General Medicine: Open Access
ISSN: 2327-5146

Liern et al., General Med 2015, 3:1
DOI: 10.4172/2327-5146.1000160

Research Article Open Access

Use of Rapamycin in Pediatric Patients with Autosomal Dominant Polycystic Kidney Disease

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Rec date: June 10, 2014 **Acc date:** Jan 20, 2015 **Pub date:** Jan 26, 2015

Abstract

Background: Autosomal dominant polycystic disease (ADPKD) is characterized by the development of renal cysts.

Objectives: 1) To assess the kidney and cyst volume growth in patients treated with Rapamycin compared to patients who receive standard ADPKD treatment. 2) Evaluate the occurrence of adverse effects related to the use of Rapamycin. 3) To assess the changes in blood pressure, proteinuria and estimated Glomerular Filtration rate (eGFR).

Materials and Methods: During 24 months were randomized 12 patients with ADPKD in a Rapamycin group (6 patients, received rapamycin 2-3 mg/m^2/day, max. 5mg/día) and a Control group (standard treatment).

Results: Of the 12 patients, 6 entered the Rapamycin group and had a total renal volume and cystic volume increase at study completion of 13% and 32% respectively. In the Control group (6 patients) the increases were 11% and 23% respectively. The eGFR was normal for both groups.

The proteinuria for rapamycin group and control group was initially 7.3 mg/m^2/hour and 6 mg/m^2/hour respectively, at the end was normal for both groups. 3 patients had arterial hypertension, but at the 24th month, were normal. The adverse effects were: Anemia, diarrhea and oral sores.

Conclusions: Rapamycin not decrease the kidney volume and cystic. There was no significant increase in proteinuria or eGFR decrease.

Mean blood pressure remained normal.

Keywords: Rapamycin; Autosomal dominant polycystic disease; Chronic renal failure

Introduction

Autosomal dominant polycystic kidney disease is the most common hereditary kidney disorder, characterized by the progressive, bilateral development and enlargement of focal cysts. Affected individuals usually present in the 3rd and 4th decade of life, progressing to end stage renal disease (ESRD) within 5–10 years after the development of renal insufficiency [1].

ADPKD occurs in 1/500 to 1/1000 of live births. Although all races and both sexes are equally affected, the renal phenotype may be more severe in males [2].

Most, if not all, cases of ADPKD are caused by mutations in two genes, PKD1 (located on the short arm of chromosome 16) and PKD2 (located on the long arm of chromosome 4). Of affected families, 85% have mutations in PKD, which encodes for Polycystin, the rest have mutations in PKD2 which encodes for Polycystin 2.

A defect in either protein leads to renal cyst formation and an overlapping clinical phenotype [3,4].

The phenotype of PKD1 phenotype is significantly more severe than PKD2, with an increased number of cysts forming at an earlier time point and an average age of ESRD onset occurring 20 years earlier than in patients with PKD2 [5,6].

The evidence indicates that the progressive increase in kidney volume in patients with ADPKD is primarily due to the accumulation of fluid within innumerable cysts and the proliferation of renal epithelial cells, a dysregulated tubular epithelial cell growth [7-9].

Objectives

The mainstay of treatment is controlling hypertension, reducing proteinuria, and treating infections [10-12].

Rapamycin (sirolimus), an inhibitor of the mammalian target of rapamycin (mTOR) –with antiproliferative and growth inhibiting effects, was shown to significantly inhibit cyst growth in animal models. It is hypothesized that Rapamycin also significantly inhibits

cyst growth and preserves renal function in patients with ADPKD [13-15].

Primary

Assess the kidney and the cyst volume growth in patients with ADPKD treated with Rapamycin compared to patients who receive standard ADPKD treatment.

Secondary

Evaluate the occurrence of adverse effects (AE) related to the use of Rapamycin.

Assess the changes in blood pressure (BP), proteinuria, and eGFR related to disease progression.

Materials and Methods

Between March 2010 and March 2012 we enrolled 12 patients with a diagnosis of ADPKD, usually seen at the Pediatric Nephrology Section of our hospital.

Patients had a positive family history of ADPKD in more than a generation (Table 1) and were diagnosed by renal ultrasound using the modified Ravine criteria [16].

