the PCK male and female compared with gender-matched +/+ rats (Fig. 1A).

**Fig. 3.** Effect of PIO on phosphorylated ERK1/2 (pERK) in PCK and +/+ kidneys. A: immunoblots were probed with an antibody to pERK or total ERK1/2 (ERK). B: ratio of pERK/ERK was determined from density analysis of the bands. C: kidney sections were stained by immunohistochemistry with an antibody to pERK. D: percentage of pERK-positive cells was determined in cystic and noncystic tubules in PCK kidney sections and in normal tubules in +/+ kidney sections. All renal tubular cells were positive for total ERK (data not shown); the total number of ERK-positive cells ranged from 547 to 892 in 5 random fields of a kidney section from each rat \(( n = 5 \) rats). Comparison between vehicle-treated and PIO-treated PCK and +/+ rats (female or male), *\( P < 0.05 \), **\( P < 0.01 \).

**Fig. 4.** Effect of PIO on phosphorylated S6 (pS6) in PCK and +/+ kidneys. A: kidney sections were stained with an antibody to pS6. B: percentage of pS6-positive cells was determined within cystic and noncystic tubules in kidney sections. Comparison between vehicle-treated and PIO-treated PCK and +/+ rats (female or male), **\( P < 0.01 \).
Daily PIO treatment for 16 wk decreased %KB by 21 and 22% in PCK male and female rats, respectively, compared with untreated littermates, but did not affect %KB in +/- rats (Fig. 1A). SUN levels in PCK male and female rats were higher than in gender-matched +/- rats (male $P < 0.01$, female $P < 0.01$, respectively) even though the levels in PCK rats were not indicative of renal insufficiency. There was a significant reduction in SUN in PIO-treated PCK males and females (Fig. 1B), consistent with improved overall renal function. As seen in kidney sections stained with HE, cysts were smaller in the PIO-treated PCK rats compared with control-treated PCK rats (Fig. 1, C and D). Cystic area was significantly decreased in both male and female PCK rats by PIO treatment (Fig. 1E). Body weight (Fig. 1F) and food and water intake (data not shown) of gender-matched PCK and F1 rats did not differ at 20 wk of age and was unaffected by treatment with PIO.

**Effect of PIO on renal cell proliferation and fibrosis.** Immunohistochemical analysis revealed that the number of cells positive for Ki67, a cell proliferation marker, was significantly higher in PCK rat kidneys compared with age- and gender-matched +/+ rats (Fig. 2). Interestingly, PIO also decreased the number of Ki67-positive cells in noncystic tubule cells in PCK rats, suggesting that PIO may inhibit early events in cyst formation. In +/- rat kidneys, the number of Ki67-positive cells was relatively low and was unaffected by PIO treatment.

Kidney sections were stained with picrosirius red to examine interstitial fibrosis. We did not detect a significant effect of PIO treatment on fibrosis in PCK kidneys, although there was a downward trend (Fig. 1G).

**Effect of PIO on the ERK, mTOR, and TGF-β-signaling pathways in PCK kidneys.** To determine whether inhibition of renal cell proliferation by PIO could be mediated by a reduction in the activity of the MEK/ERK-signaling pathway, we measured the abundance of proteins reacting with antibodies to phosphorylated ERK1/2 (pERK) and total ERK1/2 (ERK) using immunoblot analysis, and we determined the number of cells staining positive for pERK and ERK using immunohistochemical analysis.

PCK kidneys of both genders had a higher ratio of pERK per total ERK compared with +/+ kidneys, consistent with a previous report (21). Treatment with PIO decreased the pERK/ERK ratio (Fig. 3, A and B). We also found that there was a higher percentage of pERK-positive cells in renal epithelial cells of PCK kidneys compared with normal +/- kidneys (Fig. 3, C and D). PIO treatment decreased the percentage of pERK-positive cells in dilated tubules and cysts in both male and female PCK rats (Fig. 3, C and D), whereas the percentage of cells positive for total ERK was unaffected (data not shown).

