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Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease

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In autosomal dominant polycystic kidney disease (ADPKD), abnormal proliferation of tubular cells drives cyst development and growth. Sirolimus, an inhibitor of the protein kinase mammalian target of rapamycin (mTOR) and a potent anti-proliferative agent, decreases cyst growth in several genetically distinct rodent models of polycystic kidney disease (PKD). We determined here the effect of sirolimus on renal cyst growth in Pkd2WS25/− mice; an ortholog of human ADPKD involving mutation of the *Pkd2* gene. In Pkd2WS25/− mice treated with sirolimus, both the two kidney/total body weight (2K/TBW) ratio and the cyst volume density (CVD) were significantly decreased by over half compared with untreated mice suffering with PKD. However, there was no effect on the increased blood urea nitrogen (BUN) levels as an index of kidney function. There are two distinct complexes containing mTOR depending on its binding partners: mTORC1 and mTORC2. Western blot analysis of whole kidney lysates and immunohistochemistry of the cysts found that phospho-S6 ribosomal protein, a marker of mTORC1 activity, was increased in Pkd2WS25/− mice and its phosphorylation was decreased by sirolimus treatment. Phospho-Akt at serine 473, a marker associated with mTORC2 activity, was not different between Pkd2WS25/− mice and normal littermate controls. Hence, our study found that inhibition of mTORC1 by sirolimus correlated with decreased renal cyst growth in this model of human ADPKD but had no effect on the decline in renal function.

Kidney International (2010) **78,** 754–761; doi:10.1038/ki.2010.250; published online 4 August 2010

KEYWORDS: polycystic kidney disease; signaling; sirolimus polycystic kidney mouse

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Received 8 January 2010; revised 9 April 2010; accepted 11 May 2010; published online 4 August 2010

We have previously shown in the Han:SPRD rat model of polycystic kidney disease (PKD) that sirolimus treatment decreases proliferation in cystic and non-cystic tubules, markedly inhibits renal enlargement and cystogenesis and prevents the loss of kidney function.[1] Subsequently two other studies have shown that mammalian target of rapamycin (mTOR) inhibition with sirolimus[2] or everolimus[3] reduces cyst formation and renal failure in the Han:SPRD rat. It has also recently been shown that sirolimus decreases cyst formation and renal failure in the orpk-rescue mouse (defective cilia protein polaris) and the bpk mouse (over-expressing myelin and lymphocyte protein) models of PKD.[4] Autosomal dominant polycystic kidney disease (ADPKD) in humans is caused by a mutation in the *Pkd1* or *Pkd2* gene. The Han:SPRD rat, orpk, and bpk mouse do not have primary abnormalities of the *Pkd1* or *Pkd2* genes as the cause of the PKD. In this study, we tested the hypothesis that sirolimus therapy would decrease PKD caused by a mutation in the *Pkd2* gene, in the Pkd2WS25/− mouse model. These mice were engineered to have one of the *Pkd2* alleles knocked out and the other *Pkd2* allele capable of undergoing high rates of recombination with one of the recombination products resulting in the loss of a functional gene. This study in Pkd2WS25/− mice is important as not all therapies that ameliorate murine cystic disease in diverse models may be effective in disease caused by *Pkd* gene mutations and the Pkd2WS25/− mice model closely resembles human ADPKD.

The mTOR exists in association with two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTORC1 is a complex of mTOR and Raptor (regulatory associated protein of mTOR) whereas mTORC2 is a complex of mTOR and Rictor (sirolimus-independent companion of mTOR). The mTORC1 complex regulates cell growth (size), proliferation, apoptosis, and autophagy. Activation of mTORC1 has been shown in PKD in rodents[3–5] and in humans.[6] The effect of PKD and sirolimus on mTORC2 is not known. The mTORC2 increases proliferation, inhibits apoptosis, regulates the actin cytoskeleton, and can phosphorylate Akt at serine 473.[7] As proliferation and apoptosis[8,9] cytoskeleton abnormalities[10,11]

and activation of Akt[3] are features of PKD, the degree of mTORC2 activation was determined.

In this study, we tested the hypotheses that there would be increased mTORC1 and mTORC2 activation in Pkd2WS25/− mouse kidneys and that the mTORC1 inhibitor, sirolimus would decrease PKD in Pkd2WS25/− mice.

RESULTS

Effect of sirolimus on body weight, kidney weight, 2K/TBW, CVD, and BUN

Sirolimus had no effect on the body weight (Table 1). This is in contrast to previous reports showing that short-term (5 week) 0.2 mg/kg/d sirolimus treatment in Han:SPRD rats and wild-type male rats resulted in a 22% loss of body weight.[1] In this study, mice received a higher dose of sirolimus (that is, 0.5 mg/kg/d) for a longer period of time (that is, 8–12 weeks) but did not have weight loss.

Representative kidney sections of vehicle-treated Pkd2WS25/− mice and sirolimus-treated Pkd2WS25/− mice, stained with hematoxylin-eosin, at the same magnification are shown in Figure 1a and b. These representative sections show that the percentage of the kidney that is occupied by cysts is markedly reduced in the kidney obtained from the sirolimus-treated Pkd2WS25/− mice.