Group	Age in years	Gender	Affected relative	Time since diagnosis (years)	First symptom diagnosed					HTN	
Rapamycin					N	H	PrU	HTN	Pain	Initial	Final
1	8	f	Father	3		X	X			No	No
2	19	m	Father	14			X	X		Yes	No
3	11	f	Mother	8		X		X		Yes	No
4	9	m	Mother	4					X	No	No
5	12	f	Father	8			X			No	No
6	15	f	Father	10	X					No	No
Control											
1	14	f	Mother	11		X			X	No	No
2	17	f	Mother	13			X	X		Yes	No
3	16	m	Father	13	X					No	No
4	15	m	Father	10		X				No	No
5	9	f	Mother	4			X		X	No	No
6	11	f	Father	2	X					No	No

Table 1: Characteristics of the Patients at Randomization. References: m: masculine; f: femenine;N: none; H: hematuria; PrU:proteinuria; HTN: Hypertension

a) Inclusion criteria

- Increase of the Kidney volume greater than 5.5% in the last 12 months.
- eGFR higher than 60 ml/min/, 73m^2
- Negative pregnancy test

b) Exclusion criteria

- Leukopenia (White blood cell count less than 4.000 white cells/mm^3)
- Chronic hepatic disease
- Systemic illness: Diabetes Mellitus, hypothyroidism, thalassemia, etc.
- Coagulation disorders
- Tumors
- Uni or bilateral hydronephrosis
- Renal vascular disease (stenosis and/or insufficiency)
- Prolonged treatment for other diseases
- Use of immunosuppressive drugs in the last 3 months
- c) Discontinuation criteria
- eGFR decline greater than 30% of the initial value.
- Positive pregnancy test
- White blood cells (WBC) count less than 4000/mm^3 or less than 50% of the initial value.
- Lympho-proliferative disorders or tumors
- Infections compromising at least one vital organ (lungs, heat, brain) and/or positive blood cultures.
- Coagulation disorders

- Inflammatory, infectious or neoplastic hepatic disease

d) Patients were randomized into

Rapamicyn group: 6 patients received an initial dose of this drug of 2-3 mg/m^2/day (maximum 5mg/day), once a day, adjusted according to blood levels and the expected response of growth retardation and cystic renal volume

Control group (standard care only) 6 patients.

e) Patients returned every 30 days for scheduled efficacy/ safety evaluations until the end of the study. These studies included

At the beginning and the end of the study (24th month)

Magnet resonance image (MRI): General Electric Healthcare Signa HDxt 1.5 T. The sequences performed were FIESTA, T1 and T2 without contrast with axial, sagittal and coronal planes. In order to estimate cyst volume, cysts were measured on each side, in 3D axis. The total cyst volume (TCV) was the addition of all cysts measured in each patient. Total kidney volume (TKV) was measured in T, while cyst volume in T2.

At the beginning of the study and every six months

Renal ultrasonography(US) performed always by the same sonographer with: Samsung Medison MySono U5 ultrasound machine. Kidney volume was the summation of: longitudinal, transverse and anteroposterior measurements x 0.5233.

At the beginning of the study and monthly

Height, weight and blood pressure (BP). Blood pressure was assessed according to age, gender and height blood and pressure percentiles

24 hours urinary protein excretion: enzyme immunoassays test (EIA), expressed in mg/m^2/hour.

Serum albumin: EIA, expressed in gr/dl.

Total cholesterol: EIA, expressed in mg/dl.

Serum creatinine: EIA, expressed in mg/dl.

Cell blood count

Rapamycin levels:

Determined in whole-blood samples, that were taken 12 hours after the last dose, and analyzed by immunoassay, expressed in ng/ml. The first measurement was performed 72 hours after initial dose. Rapamycin was always taken at the same time daily in order to diminish variation in the drug bioavailability.

The eGFR was assessed using the modified Schwartz formula for pediatric patients, expressed in ml/min/, 73 m^2 [17].

A pregnancy test was performed on all women prior to enrollment in the study, and every 3 months during the study.

f) Study design

Single-center, randomized controlled trial, no blinded, Parallel-group, phase 3, off-label, to evaluate the efficacy and safety of the Rapamycin treatment in ADPKD.

1f) Outcome variables

Primary: Percent rate of TKV and TCV increase in two years as assessed by MRI.

Secondary: Change in renal function defined by eGFR, hypertension changes in proteinuria.

2f) Statistical analysis

Patients were randomized into the Control Group (6 patients) or Rapamycin Group (6 patients) with the statistical software EPIDAT 3.0.

Relative Risk (RR) greater than , exposed risk (Rapamycin Group) and non-exposed risk (Control Group) were used to statistically assess the total kidney volume and total cyst volume changes as a primary outcome related with the treatment with Rapamycin or standard of care treatment (i.e. hypertension control, proteinuria control, etc) .

Chi-square test (Yates correction) was used as continuous probability distribution to assess the effect of a therapeutic intervention between 2 paired means of a population.

P <0,05 was considered statistically significant.

The power accepted to detecta association between the use of Rapamycin and the reduction of the kidney and cyst volumen was 80%.