Ribosomal protein S6 is a substrate for p70S6 kinase, a downstream component of the AKT/mTOR signaling pathway. Increased levels of p70S6 kinase and phosphorylated S6 (pS6) have been demonstrated in the cystic kidneys of human ADPKD and rodent models of PKD (5, 8, 9, 26, 34, 37, 41, 42, 53). In the present study, we tested whether activation of the PPAR-γ pathway by PIO affects the level of pS6. We found that the percentage of pS6-positive cells was higher in PCK kidneys compared with age- and gender-matched +/- kidneys (Fig. 4), indicating that there is increased renal mTOR activity in this ARPKD rat model. The number of total pS6-positive cells was decreased in both male and female PCK rat by PIO treatment (male $P < 0.001$, female $P < 0.05$). PIO treatment decreased the percentage of pS6-positive cells in the dilated tubules and cysts in PCK kidneys of both genders, while the percentage of positive cells for total S6 was unaffected (data not shown).

**Effect of PIO on hepatic cysts and liver function.** Liver weight relative to body weight was 1.6- and 2.0-fold greater in male and female PCK rats, respectively, compared with gender-matched +/- rats (Fig. 5A). PIO treatment decreased liver weight (Fig. 5B) and food and water intake (data not shown) of gender-matched PCK and F1 rats did not differ at 20 wk of age and was unaffected by treatment with PIO.
weight by 21% in male and 16% in female PCK rats. There was also a small but significant reduction in liver weight in male +/+ rats treated with PIO, although there was no apparent effect in female +/+ rats. Blood sugar levels of gender-matched PCK and +/+ rats did not differ at 20 wk of age and were unaffected by treatment with PIO. In the liver sections from PIO-treated PCK rats, the average size of the cysts was less than in those of vehicle-treated livers; although there still were many cysts present (Fig. 5, B–E). Figure 5H shows that there was a decrease in the cyst area in both male and female PCK rats. Serum AST and total bilirubin levels were normal in both PCK genders at 20 wk and were unaffected by PIO treatment (data not shown).

**Effect of PIO on hepatic cell proliferation and fibrosis.** The number of Ki67-positive cells in PCK livers was significantly higher than that in +/+ livers (Fig. 6A). In the hepatocyte regions of the liver, no significant differences between PIO-treated and vehicle-treated PCK rats were seen (data not shown). Cholangiocytes can be classified as C-type due to a cuboidal shape or F-type due to a flattened shape (30). F-type cholangiocytes have thickened extracellular matrix (progressive fibrosis), suggesting that the two types of cholangiocytes have different characteristics. Treatment with PIO decreased the percentage of Ki67-positive C-type cholangiocytes in male and female rats (Fig. 6B). Ki67 was also decreased in F-type cholangiocytes, although this was significant only in the livers of the female rats. Interestingly, PIO also decreased the number of Ki67-positive interstitial cells of PCK livers. In +/+ rats, the number of Ki67-positive cholangiocytes, interstitial cells, and hepatocytes was unaffected by PIO treatment.

Picrosirius red staining was performed as a measure of fibrosis (Fig. 5, F and G). PIO significantly decreased the overall fibrotic area per total liver area in male and female PCK rats (Fig. 5I), although we do not know whether the thickness per se of these fibrotic areas decreased.

**Effect of PIO on the ERK, mTOR, and TGF-β-signaling pathways in PCK livers.** To determine the effects of PIO on cellular signaling events associated with proliferation and fibrosis in the livers of PCK rats, we measured pERK, phosphorylated ERK5 (pERK5), and TGF-β. There was a higher ratio of pERK to total ERK in PCK livers compared with +/+ livers in both genders (Fig. 7, A and B), consistent with other models of PKD (20, 22, 35). Treatment with PIO decreased the ratio of pERK to total ERK in these livers. The number of pERK-positive cells in PCK livers was significantly higher than in the livers of +/+ rats (Fig. 7C). PIO treatment decreased the percentage of total pERK-positive cells in both PCK genders (male \( P < 0.05 \), female \( P < 0.05 \)), specifically in the C-type cholangiocytes (Fig. 7D).

