



Article

Effects of Suramin on Polycystic Kidney Disease in a Mouse Model of Polycystin-1 Deficiency

Ming-Yang Chang ^{*}, Shen-Hsing Hsu, Li-Yi Ma, Li-Feng Chou, Cheng-Chieh Hung, Ya-Chung Tian and Chih-Wei Yang

Kidney Research Center, Department of Nephrology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan 333, Taiwan; d938208@gmail.com (S.-H.H.); maliyi24@gmail.com (L.-Y.M.); d928208@gmail.com (L.-F.C.); cchung@adm.cgmh.org.tw (C.-C.H.); dryctian@adm.cgmh.org.tw (Y.-C.T.); cwyang@ms1.hinet.net (C.-W.Y.)

* Correspondence: mingyc@adm.cgmh.org.tw; Tel.: +886-3-3281200; Fax: +886-3-3282173

Abstract: The aberrant activation of the purinergic signaling pathway has been shown to promote cyst growth and fluid secretion in autosomal dominant polycystic kidney disease (ADPKD). Suramin is an anti-parasitic drug that has strong anti-purinergic properties. Whether suramin could have a therapeutic effect on ADPKD has not been fully investigated. We examined the effect of suramin on cyst progression in a *Pkd1* microRNAs transgenic mouse model that presented stable *Pkd1* knockdown and moderate disease progression. The *Pkd1*-deficient mice were treated with suramin (60 mg/kg) by intraperitoneal injection twice a week from postnatal days 35 to 90. Kidney-to-body weight ratios, cyst indices, and blood urea nitrogen (BUN) levels were measured. Cell proliferation and macrophage infiltration were determined by immunohistochemistry. The suramin-treated group had significantly lower renal cyst densities, cell proliferation, and macrophage infiltration compared with saline-treated controls. Suramin significantly inhibited ERK phosphorylation and the expression of *Il1b*, *Il6*, *Nlrp3*, *Tgfb*, *Fn1*, *P2rx7*, and *P2ry2* mRNAs in the kidneys. However, BUN levels remained high despite the reduction in cyst growth. Furthermore, plasma cystatin C and neutrophil gelatinase-associated lipocalin (NGAL) levels were significantly higher in the suramin-treated group compared with the control group. Periodic acid-Schiff staining revealed degenerative changes and epithelial cell vacuolation in the non-cystic renal tubules, which indicated phospholipidosis following suramin treatment. These results suggest that suramin may reduce renal cyst growth and inflammation, but the associated tubular cell injuries could limit its therapeutic potential. Other purinergic receptor antagonists with less nephrotoxicity may deserve further investigation for the treatment of ADPKD.

Keywords: polycystic kidney disease; purinergic signaling; suramin



Citation: Chang, M.-Y.; Hsu, S.-H.; Ma, L.-Y.; Chou, L.-F.; Hung, C.-C.; Tian, Y.-C.; Yang, C.-W. Effects of Suramin on Polycystic Kidney Disease in a Mouse Model of Polycystin-1 Deficiency. *Int. J. Mol. Sci.* **2022**, *23*, 8499. <https://doi.org/10.3390/ijms23158499>

Academic Editor: Ivano Condò

Received: 10 July 2022

Accepted: 28 July 2022

Published: 31 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease characterized by the formation of multiple renal cysts and the progressive loss of kidney function, leading to end-stage kidney failure in about 50% of affected patients over the age of 60 [1,2]. The majority of ADPKD patients (~85%) are caused by mutations in *PKD1* and the remaining are caused by *PKD2* and other novel genes, such as *GANAB* and *DNAJB11* [3,4]. The pathogenesis of ADPKD involves excessive cell proliferation and the fluid secretion of the cyst-lining epithelium, which has been associated with the aberrant activation of cyclic adenosine monophosphate (cAMP), mTOR (mammalian target of rapamycin), and other pro-proliferative signaling pathways [5,6]. Tolvaptan, a vasopressin V2 receptor antagonist, is the first FDA-approved treatment for ADPKD [7]. However, the potential liver toxicity and severe aquaretic side effects have limited its clinical use in ADPKD patients with rapid disease progression [8]. Therefore, other potential treatments targeting different signaling pathways warrant further investigation [9].

The purinergic P2 receptor signaling pathway has been found to enhance fluid secretion and cell proliferation in ADPKD [10,11]. High levels of adenosine-5'-triphosphate (ATP) in renal cysts reduce the sodium reabsorption mediated by epithelial sodium channels (ENaC) and promote cyst expansion [12]. A synergistic effect of cAMP and ATP on enhancing fluid secretion and cyst growth has also been found in the Madin-Darby canine kidney (MDCK) cell model [13]. An in vivo study showed that P2X7 receptor knockout attenuates cyst growth in a rat model of autosomal recessive PKD (ARPKD) [14]. P2X7 receptor inhibition also reduces cell proliferation and pronephros cyst formation in a *Pkd2*-deficient zebrafish model [15]. Furthermore, the P2Y2 receptors are transcriptionally regulated by hypoxia-inducible factor (HIF)-1 α and promote ATP-dependent cyst growth [16]. These data suggest a potential benefit of blocking purinergic P2 receptors in slowing cyst growth in ADPKD.

Suramin is a 100-year-old drug that has been primarily used for the treatment of African trypanosomiasis [17,18]. It is a non-selective P2 receptor antagonist (P2Xs and P2Ys) that has multitarget and pleiotropic effects [19–22]. Suramin was initially investigated as an antitumor and anti-HIV (human immunodeficiency virus) agent but has demonstrated limited efficacy in previous clinical trials [23,24]. Suramin has been repurposed to improve the metabolic abnormalities in autism spectrum disorder (ASD) via the inhibition of P2X7 receptors [25]. Furthermore, single-dose suramin reduced the deposition of extracellular matrix and interstitial leukocyte infiltration in a mouse model of unilateral ureteral obstruction (UUO), suggesting its therapeutic potential for chronic kidney diseases [26]. An in vitro study showed that suramin reduces cyst formation in a three-dimensional microcyst model in MDCK cells [13]. Therefore, we hypothesized that suramin might suppress ADPKD by inhibiting purinergic receptor pathways in vivo.

In this study, we determined the effects of suramin on cyst growth and disease progression in an established *Pkd1* hypomorphic model of ADPKD. We examined if suramin treatment could ameliorate the cystic burden of *Pkd1* knockdown mice and suppress renal inflammation and cell proliferation. We also evaluated the degree of renal tubular injury and the overall kidney function following suramin treatment.

2. Results

2.1. Increased Expression of P2X7 Receptors in *Pkd1*-miR Tg Mice

This study investigated the effects of suramin on cystogenesis in an established mouse model of *Pkd1* knockdown that recapitulates the chronic disease progression in humans [27]. First, we examined whether the expression of P2X7 receptors is upregulated in *Pkd1*-miR Tg mice as has been previously shown in the *Pkd1*^{RC/RC} mouse model [12]. In wild-type mice, we found a strong expression of P2X7 receptors in the distal tubules and a weaker expression in the proximal tubules by immunohistochemistry, whereas the signal was undetectable in the glomeruli (Figure 1A). In *Pkd1*-miR Tg mice, the immunostaining of P2X7 receptors was most evident in the cyst lining epithelium both at the ages of postnatal day 30 (P30) and P60 (Figure 1B,C). Furthermore, *Pkd1*-miR Tg mice had significantly higher levels of renal *P2rx7* mRNA expression than wild-type mice by 2.5-fold at P30 ($p < 0.05$) and 4.4-fold at P60 ($p < 0.01$), respectively. This was associated with an increased mRNA expression of *Il1b*, *Tnf*, *Mcp1*, *Fn1*, and *Col1a2* genes in the cystic kidneys of *Pkd1*-miR Tg mice (Figure 1D).