The Spearman correlation coefficient was used to assess the relation between non-parametrics continuous variables (Kidney volume and cyst volume increase in the same kidney).

Data are presented as means ±SD and were analyzed by statistical software EPIDAT 3.0 and GraphPad.

The study took place for 24 months and was approved by the Ethics Committee of our hospital.

Informed consent was obtained from the children and their parents. The study was carried out in accordance with the ethical standard laid down in the Declaration of Helsinki

Results

12 patients with ADPKD were enrolled (8 female patients), mean age: 13 years (range 8-19 years), and randomized into two groups: Rapamycin (6 patients) and Control (6 patients).

With MRI the TKV increase was 13% in Rapamycin group (Exposed risk 46%) and 11% in the Control group (Non-exposed risk 47%). Relative Risk (RR): 0.98 (95% Confidence interval: 0.89-1.07).

Chi-squared test (Yates correction): 0, 1348. p 0.7135 (Non-significant).

TCV increase was 32% in Rapamycin group (Exposed risk 46%) and 23% in Control group (Non exposed risk 48%). RR 0.96 (95% CI 0.81-1.15) (Table 2).

Chi-squared test (Yates correction): 0.0713, p 0.78 (Non-significant).

Spearman's rank correlation coefficient between renal and cyst volume was r=0. 71 for Rapamycin group (p: 0.02 significant), and r = 0.6871 for Control group (p: 0.03, significant).

Rapamycin group N: 6 patients	**0 month**	**24th month**	**P value**
Total kidney volume (mean)	502 cm3	572 cm3	0.71(NS)
SD	114 cm3	130 cm3	
TKV Percent increase		13%	
Total cyst volume (mean)	128 cm3	149 cm3	
SD	28 cm3	34 cm3	
TCV percent increase		32%	0.78(NS)
Control group N: 6 patients			
Total kidney volume (mean)	448 cm3	492 cm3	0.71(NS)
SD	144 cm3	146 cm3	
TKV percent increase (mean)		11%	
TCV	143 cm3	157 cm3	0.78(NS)
SD	31 cm3	38 cm3	
TCV percent increase		23%	

Table 2: Total kidney volume and total cyst volume increase in Rapamycin and Control Group

With Renal ultrasonography (at the beginning of the study, the average TKV for the Rapamycin group was 480 cm^3 (SD 92 cm^3) and for the Control group: 472 cm^3(SD 134 cm3). At the end of study, TKV was 592 cm^3 (SD 115 cm^3) and 484 cm^3 (SD 135 cm^3), respectively.

Rapamycin mean dosage: 4 mg/day (range 3-5 mg/day) with average blood levels of 8.8 ng/ml (range: 7.9-9.5 ng/ml) (Table 2).

3 patients (2 of Rapamycin group and 1 of Control group) had blood pressure values between 95 and 99 percentiles (average systolic blood pressure (SBP) 138 mmHg and diastolic (DBP) 88 mmHg), but at the 24th month time point, the blood pressure was under the 90th percentile in these 3 patients (SBP 110 mmHg y DBP 68 mmHg). We calculated an average initial eGFR of 100 ml/min/,73 m^2 in Rapamycin group, and 101 ml/min/,73 m^2 in Control group, and at 24th month the values were 99 ml/min/,73 m^2 and 102 ml/min/, 73 m^2, respectively (Figure 1).

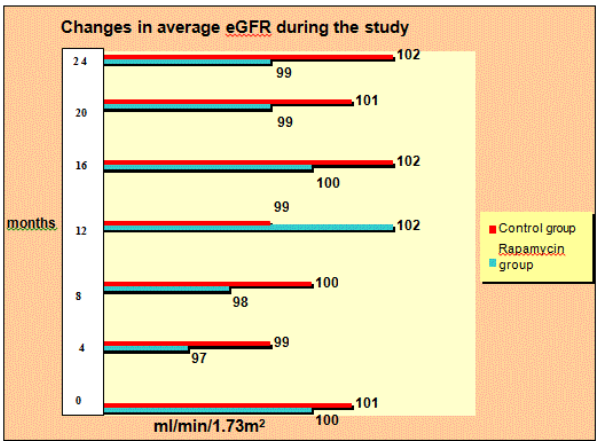

Figure 1: During the study the eGFR average does not vary significantly. n: 12 patients

The average urinary protein excretion for the Rapamycin group was 7.3 mg/m^2/hour at study initiation and at the end of the study, was reduced to 3.9 mg/m2/hour. The 3 patients with significant proteinuria received an average dose of enalapril 0,22 mg/kg/day (range 0,18-0,32 mg/kg/day) and average dose of losartan 1.1 mg/kg/day (range:1-1.2 mg/kg/day). The treatment was effective in reducing proteinuria in all 3 patients but remained above desired levels in 1 of the 3.