Sato et al. (29) showed that there was an elevation of ERK5 activity in polycystic livers of PCK rats in addition to increased ERK1/2 activity. Similarly, we found that the percentage of pERK5-positive cells in PCK livers was significantly higher than in +/+ livers (Fig. 8, A and B). PIO decreased the percentage of pERK5-positive cells in livers of both male and female PCK rats. The effect was most predominant in the C-type cholangiocytes (Fig. 8B). Total ERK5 was found in C-type and F-type cholangiocytes and interstitial cells of PCK liver and in bile duct epithelial cells and interstitial cells in +/+ rats. The number of cells positive for total ERK5 was not altered by PIO treatment in +/+ rats (data not shown).

Phosphorylated S6 (pS6), a readout for mTOR pathway activation, was detected in F-type cholangiocytes but not in...
C-type cholangiocytes in untreated PCK rats; however, there was no apparent effect of PIO treatment on pS6 levels.

TGF-β is associated with progression of hepatic fibrosis in PCK rats (30). We found that the percentage of TGF-β-positive cells in livers of PCK rats was significantly higher than in livers of +/- rats (Fig. 9, A and B) and that treatment with PIO decreased this percentage in both genders (male \( P < 0.001 \), female \( P < 0.002 \)). This effect of PIO was primarily on TGF-β-positive interstitial cells (Fig. 9 B), suggesting that PIO may inhibit both inflammation and fibrosis in PCK livers.

**DISCUSSION**

PIO, a thiazolidinedione, activates PPAR-γ and downstream signaling pathways involved in the regulation of many cellular processes, including glucose homeostasis, cell proliferation, and differentiation. PPAR-γ ligands have been shown to inhibit Akt/mTOR/S6K activity, TGF-β activity, fibrosis, and matrix production in fibroblasts and heart tissue and to decrease ERK activity in endothelial cells (4, 12, 16, 54). The activation of PPAR-γ can inhibit several components of the cell cycle (43) and has an anti-proliferative effect on epithelial cancer cells (1, 6, 38). Because of their anti-proliferative and anti-fibrotic effects, PPAR agonists have emerged as promising pharmacological agents for the treatment of a variety of clinical disorders, including cancer.

Cysts share many features with epithelial cell cancers, including incomplete cellular differentiation, aberrant expression of transcription factors, abnormal cell proliferation, loss of planar cell polarity, and overexpression of extracellular matrix molecules (10, 39). Mutations in PKD genes confer a cellular phenotype characterized by cAMP-dependent ERK activation and renal cell proliferation that is normally not typical of renal tubule cells. This has been observed in both ADPKD and ARPKD (20, 24, 32, 50, 51). This stimulatory effect of cAMP is equally potent and complementary to growth factor stimulation. Elevated activities of components of Akt/mTOR/S6K signaling in PKD kidneys and liver indicate that this pathway might also be involved in cyst formation and progressive fibrosis. Treatment with rapamycin, an mTOR inhibitor, reduced renal disease progression in pcy mice, Cy rats, and mice with a conditionally targeted \( Pkd1 \) mutation (9, 34, 37, 42). In retrospective studies involving ADPKD patients who had received a renal transplant, rapamycin used for immunosuppression appeared to be sufficient to reduce mTOR activity and cyst
growth in both the kidneys and liver (26). However, in two recent clinical trials, mTOR inhibitors did not appear to be as effective in treating PKD in ADPKD patients (31, 44, 49), although there are considerations about dosage and timing of the treatments.