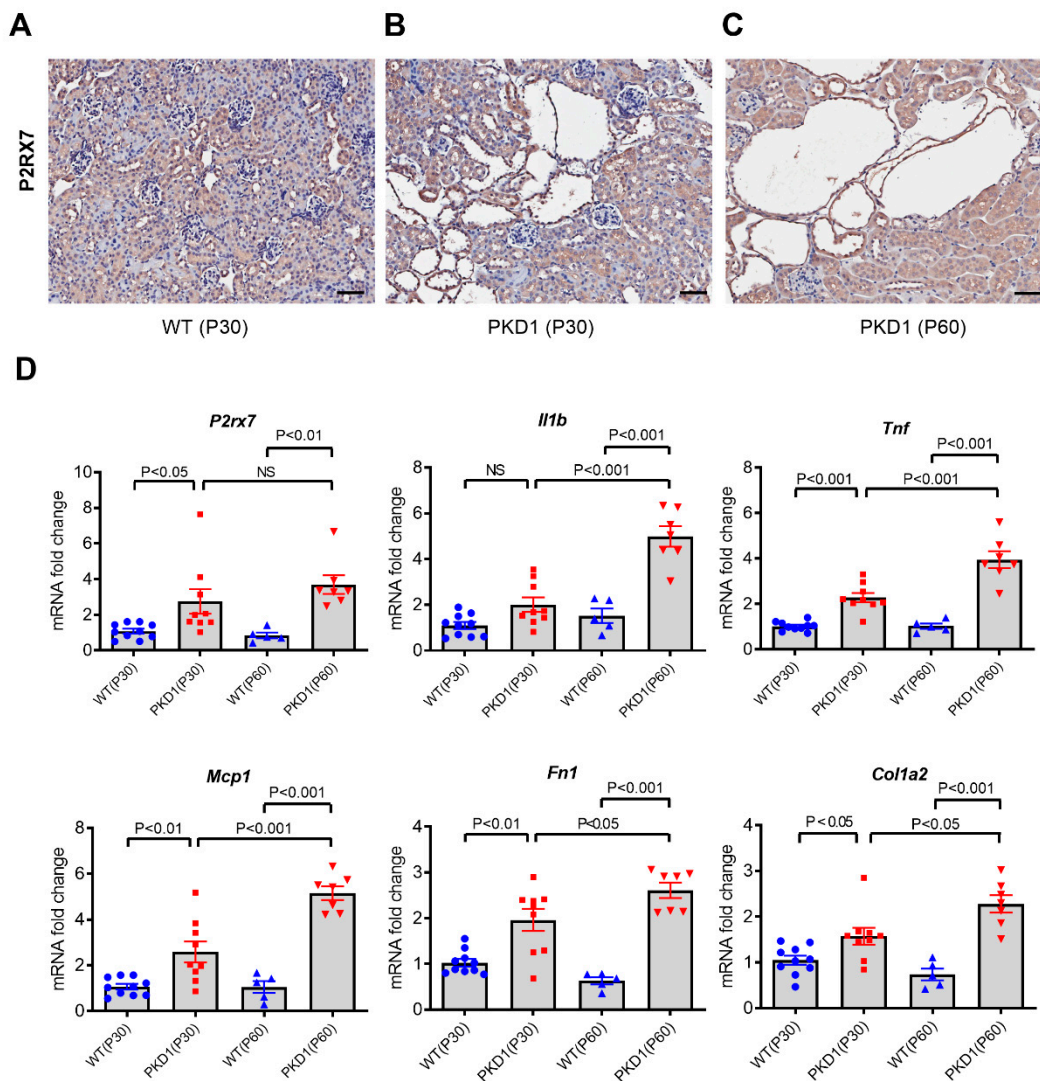


Figure 1. The expression of P2X7 receptors in wild-type and *Pkd1*-miR Tg mice. (A) Representative images of kidney sections showing diffuse immunostaining for P2X7 receptors in renal tubules in wild-type mice (n = 5). (B) Strong staining for P2X7 receptors is noted in the cyst-lining epithelial cells in the *Pkd1* miR Tg mice at 30 days of postnatal age (n = 5). (C) Diffuse staining for P2X7 receptors in cyst walls and non-cystic tubules in the *Pkd1*-miR Tg mice at 60 days of age (n = 5). (D) Comparison of the renal mRNA expression of *P2rx7* and other pro-inflammatory and pro-fibrotic genes in the kidneys of wild-type and *Pkd1* miR Tg mice according to different postnatal ages (n = 10, 9, 5, 7, respectively). Data are mean \pm SEM. Scale bar, 50 μ m.

2.2. Suramin Suppressed Cystogenesis in *Pkd1*-miR Tg Mice

We then investigated the effects of suramin on cyst formation in *Pkd1*-miR Tg mice (Figure 2A). The dosage of suramin (60 mg/kg twice weekly i.p.) was selected according to previous studies that effectively reduce myocardial fibrosis, inflammation, and myonecrosis in the mdx mouse model of Duchenne muscular dystrophy [28–30]. As shown in Figure 2B and Supplementary Table S1, the suramin-treated *Pkd1*-miR Tg mice tended to have reduced kidney weights compared with saline-treated mutant mice, but the kidney-to-body weight ratios were not significantly different between the control and treatment groups (Figure 2C), possibly due to lower body weights found in the suramin-treated mice. Suramin treatment could cause systemic adverse effects that may affect the normal growth and metabolism in mice [31]. We also performed a histomorphological analysis on whole kidney sections for the comparison of cyst indices among groups. Indeed, *Pkd1*-miR Tg mice treated

with suramin had significantly reduced cyst indices (21.4 % reduction) compared with vehicle-treated mutant mice ($p < 0.001$) (Figure 2D). However, the elevated BUN levels in *Pkd1*-miR Tg mice were not ameliorated after treatment with suramin (Figure 2E). These results indicated that suramin suppressed renal cyst formation in *Pkd1*-miR Tg mice but did not slow the decline of kidney function.

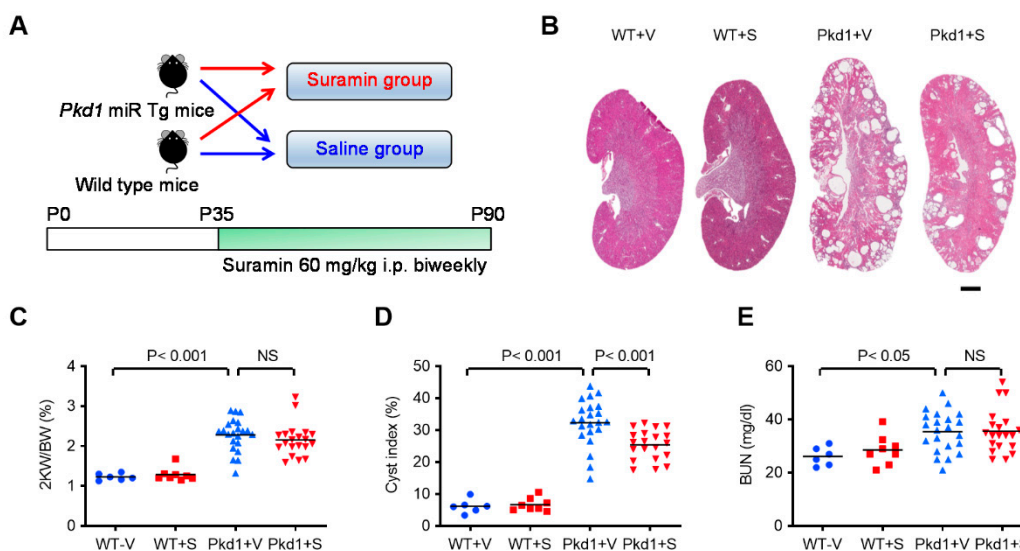


Figure 2. Suramin treatment reduces cyst burden in *Pkd1* miR Tg mice. (A) Schematic illustration of the experimental groups and the time frame of suramin administration. (B) Representative images of whole kidney sections (H&E stain) in the suramin-treated *Pkd1*-miR Tg mice compared to the vehicle-treated controls. (C) Kidney-to-body weight ratios, (D) cystic indices, and (E) BUN levels in different experimental groups as indicated ($n = 6, 8, 22,$ and 20 per group, respectively). Data are mean \pm SEM. S, suramin; V, vehicle. Scale bar, 1 mm.

2.3. Effect of Suramin on Cell Proliferation

Because suramin has been shown to suppress tumor growth in earlier research [32], we next sought to determine whether suramin could reduce the proliferation of cyst-lining epithelial cells. We used in vivo BrdU labeling assays to quantify cells in the S-phase of cell cycle progression. A significant reduction (44.7%) in the number of BrdU-labeled cyst-lining cells was detected in *Pkd1*-miR Tg mice with suramin treatment compared with the saline-treated group ($p < 0.01$) (Figure 3). This result suggests that suramin could reduce cyst densities by inhibiting cell proliferation in the cyst-lining epithelium.