The two kidney/total body weight ratio (2K/TBW), which corrects for differences in body weight, was nearly double in Pkd2WS25/− mice compared with vehicle-treated +/+ mice. Sirolimus resulted in a 61% decrease in 2K/TBW (Table 1).

The increase in kidney weights in the Pkd2WS25/− mice was directly paralleled by increases in the measured cyst volume density (CVD) in the kidneys. Mean CVD percentage was 39% in Pkd2WS25/− mice treated with vehicle and 20% in Pkd2WS25/− mice treated with sirolimus. Sirolimus resulted in a 51% decrease in CVD (Table 1).

The blood urea nitrogen (BUN) level was increased in vehicle-treated Pkd2WS25/− mice and sirolimus-treated Pkd2WS25/− mice compared with vehicle-treated +/+ mice and sirolimus-treated +/+ mice. BUN concentration was not different between vehicle-treated Pkd2WS25/− mice and sirolimus-treated Pkd2WS25/− mice (Table 1).

Female Pkd2WS25/− mice have less severe PKD than that that in males.[12] Only two of the ten Pkd2WS25/− vehicle and two of the ten Pkd2WS25/−sirolimus-treated mice were female. The two female Pkd2WS25/− mice treated with sirolimus had a decrease in 2K/TBW (%) and CVD compared with female littermate controls.

In the Pkd2WS25/− model, liver cyst formation increases significantly after 16 weeks of age.[12] This study ended at 16 weeks of age. At the end of the study, liver weight was identical (1.6 g) in Pkd2Ws25/− treated with vehicle and Pkd2Ws25/− treated with sirolimus.

Renal fibrosis

To evaluate renal fibrosis, quantitation of collagen deposition by Masson's trichrome staining was carried out. As shown in Figure 2, sirolimus reduced collagen staining. The renal failure in PKD has been linked to fibrosis. However, despite the reduction in collagen staining by sirolimus, there was no effect on kidney function by sirolimus.

Apotosis and caspase-3

The number of terminal deoxynucleotidyltransferase (TdT)-mediated dUTP nick-end labeling (TUNEL)-positive apoptotic cells per cyst was 0.15 ± 0.06 in Pkd2WS25/− mice treated with the vehicle and 0.26 ± 0.09 in Pkd2WS25/− mice treated with sirolimus (P = not significant vs vehicle-treated, $N = 4$) (Figure 3). Representative pictures of TUNEL staining are shown in Figure 3.

Caspase-3 is the major mediator of apoptosis. In support of the data that there is no significant difference in apoptosis with sirolimus treatment, caspase-3 activity was not

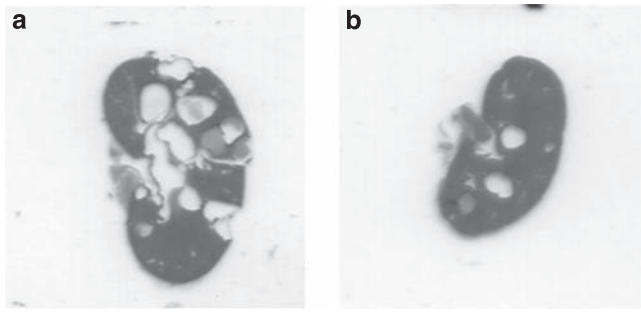

Figure 1 | Effects of sirolimus on polycystic kidney disease (PKD) in Pkd2WS25/− mice. Representative sections at the same magnification, stained with hematoxylin and eosin, show more renal cysts in (**a**) vehicle-treated compared with (**b**) sirolimus-treated Pkd2WS25/− mice.

Table 1 | Effects of sirolimus in Pkd2WS25/− mice

	+/+ vehicle (n=24)	+/+ sirolimus (n=8)	Pkd2WS25/− vehicle (N=10)	Pkd2WS25/− sirolimus (N=10)
Body weight (g)	22.8 ± 3.1	23.4 ± 0.7	27.4 ± 1	27.6 ± 1
Kidney weight (g)	0.31 ± 0.01	0.27 ± 0.02	0.60 ± 0.11*	0.44 ± 0.04**
2K/TBW (%)	1.24 ± 0.02	1.15 ± 0.05	2.20 ± 0.4*	1.63 ± 0.1**
CVD (%)	0.5 ± 0.2	0.5 ± 0.2	38.8 ± 3.7†	20.4 ± 5.1††
BUN (mg/dl)	16.7 ± 0.9	17.5 ± 0.9	33.8 ± 4.2*	29.2 ± 1.5‡

Abbreviations: BUN, blood urea nitrogen; CVD, cystic volume density; NS, not significant; 2K/TBW, two kidney/total body weight.
*P<0.01 vs +/+ vehicle and +/+ sirolimus, **P<0.05 vs Pkd2WS25/− vehicle, NS vs +/+ vehicle and +/+ sirolimus. $^{\dagger}P$<0.001 +/+ vehicle and +/+ sirolimus, $^{\dagger\dagger}P$<0.01 vs Pkd2WS25/− vehicle, ‡NS vs Pkd2WS25/− vehicle. The P-value for the interaction between sirolimus and genotype was NS for kidney weight, NS for 2K/TBW (%), 0.009 for CVD (%) and NS for BUN.