In the current study, we found that administration of 10 mg·kg⁻¹·day⁻¹ PIO for 16 wk significantly reduced polycystic kidney and liver disease in a model of human ARPKD. Our findings suggest that PIO reduces disease progression by targeting two key components of ARPKD. First, PIO appears to have an anti-proliferative effect by inhibition of the ERK and mTOR pathways in renal cysts and by inhibition of ERK and ERK5 activity in cholangiocytes; and second, PIO appears to have anti-fibrotic and anti-inflammatory effects by decreasing TGF-β levels in interstitial cells adjacent biliary ducts within the liver. We observed that the noncystic tubule cells had an increase in their proliferation index but not in their pERK status, suggesting that noncystic and cystic epithelial cells may utilize distinct proliferative pathways.

Previously, PIO had been tested in ADPKD mice with mutations in Pkd1. Muto et al. (19) found that treatment of Pkd1+/- pregnant mice with 80 mg·kg⁻¹·day⁻¹ PIO during...
embryos up to day E18.5. The mechanism for this beneficial effect was unclear; however, the drug seemed to decrease the levels of tyrosine phosphorylation of epidermal growth factor receptor (19). Raphael et al. (27) found that PIO (~4.8 mg·kg−1·day−1) improved survival of PC-Pkd1-KO mice, an ADPKD model in which Pkd1 is knocked out in the principal cells of renal collecting ducts. There was no improvement of renal function, cell proliferation, apoptosis, or cyst formation with PIO treatment; however, the PKD animals receiving drug had lower blood pressure, suggesting that it had an antihypertensive effect that might have improved survival. The differences in the effects of PIO on cyst formation in the two studies may be due to differences in the doses of PIO. In humans, the maximal dose delivered in clinical trials for type 2 diabetes was 45 mg·kg−1·day−1; whereas in preclinical studies for type 2 diabetes, 10 mg·kg−1·day−1 PIO was shown to effectively reduce disease progression in KK/Ta and ob/ob mice (15, 36). Therefore, in the current study, a dose of 10 mg·kg−1·day−1 was selected. Although 10 mg·kg−1·day−1 PIO reduced liver weight in wild-type males, there was no obvious effect on liver function or histology, suggesting that this concentration is well-tolerated by the rats.

Because PCK rats have polycystic kidney and liver disease over a 1.5-yr life span, it may be a more useful human disease model to evaluate long-term anti-proliferative, anti-fibrotic, and anti-inflammatory effects of PIO compared with other animal models that are embryonic lethal or have shorter average life spans (19, 27). In a model of slowly progressive ADPKD, the Cy rat, rosiglitazone attenuated disease progression and prolonged survival by decreasing the levels of β-catenin, TGF-β, and MCP-1 (3). In the current study, we were unable to demonstrate a therapeutic effect of PIO on renal fibrosis since the degree of fibrosis and TGF-β expression in the PCK kidneys were not markedly elevated compared with normal animals; however, we were able to show anti-fibrotic effects of PIO in the livers. As such, the anti-fibrotic effects of PIO on the progression of polycystic/fibrocystic liver disease may be important for ARPKD patients.

We classified C-type and F-type cholangiocytes by cellular shape and by the ratio of progressive fibrosis around bile ducts, according to the histological classification of Sato et al. (30). We found that C-type cholangiocytes were the predominant type to form biliary cysts, whereas progressive hepatic fibrosis was principally associated with F-type bile ducts. PIO treatment decreased the expression of cell proliferation markers Ki67, pERK, and pERK5 in C-type but not in F-type cholangiocytes in PCK rats. These findings suggest that the anti-proliferative effects of PIO may be primarily manifested in C-type cholangiocytes of the cystic bile ducts.

In conclusion, the PPAR-γ agonist PIO decreases PKD in association with inhibition of the ERK and mTOR-signaling pathways, and fibrocystic liver disease in association with inhibition of the ERK and TGF-β-signaling pathways in PCK rats, an orthologous model of human ARPKD. These data support the use of PPAR-γ agonists as therapeutic agents for the treatment of polycystic kidney and liver disease in ARPKD patients.

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DISCLOSURES
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