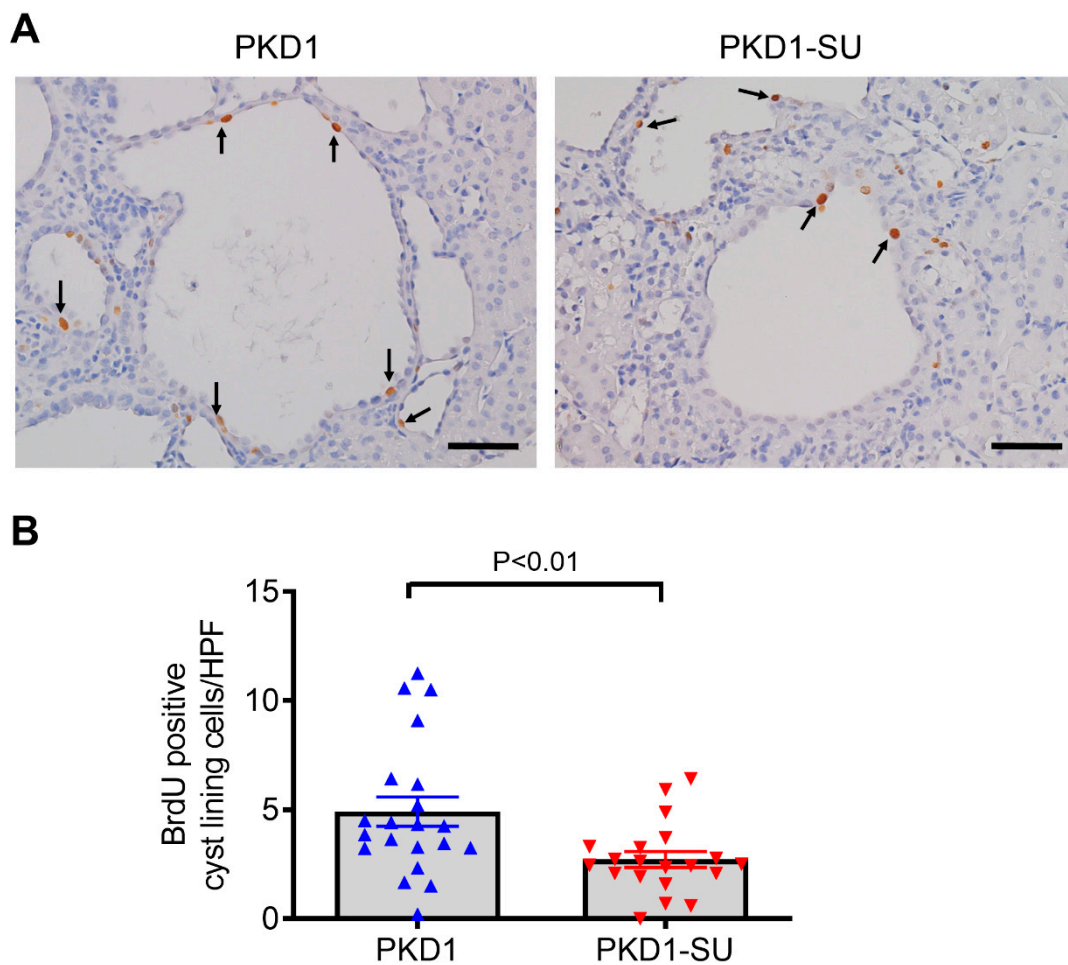


Figure 3. Suramin treatment inhibits the proliferation of cyst-lining epithelial cells in *Pkd1* miR Tg mice. (A) Immunostaining for BrdU incorporation in kidney sections from *Pkd1*-miR Tg mice in vehicle- and suramin-treated groups. Arrows indicate BrdU-positive cyst lining epithelial cells. Scale bar, 50 μ m. (B) Quantitative image analysis showed a significant reduction ($p < 0.01$) in the number of BrdU-positive cells in the suramin-treated *Pkd1*-miR Tg mice ($n = 20$) compared with the vehicle-treated mutant mice ($n = 21$) using an unpaired, two-tailed *t*-test. Data are mean \pm SEM.

2.4. Suramin Inhibited Renal Inflammation and Macrophage Infiltration

Next, we determined if suramin could reduce renal inflammation in *Pkd1*-miR Tg mice as has been shown in other kidney disease models [33–35]. Immunohistochemical staining revealed a significant reduction (53.9%) in the percentage of F4/80 positive area in the kidneys of suramin-treated *Pkd1*-miR Tg mice compared with those treated with vehicle ($p < 0.01$) (Figure 4A). Furthermore, *Pkd1*-miR Tg mice treated with suramin also showed a significant decrease in the renal mRNA expression of *Il1b*, *Il6*, *Nlrp3*, *Emr1* (F4/80), *Tnf*, *Mcp1*, *P2ry2*, and *P2rx7* compared with vehicle-treated mutant mice (Figure 4B). These results suggest that suramin exerted strong anti-inflammatory effects on the cystic kidneys, which could contribute to the reduced cyst burden.

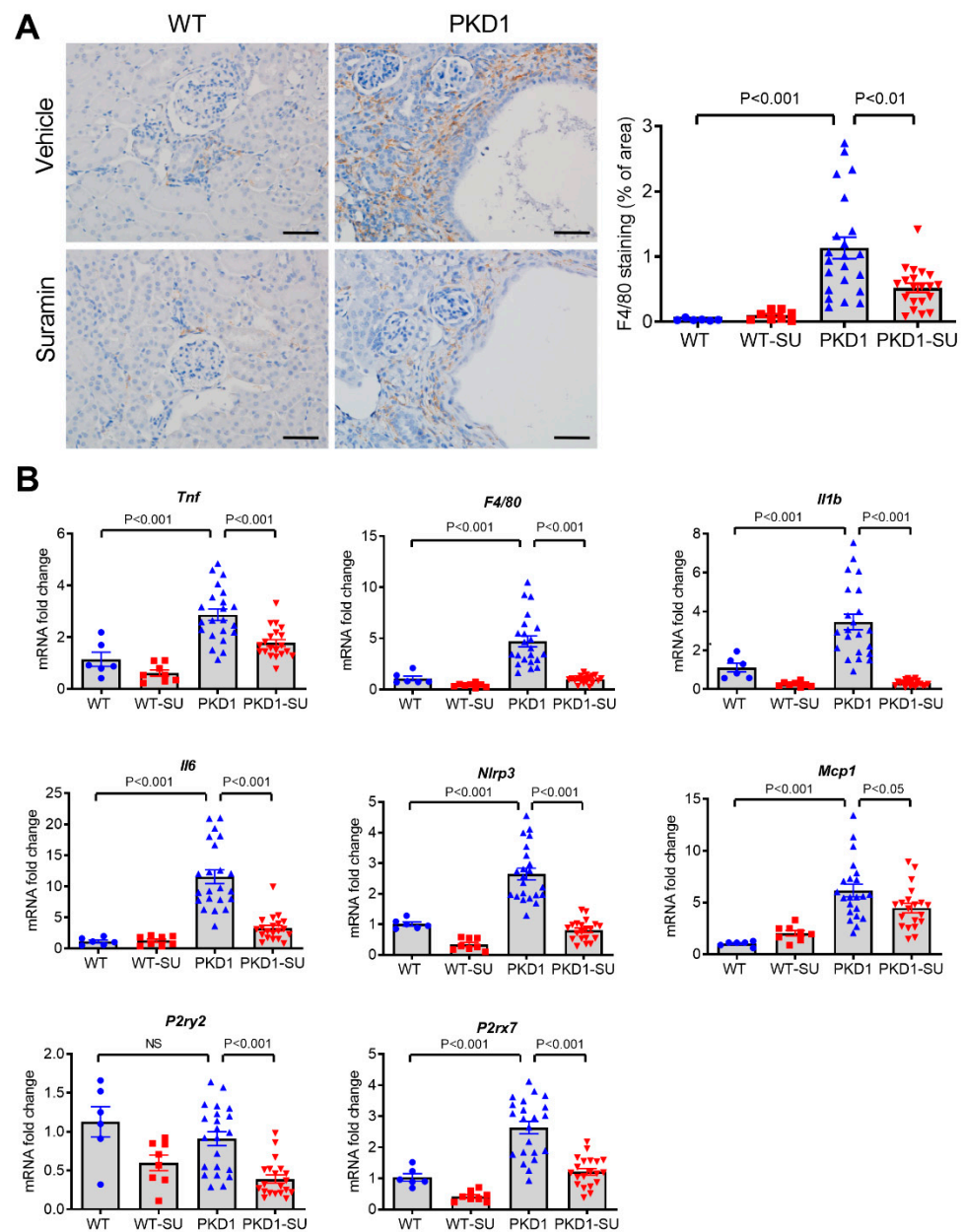


Figure 4. Suramin treatment inhibited renal inflammation and macrophage infiltration. **(A)** Immunostaining of kidney sections for F4/80 in different treatment groups as indicated. Quantitative image analysis showed a significant decrease in the percentage of F4/80 positive cells in the suramin-treated *Pkd1*-miR Tg mice compared with the vehicle-treated controls. Scale bar, 50 μ m. **(B)** Suramin treatment reduced the mRNA expression of pro-inflammatory genes and purinergic receptor genes in the kidneys, as determined by qRT-PCR ($n = 6, 8, 22,$ and 20 per group, respectively). Data are mean \pm SEM.

2.5. Effects of Suramin on Renal Fibrosis

Because a previous study demonstrated that a single dose of suramin suppressed renal fibrosis after acute injury in the mouse UUO model [26], we determined the effect of suramin on renal fibrosis in PKD. We found that the renal mRNA levels of *Fln1*, *Col1a2*, and *Tgfb1* were significantly lower in the suramin-treated group when compared with the vehicle-treated group (Figure 5A). However, immunohistochemical staining showed no discernible difference in the expression of fibronectin in the kidneys of suramin- or vehicle-

treated *Pkd1*-miR Tg mice (Figure 5B). These results indicate that suramin had no obvious antifibrotic effect in the current PKD model despite its strong anti-inflammatory activity.

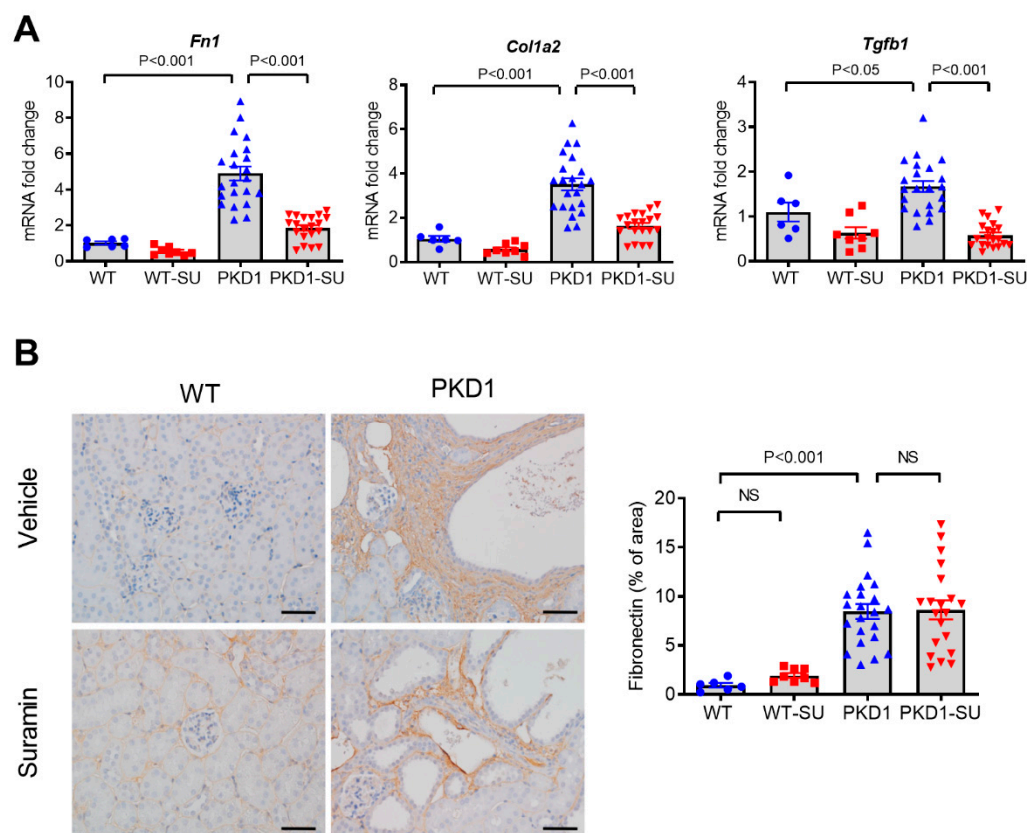


Figure 5. Effect of suramin treatment on renal fibrosis in *Pkd1*-miR Tg mice. (A) Comparison of the renal *Fn1*, *Col1a2*, and *Tgfb1* mRNA expression in different study groups as indicated. The internal control gene used for normalization was rodent 18S ribosomal RNA. (B) Immunohistochemistry staining for fibronectin in the kidneys of wild-type and *Pkd1*-miR Tg mice. Quantitative analysis showed increased fibronectin deposition in the cystic kidneys of *Pkd1*-miR Tg mice, but no significant changes were noted after treatment with suramin. Scale bar, 50 μ m. Data are mean \pm SEM. n = 6, 8, 22, and 20 per group, respectively.

2.6. Suramin Reduces ERK Phosphorylation in Cystic Kidneys

The activation of the ERK pathway is essential for cAMP-dependent cyst growth in ADPKD [36]. Furthermore, the ERK pathway is one of the downstream effectors of the purinergic receptors [37]. Therefore, we determined whether the increased ERK phosphorylation in cystic kidneys could be suppressed by suramin treatment. Indeed, Western blot analysis showed that the levels of phosphorylation of ERK1/2 in the kidneys of *Pkd1*-miR Tg mice were significantly reduced in the suramin group compared with those in the vehicle group ($p < 0.01$) (Figure 6 and Supplementary Figure S1). These results suggest that suramin could suppress cyst growth through the inhibition of ERK-dependent signaling pathways.

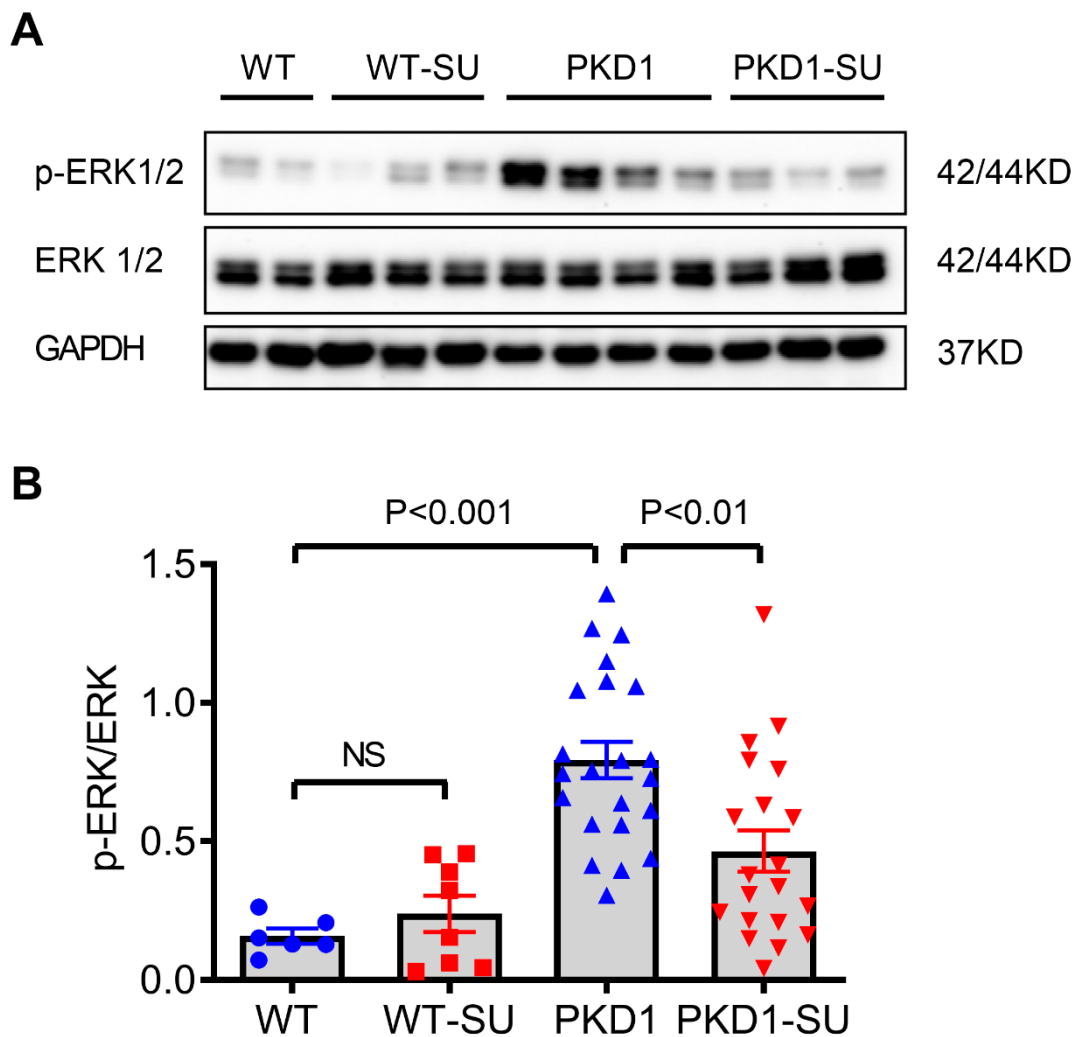


Figure 6. Suramin reduces the phosphorylation of ERK1/2 in *Pkd1*-miR Tg mice. Kidney lysates from *Pkd1*-miR Tg mice and wild-type mice with or without suramin treatment were analyzed by Western blotting analysis. (A) Representative immunoblots of phosphorylated ERK1/2, total ERK1/2, and GAPDH. (B) The densitometric analysis of p-ERK1/2 normalized to total ERK1/2 protein expression. GAPDH was used for loading control. Data are mean \pm SEM (n = 6, 8, 22, and 20 per group respectively).

2.7. Effect of Suramin on Renal Function and Kidney Injury Markers

Despite the reduction of cyst burden and macrophage infiltration, the elevated BUN levels in *Pkd1*-miR Tg mice were not improved after treatment with suramin. Furthermore, the plasma cystatin-C and Ngal levels in the suramin-treated *Pkd1*-miR Tg mice were significantly higher than those in the vehicle controls by 1.4- and 2.4-fold, respectively ($p < 0.001$) (Figure 7). These unexpected findings suggested that suramin could have caused a nephrotoxic injury. Further histological examination with PAS staining showed multiple vacuolar degenerative changes in the proximal renal tubules in suramin-treated *Pkd1*-miR Tg mice. These results suggest that the accumulation of suramin could induce renal tubular injury and phospholipidosis, which may counteract its beneficial effects on reducing cyst growth and kidney inflammation.

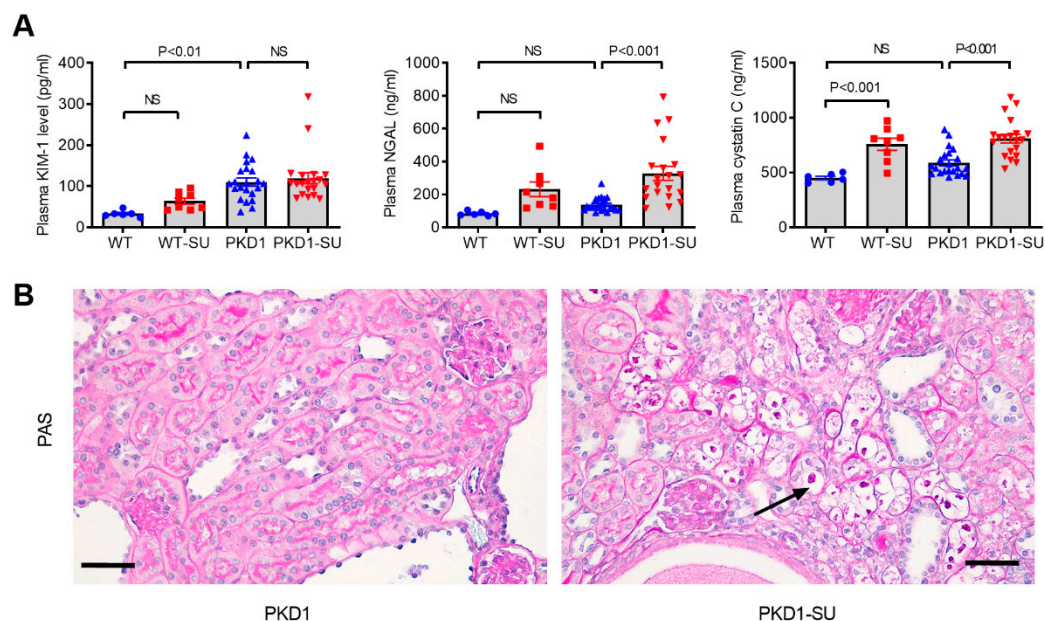


Figure 7. Suramin induces cytoplasmic vacuolation and kidney injuries in non-cystic tubules. (A) Plasma levels of kidney injury markers (KIM-1, NGAL, cystatin C) were increased in the suramin-treated *Pkd1*-miR Tg mice compared to the vehicle-treated mutant mice. Data are mean \pm SEM (n = 6, 8, 22, and 20 per group respectively). (B) Representative periodic acid-Schiff (PAS) staining in the kidney sections from the vehicle- and suramin-treated *Pkd1*-miR Tg mice (n = 3 per group). Arrow indicates the cytoplasmic vacuolation and degeneration of proximal tubules. Scale bar, 50 μ m.

3. Discussion

This study determined the effects of suramin, a pan-purinergic receptor antagonist, on the disease progression of PKD in a *Pkd1*-knockdown mouse model. To the best of our knowledge, this is the first in vivo study examining the role of suramin in ADPKD progression. We showed that suramin significantly reduced cyst growth and suppressed renal macrophage infiltration in *Pkd1*-deficient mice. Our results are in line with previous in vitro studies which show an inhibitory effect of suramin on cyst growth in ADPKD [13,38]. A recent bio-chemoinformatic analysis also identified suramin as a candidate drug for ADPKD [39]. However, we found that the administration of suramin also induced degenerative kidney injuries and phospholipidosis in the non-cystic tubules. These results revealed a novel dual role of suramin in modulating PKD progression; it may inhibit the purinergic pathway and cyst growth but can also induce phospholipidosis and tubular cell injuries.

Suramin has demonstrated potent anti-inflammatory and antifibrotic properties in various kidney disease models but has not been tested in ADPKD [26,33,35,40]. Indeed, we found that suramin markedly inhibited inflammatory cytokines and macrophage infiltration in the cystic kidneys, which was associated with reduced cyst growth. These findings support the emerging concept that the inhibition of macrophage infiltration surrounding kidney cysts could modify the cyst microenvironment and reduce the proliferation of cyst-lining epithelial cells [41–43]. However, we did not observe a significant reduction of renal fibrosis after treatment with suramin. The discrepancy could be attributed to different disease-specific mechanisms of renal injury and fibrosis in these animal models and the time frame of suramin administration (i.e., single-dose vs repeated doses). The defective lysosomal degradation of extracellular matrix proteins in ADPKD could be further aggravated by suramin-induced lysosomal damage, which may counteract its potential anti-fibrotic effect [44–46].

One important finding of the current study is the unexpected tubular injury following suramin treatment. These findings are consistent with earlier clinical trials, which have demonstrated that suramin can cause neurotoxicity and nephrotoxicity in patients with

prostate cancer [47]. Acute non-oliguric kidney injury has been reported in a patient with prostate cancer following suramin treatment [48]. Suramin is highly protein-bound and has a long elimination half-life (36 to 54 days) from urinary excretion [49]. In animal experiments, suramin has been shown to induce glycosaminoglycan and sphingolipid accumulation in the renal tubular lysosome [48,50]. A recent study showed that suramin may induce phospholipidosis via the inhibition of lysosomal phospholipase A2 [51]. The dose of suramin used in the current study is still within the non-cytotoxic concentration range according to previous studies in mouse models of cardiac fibrosis and lung injury [28–30]. Although further studies using other animal models for PKD and different dosing schedules are needed to confirm our results, our findings raised an important safety concern about using suramin in the setting of PKD due to drug-induced kidney injury and lysosomal damage. The results also highlight the importance to evaluate the effects of candidate compounds on the non-cystic tubules and glomeruli, which are responsible for maintaining normal kidney function.

4. Materials and Methods

Animals: *Pkd1* miRNA transgenic (referred to as *Pkd1*-miR Tg) mice that present stable and heritable *Pkd1* knockdown (~70%) were used to determine the effects of suramin [27]. This *Pkd1*-deficient model is characterized by a moderately progressive disease course that mimics the gradual cyst development in ADPKD [52]. Wild-type littermates (C57BL/6) were used as controls. Mice were housed under climate-controlled conditions with a 12-h light–dark cycle and free access to drinking water and standard rodent chow. The study was approved by the Animal Care and Use Committee of Chang Gung Memorial Hospital and conformed to the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Experimental design: *Pkd1*-miR Tg mice and wild-type littermates of both genders were allocated to experimental and control groups randomly. The experimental groups received twice-weekly i.p. injections of suramin (60 mg/kg, Tocris Bioscience, Bristol, UK) from P35 for eight weeks according to the effective and non-cytotoxic dose found in mouse models of heart and lung fibrosis [28–30]. The control groups received the same volume of vehicle (normal saline) by i.p. injections. Mice were sacrificed on P90 and blood samples were obtained by cardiac puncture under anesthesia. Both kidneys were harvested and weighed immediately. Tissue samples were snap-frozen in liquid nitrogen and stored at –80 °C for further experiments.

Kidney function tests: Blood urea nitrogen (BUN) levels were measured using VITROS DT60 II Chemistry System (Ortho Clinical Diagnostics, Rochester, NY, USA). The plasma levels of cystatin C, neutrophil gelatinase-associated lipocalin (NGAL) and the kidney injury molecule-1 (KIM-1) were measured using mouse ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions [53].

Histomorphometric analysis: Kidney samples were fixed in 10% neutral-buffered formalin overnight and transferred to 75% ethanol before being embedded in paraffin for sectioning. Hematoxylin and eosin (H&E) staining and periodic acid-Schiff (PAS) staining were performed on transverse kidney sections according to the standard methods. The cystic index was calculated by dividing the area of the cystic lumen by the total kidney area using MetaMorph software (Universal Imaging, West Chester, PA, USA) [54]. All analyses were performed on coded slides in a single-blinded manner.

Immunohistochemistry: Kidney sections (4- μ m thick) were dewaxed, rehydrated, and boiled in citrate buffer (pH 6.0) for antigen retrieval. The slides were blocked with Dako Antibody Diluent (Cat. No. S3022; Dako Agilent Technologies, Santa Clara, CA, USA). Renal macrophages were recognized using a rat anti-mouse F4/80 antibody (1:100, MCA497R; Serotec, Oxford, UK) overnight at 4°C, followed by a biotin-conjugated goat anti-rat light chain antibody (1:1000, AP202B; Sigma-Aldrich, Saint Louis, MO, USA) and streptavidin–horseradish peroxidase (HRP) label (HP604H, Biocare Medical, Concord, CA, USA) 30 min at room temperature. Fibronectin and P2X7 receptor immunostaining were performed us-

ing a rabbit anti-fibronectin antibody (1:250, ab2413; Abcam, Cambridge, UK) and a rabbit polyclonal anti-P2X7 receptor ATTO 550 antibody (1: 50, APR 004 AO; Alomone Laboratories, Jerusalem, Israel) overnight at 4°C, respectively. The signals were detected by using Rabbit-on-Rodent HRP-Polymer (RMR622H; Biocare Medical, Concord, CA, USA) for 30 min at room temperature. A positive reaction was visualized using 3,3'-diaminobenzidine chromogens (K3468; DAKO, Glostrup, Denmark). The slides were counterstained with hematoxylin (Muto Pure Chemicals, Tokyo, Japan). The percentage of F4/80 or fibronectin was calculated using five consecutively selected fields of the renal cortex and medulla at 100× magnification in the sections.

To determine the degree of cell proliferation, mice received an i.p. injection of 5-Bromo-2'-deoxyuridine (BrdU, 0.3 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) four hours before sacrifice. The kidney sections were treated with 2 N HCl for antigen retrieval. BrdU-positive cells were detected by a rabbit monoclonal anti-BrdU antibody (1:1000, ab6326, Abcam, Cambridge, UK). The proliferative index was determined by counting the number of BrdU-positive epithelial cells in sequentially selected fields in each renal section at 200× magnification.

Quantitative polymerase chain reaction: Total RNA was extracted from snap-frozen kidney tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was obtained from the total RNA using a Transcriptor First Strand synthesis kit (Roche, Indianapolis, IN). A quantitative real-time polymerase chain reaction (qRT-PCR) was performed using an ABI ViiA7 sequence detection system (Applied Biosystems, Foster City, CA, USA) with TaqMan assays or SYBR Green primers as listed (Supplementary Table S2). The reactions for each sample were performed in duplicate. The relative mRNA expression levels were calculated using the 2- $\Delta\Delta C_t$ method. Rodent 18S ribosomal RNA was used as an endogenous control.

Western blotting: Proteins were isolated from homogenized frozen kidney specimens. Equal amounts of total protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA). After blocking in 5% fat-free milk, the membrane was incubated individually with the primary antibodies, including rabbit polyclonal anti-phospho-extracellular signal-regulated kinase (ERK1/2) (Thr202/Tyr204) (1:1000, #9101), rabbit monoclonal anti-ERK1/2 (137F5) (1:1000, #4695; Cell Signaling Technology, Danvers, MA, USA), and rabbit monoclonal anti-GAPDH (EPR16891) (1:10000, ab181602; Abcam, Cambridge, UK), at 4 °C overnight. The membranes were further incubated with HRP-conjugated secondary antibodies at room temperature and the signal was detected through the enhanced chemiluminescence method.

Statistical analysis: Values are expressed as mean \pm SEM. Between-group comparisons were performed using one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test. *p* values < 0.05 were considered statistically significant. All analyses were performed using GraphPad Prism 9 (GraphPad, La Jolla, CA, USA).

Conclusions: In summary, the study shows that suramin reduced cyst growth and suppressed renal inflammation in a *Pkd1*-deficient mouse model of ADPKD. However, suramin also induced kidney injuries in non-cystic tubules and overall did not slow the decline of renal function. Our findings do not support a repurposed role of suramin in the treatment of ADPKD progression. Further studies using other purinergic receptor antagonists with less nephrotoxicity may help to elucidate the therapeutic potential of targeting purinergic signaling in ADPKD.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms23158499/s1>.

Author Contributions: Conceptualization, M.-Y.C.; Investigation, S.-H.H., L.-Y.M., L.-F.C.; Data analysis, M.-Y.C., S.-H.H., L.-F.C.; Methodology, S.-H.H., L.-F.C., C.-C.H., Y.-C.T.; Resources, C.-C.H., Y.-C.T.; Supervision, C.-W.Y., and M.-Y.C.; Writing—original draft, M.-Y.C., Writing—review and editing, S.-H.H., L.-F.C.; Funding acquisition, M.-Y.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the Chang Gung Memorial Hospital (CMRPG3D0651, CMRPG3E2001, CMRPG3L0711) and the Ministry of Science and Technology of Taiwan (107-2314-B-182A-016, 110-2314-B-182A-056).

Institutional Review Board Statement: The study was approved by the Animal Care and Use Committee of Chang Gung Memorial Hospital (IACUC No.2013090301, 16 October 2013) and conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Hui-Wen Chiang, Yi-Ching Ko, Chiung-Tzeng Huang, and Jing-Shiuan Liu for their excellent technical assistance. We also thank Si-Tse Jiang for providing the *Pkd1*-miR Tg mice and Taiwan National Laboratory Animal Center for their technical support in breeding and testing services.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cornec-Le Gall, E.; Alam, A.; Perrone, R.D. Autosomal dominant polycystic kidney disease. *Lancet* **2019**, *393*, 919–935. [[CrossRef](#)]
2. Dong, K.; Zhang, C.; Tian, X.; Coman, D.; Hyder, F.; Ma, M.; Somlo, S. Renal plasticity revealed through reversal of polycystic kidney disease in mice. *Nat. Genet.* **2021**, *53*, 1649–1663. [[CrossRef](#)] [[PubMed](#)]
3. Zhou, J.X.; Fan, L.X.; Li, X.; Calvet, J.P. TNF α signaling regulates cystic epithelial cell proliferation through Akt/mTOR and ERK/MAPK/Cdk2 mediated Id2 signaling. *PLoS ONE* **2015**, *10*, e0131043. [[CrossRef](#)]
4. Mallawaarachchi, A.C.; Furlong, T.J.; Shine, J.; Harris, P.C.; Cowley, M.J. Population data improves variant interpretation in autosomal dominant polycystic kidney disease. *Genet. Med.* **2019**, *21*, 1425–1434. [[CrossRef](#)] [[PubMed](#)]
5. Olsan, E.E.; Mukherjee, S.; Wulkersdorfer, B.; Shillingford, J.M.; Giovannone, A.J.; Todorov, G.; Song, X.; Pei, Y.; Weimbs, T. Signal transducer and activator of transcription-6 (STAT6) inhibition suppresses renal cyst growth in polycystic kidney disease. *Proc. Natl. Acad. Sci. USA.* **2011**, *108*, 18067–18072. [[CrossRef](#)] [[PubMed](#)]
6. Yheskel, M.; Lakhia, R.; Cobo-Stark, P.; Flaten, A.; Patel, V. Anti-microRNA screen uncovers miR-17 family within miR-17-92 cluster as the primary driver of kidney cyst growth. *Sci. Rep.* **2019**, *9*, 1920. [[CrossRef](#)] [[PubMed](#)]
7. Torres, V.E.; Gansevoort, R.T.; Czerwiec, F.S. Tolvaptan in Later-Stage Polycystic Kidney Disease. *N. Engl. J. Med.* **2018**, *378*, 489–490. [[CrossRef](#)]
8. Torres, V.E.; Gansevoort, R.T.; Czerwiec, F.S. Tolvaptan in autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* **2013**, *368*, 1259. [[CrossRef](#)] [[PubMed](#)]
9. Nowak, K.L.; Hopp, K. Metabolic Reprogramming in Autosomal Dominant Polycystic Kidney Disease: Evidence and Therapeutic Potential. *Clin. J. Am. Soc. Nephrol.* **2020**, *15*, 577–584. [[CrossRef](#)]
10. Verschuren, E.H.J.; Rigalli, J.P.; Castenmiller, C.; Rohrbach, M.U.; Bindels, R.J.M.; Peters, D.J.M.; Arjona, F.J.; Hoenderop, J.G.J. Pannexin-1 mediates fluid shear stress-sensitive purinergic signaling and cyst growth in polycystic kidney disease. *FASEB J.* **2020**, *34*, 6382–6398. [[CrossRef](#)]
11. Turner, C.M.; Ramesh, B.; Srail, S.K.; Burnstock, G.; Unwin, R.J. Altered ATP-sensitive P2 receptor subtype expression in the Han:SPRD cy/+ rat, a model of autosomal dominant polycystic kidney disease. *Cells Tissues Organs* **2004**, *178*, 168–179. [[CrossRef](#)]
12. Arkhipov, S.N.; Pavlov, T.S. ATP release into ADPKD cysts via pannexin-1/P2X7 channels decreases ENaC activity. *Biochem. Biophys. Res. Commun.* **2019**, *513*, 166–171. [[CrossRef](#)] [[PubMed](#)]
13. Buchholz, B.; Teschemacher, B.; Schley, G.; Schillers, H.; Eckardt, K.U. Formation of cysts by principal-like MDCK cells depends on the synergy of cAMP- and ATP-mediated fluid secretion. *J. Mol. Med. (Berl.)* **2011**, *89*, 251–261. [[CrossRef](#)] [[PubMed](#)]
14. Arkhipov, S.N.; Potter, D.L.; Geurts, A.M.; Pavlov, T.S. Knockout of P2rx7 purinergic receptor attenuates cyst growth in a rat model of ARPKD. *Am. J. Physiol. Ren. Physiol.* **2019**, *317*, F1649–F1655. [[CrossRef](#)] [[PubMed](#)]
15. Chang, M.Y.; Lu, J.K.; Tian, Y.C.; Chen, Y.C.; Hung, C.C.; Huang, Y.H.; Chen, Y.H.; Wu, M.S.; Yang, C.W.; Cheng, Y.C. Inhibition of the P2X7 receptor reduces cystogenesis in PKD. *J. Am. Soc. Nephrol.* **2011**, *22*, 1696–1706. [[CrossRef](#)]

16. Kraus, A.; Grampp, S.; Goppelt-Struebe, M.; Schreiber, R.; Kunzelmann, K.; Peters, D.J.; Leipziger, J.; Schley, G.; Schodel, J.; Eckardt, K.U.; et al. P2Y2R is a direct target of HIF-1 α and mediates secretion-dependent cyst growth of renal cyst-forming epithelial cells. *Purinergic Signal*. **2016**, *12*, 687–695. [[CrossRef](#)] [[PubMed](#)]
17. Abdeen, S.; Salim, N.; Mammadova, N.; Summers, C.M.; Goldsmith-Pestana, K.; McMahon-Pratt, D.; Schultz, P.G.; Horwich, A.L.; Chapman, E.; Johnson, S.M. Targeting the HSP60/10 chaperonin systems of *Trypanosoma brucei* as a strategy for treating African sleeping sickness. *Bioorganic Med. Chem. Lett.* **2016**, *26*, 5247–5253. [[CrossRef](#)] [[PubMed](#)]
18. Zeelen, J.; van Straaten, M.; Verdi, J.; Hempelmann, A.; Hashemi, H.; Perez, K.; Jeffrey, P.D.; Halg, S.; Wiedemar, N.; Maser, P.; et al. Structure of trypanosome coat protein VSG_{sur} and function in suramin resistance. *Nat. Microbiol.* **2021**, *6*, 392–400. [[CrossRef](#)] [[PubMed](#)]
19. Stein, C.A. Suramin: A novel antineoplastic agent with multiple potential mechanisms of action. *Cancer Res.* **1993**, *53*, 2239–2248. [[PubMed](#)]
20. Wiedemar, N.; Hauser, D.A.; Maser, P. 100 Years of Suramin. *Antimicrob Agents Chemother* **2020**, *64*, e01168-19. [[CrossRef](#)]
21. Wang, Y.; Qing, J.; Sun, Y.; Rao, Z. Suramin inhibits EV71 infection. *Antivir. Res.* **2014**, *103*, 1–6. [[CrossRef](#)]
22. Kavvadas, P.; Abed, A.; Poulain, C.; Authier, F.; Labejof, L.P.; Calmont, A.; Afieri, C.; Prakoura, N.; Dussaule, J.C.; Chatziantoniou, C.; et al. Decreased Expression of Connexin 43 Blunts the Progression of Experimental GN. *J. Am. Soc. Nephrol.* **2017**, *28*, 2915–2930. [[CrossRef](#)] [[PubMed](#)]
23. Rees, S.; Constantopoulos, G.; Brady, R.O. The suramin-treated rat as a model of mucopolysaccharidosis. Variation in the reversibility of biochemical and morphological changes among different organs. *Virchows Archiv. B Cell Pathol. Incl. Mol. Pathol.* **1986**, *52*, 259–272. [[CrossRef](#)] [[PubMed](#)]
24. Guo, C.; Wu, Z.; Lin, W.; Xu, H.; Chang, T.; Dai, Y.; Lin, D. Suramin Targets the Conserved Ligand-Binding Pocket of Human Raf1 Kinase Inhibitory Protein. *Molecules* **2021**, *26*, 1151. [[CrossRef](#)] [[PubMed](#)]
25. Naviaux, R.K.; Zolkipli, Z.; Wang, L.; Nakayama, T.; Naviaux, J.C.; Le, T.P.; Schuchbauer, M.A.; Rogac, M.; Tang, Q.; Dugan, L.L.; et al. Antipurinergic therapy corrects the autism-like features in the poly(IC) mouse model. *PLoS ONE* **2013**, *8*, e57380. [[CrossRef](#)]
26. Liu, N.; Tolbert, E.; Pang, M.; Ponnusamy, M.; Yan, H.; Zhuang, S. Suramin inhibits renal fibrosis in chronic kidney disease. *J. Am. Soc. Nephrol.* **2011**, *22*, 1064–1075. [[CrossRef](#)] [[PubMed](#)]
27. Wang, E.; Hsieh-Li, H.M.; Chiou, Y.Y.; Chien, Y.L.; Ho, H.H.; Chin, H.J.; Wang, C.K.; Liang, S.C.; Jiang, S.T. Progressive renal distortion by multiple cysts in transgenic mice expressing artificial microRNAs against Pkd1. *J. Pathol.* **2010**, *222*, 238–248. [[CrossRef](#)]
28. De Oliveira Moreira, D.; Pereira, J.A.; Taniguti, A.P.; Matsumura, C.Y.; Ramos, L.A.; Areas, M.A.; Neto, H.S.; Marques, M.J. Suramin attenuates dystrophin-deficient cardiomyopathy in the mdx mouse model of duchenne muscular dystrophy. *Muscle Nerve* **2013**, *48*, 911–919. [[CrossRef](#)]
29. Taniguti, A.P.; Pertille, A.; Matsumura, C.Y.; Santo Neto, H.; Marques, M.J. Prevention of muscle fibrosis and myonecrosis in mdx mice by suramin, a TGF- β 1 blocker. *Muscle Nerve* **2011**, *43*, 82–87. [[CrossRef](#)] [[PubMed](#)]
30. Lossos, I.S.; Izbicki, G.; Or, R.; Goldstein, R.H.; Breuer, R. The effect of suramin on bleomycin-induced lung injury. *Life Sci.* **2000**, *67*, 2873–2881. [[CrossRef](#)]
31. Kuo, S.C.; Wang, Y.M.; Ho, Y.J.; Chang, T.Y.; Lai, Z.Z.; Tsui, P.Y.; Wu, T.Y.; Lin, C.C. Suramin treatment reduces chikungunya pathogenesis in mice. *Antivir. Res.* **2016**, *134*, 89–96. [[CrossRef](#)] [[PubMed](#)]
32. Takano, S.; Gately, S.; Neville, M.E.; Herblin, W.F.; Gross, J.L.; Engelhard, H.; Perricone, M.; Eidsvoog, K.; Brem, S. Suramin, an anticancer and angiostatic agent, inhibits endothelial cell binding of basic fibroblast growth factor, migration, proliferation, and induction of urokinase-type plasminogen activator. *Cancer Res.* **1994**, *54*, 2654–2660. [[PubMed](#)]
33. Dupre, T.V.; Doll, M.A.; Shah, P.P.; Sharp, C.N.; Kiefer, A.; Scherzer, M.T.; Saurabh, K.; Saforo, D.; Siow, D.; Casson, L.; et al. Suramin protects from cisplatin-induced acute kidney injury. *Am. J. Physiol. Ren. Physiol.* **2016**, *310*, F248–F258. [[CrossRef](#)] [[PubMed](#)]
34. Korrapati, M.C.; Howell, L.A.; Shaner, B.E.; Megyesi, J.K.; Siskind, L.J.; Schnellmann, R.G. Suramin: A potential therapy for diabetic nephropathy. *PLoS ONE* **2013**, *8*, e73655. [[CrossRef](#)] [[PubMed](#)]
35. Liu, N.; He, S.; Tolbert, E.; Gong, R.; Bayliss, G.; Zhuang, S. Suramin alleviates glomerular injury and inflammation in the remnant kidney. *PLoS ONE* **2012**, *7*, e36194. [[CrossRef](#)]
36. Yamaguchi, T.; Reif, G.A.; Calvet, J.P.; Wallace, D.P. Sorafenib inhibits cAMP-dependent ERK activation, cell proliferation, and in vitro cyst growth of human ADPKD cyst epithelial cells. *Am. J. Physiol. Ren. Physiol.* **2010**, *299*, F944–F951. [[CrossRef](#)]
37. Queipo, M.J.; Gil-Redondo, J.C.; Morente, V.; Ortega, F.; Miras-Portugal, M.T.; Delicado, E.G.; Perez-Sen, R. P2X7 Nucleotide and EGF Receptors Exert Dual Modulation of the Dual-Specificity Phosphatase 6 (MKP-3) in Granule Neurons and Astrocytes, Contributing to Negative Feedback on ERK Signaling. *Front. Mol. Neurosci.* **2017**, *10*, 448. [[CrossRef](#)] [[PubMed](#)]
38. Turner, C.M.; King, B.F.; Srani, K.S.; Unwin, R.J. Antagonism of endogenous putative P2Y receptors reduces the growth of MDCK-derived cysts cultured in vitro. *Am. J. Physiol. Ren. Physiol.* **2007**, *292*, F15–F25. [[CrossRef](#)]
39. Malas, T.B.; Leonhard, W.N.; Bange, H.; Granchi, Z.; Hettne, K.M.; Van Westen, G.J.P.; Price, L.S.; 't Hoen, P.A.C.; Peters, D.J.M. Prioritization of novel ADPKD drug candidates from disease-stage specific gene expression profiles. *EBioMedicine* **2020**, *51*, 102585. [[CrossRef](#)]
40. Liu, N.; Tolbert, E.; Ponnusamy, M.; Yan, H.; Zhuang, S. Delayed administration of suramin attenuates the progression of renal fibrosis in obstructive nephropathy. *J. Pharmacol. Exp. Ther.* **2011**, *338*, 758–766. [[CrossRef](#)]
41. Xue, C.; Mei, C.L. Polycystic Kidney Disease and Renal Fibrosis. *Adv. Exp. Med. Biol.* **2019**, *1165*, 81–100. [[CrossRef](#)] [[PubMed](#)]

42. Cassini, M.F.; Kakade, V.R.; Kurtz, E.; Sulkowski, P.; Glazer, P.; Torres, R.; Somlo, S.; Cantley, L.G. Mcp1 Promotes Macrophage-Dependent Cyst Expansion in Autosomal Dominant Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* **2018**, *29*, 2471–2481. [[CrossRef](#)] [[PubMed](#)]
43. Yang, Y.; Chen, M.; Zhou, J.; Lv, J.; Song, S.; Fu, L.; Chen, J.; Yang, M.; Mei, C. Interactions between Macrophages and Cyst-Lining Epithelial Cells Promote Kidney Cyst Growth in Pkd1-Deficient Mice. *J. Am. Soc. Nephrol.* **2018**, *29*, 2310–2325. [[CrossRef](#)] [[PubMed](#)]
44. Mangos, S.; Lam, P.Y.; Zhao, A.; Liu, Y.; Mudumana, S.; Vasilyev, A.; Liu, A.; Drummond, I.A. The ADPKD genes pkd1a/b and pkd2 regulate extracellular matrix formation. *Dis. Models Mech.* **2010**, *3*, 354–365. [[CrossRef](#)] [[PubMed](#)]
45. Akanji, M.A. Rat kidney lysosomal membrane damage induced by suramin in vitro and in vivo. *Pharmacol. Toxicol.* **1988**, *62*, 318–321. [[CrossRef](#)]
46. McKleroy, W.; Lee, T.H.; Atabai, K. Always cleave up your mess: Targeting collagen degradation to treat tissue fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2013**, *304*, L709–L721. [[CrossRef](#)] [[PubMed](#)]
47. Eisenberger, M.A.; Reyno, L.M.; Jodrell, D.I.; Sinibaldi, V.J.; Tkaczuk, K.H.; Sridhara, R.; Zuhowski, E.G.; Lowitt, M.H.; Jacobs, S.C.; Egorin, M.J. Suramin, an active drug for prostate cancer: Interim observations in a phase I trial. *J. Natl. Cancer Inst.* **1993**, *85*, 611–621. [[CrossRef](#)] [[PubMed](#)]
48. Figg, W.D.; Cooper, M.R.; Thibault, A.; Headlee, D.; Humphrey, J.; Bergan, R.C.; Reed, E.; Sartor, O. Acute renal toxicity associated with suramin in the treatment of prostate cancer. *Cancer* **1994**, *74*, 1612–1614. [[CrossRef](#)]
49. Edwards, G.; Breckenridge, A.M. Clinical pharmacokinetics of anthelmintic drugs. *Clin. Pharm.* **1988**, *15*, 67–93. [[CrossRef](#)]
50. Constantopoulos, G.; Rees, S.; Cragg, B.G.; Barranger, J.A.; Brady, R.O. Experimental animal model for mucopolysaccharidosis: Suramin-induced glycosaminoglycan and sphingolipid accumulation in the rat. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 3700–3704. [[CrossRef](#)]
51. Hinkovska-Galcheva, V.; Treadwell, T.; Shillingford, J.M.; Lee, A.; Abe, A.; Tesmer, J.J.G.; Shayman, J.A. Inhibition of lysosomal phospholipase A2 predicts drug-induced phospholipidosis. *J. Lipid. Res.* **2021**, *62*, 100089. [[CrossRef](#)] [[PubMed](#)]
52. Happe, H.; Peters, D.J. Translational research in ADPKD: Lessons from animal models. *Nat. Rev. Nephrol.* **2014**, *10*, 587–601. [[CrossRef](#)] [[PubMed](#)]
53. Warner, G.; Hein, K.Z.; Nin, V.; Edwards, M.; Chini, C.C.; Hopp, K.; Harris, P.C.; Torres, V.E.; Chini, E.N. Food Restriction Ameliorates the Development of Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* **2016**, *27*, 1437–1447. [[CrossRef](#)]
54. Novalic, Z.; van der Wal, A.M.; Leonhard, W.N.; Koehl, G.; Breuning, M.H.; Geissler, E.K.; de Heer, E.; Peters, D.J. Dose-dependent effects of sirolimus on mTOR signaling and polycystic kidney disease. *J. Am. Soc. Nephrol.* **2012**, *23*, 842–853. [[CrossRef](#)] [[PubMed](#)]