In the control group, the proteinuria was 6 mg/m2/hour at study initiation and at 24th month 3.5 mg/m^2/hour. 2 patients with significant proteinuria received an average dose of enalapril of 0,26 mg/kg/day (range 0.21-0.30 mg/kg/day) and an average dose of losartan ,2 mg/kg/day. This treatment was effective in both patients but only in one patient was proteinuria reduced to normal levels (Figure 2).

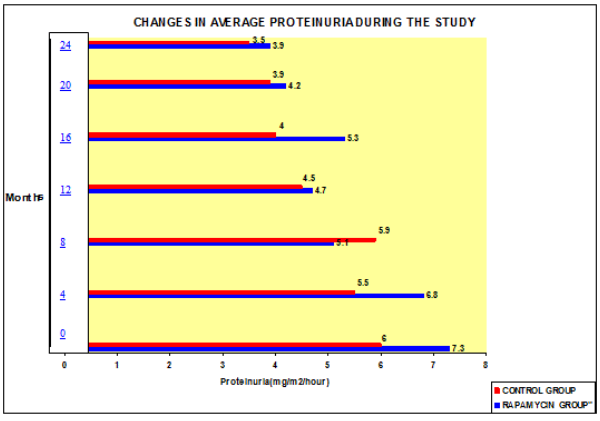

Figure 2: After the 24-month study proteinuria remained within the physiological range in all patients. n: 12 patients

The AE observed in Rapamicyn group were:

a) Anemia: 2 patients, 1 with low ferritin levels and with a good response to treatment with ferrous sulfate. The second patient with normal ferritin levels and low transferrin saturation received ferrous sulfate, folic acid and B vitamin.

b) Acute diarrhea: 4 patients. The symptoms were resolved with diet and the temporary reduction of Rapamycin.

c) Oral sores: 4 patients, resolved with sucralfate mouthwash.

Discussion

To our knowledge this is the first study evaluating the use of Rapamycin in pediatric patients with ADPKD. We assessed the efficacy of this mTOR inhibitor to reduce the total kidney volume growth in patients with ADPKD, Contrary to our hypothesis; we found no difference between the group treated with Rapamycin and the group who received standard care after 24 months.

ADPKD is a frequent inherited disease and its prevalence is 1/500 to 1/1000 live births. Statistically, half of hese patients will progress to ESRD between the fifth and sixth decade of life [18,19]. Ciliopathies comprise a group of disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of cilia. ADPKD is caused by a mutation in either of two genes, PKD1 (located on chromosome 16) or PKD2 (located on chromosome 4), which encode transmembrane proteins, polycystin 1 and polycystin 2, respectively. The hallmarks of this inherited condition are massively enlarged kidneys caused by the sustained expansion of innumerable fluid-filled cysts that derive from microscopic tubule precursors [20,21]. Both PKD1 and PKD2 mutations lead to ADPKD with nearly identical clinical manifestations [2,22].

The identification of PKD1 and PKD2 is required for molecular diagnosis of ADPKD. Positive detection rates of up to 89% have been reported [23]. Molecular diagnosis is currently not available in our hospital therefore diagnosis was made by ultrasonography using the modified Ravine Criteria, and a positive family history of ADPKD.

The renal US used to measure kidney volumes is two-dimensional in nature, is subject to operator dependence, and uses geometric assumptions about the shape of the kidney to estimate kidney volumes [24-26]. We attempted to minimize the operator dependence bias by using the same US operator across all patients. In contrast, magnetic resonance imaging (MRI) has the benefit of acquiring true tomographic data along any orientation, without the constraints of ionizing radiation and nephrotoxic contrast burden. We therefore used MRI to confirm US diagnosis as well as to measure TKV at baseline and follow up and we specifically determined changes in both total kidney volume and total cyst volume [27-29].

In agreement with the CRISP study, we found a correlation between increases in kidney and cyst volumes [30]. However, there was no decline in eGFR in our cohort, despite increases in both TKV and TCV. We hypothesize that this difference was caused not only by the growth of each cyst, but also by the addition of new cysts which developed during the 2 years that this study took place. The mTOR pathway is inappropriately activated in cyst-lining epithelial cells in human ADPKD and ARPKD. We selected Rapamycin for treatment because it is an immunosuppressant drug with antiproliferative and growth inhibiting effects. It binds to FK Binding Protein-12 (FKBP-12) and inhibits the activation of the mTOR. In regard of these effects, we hypothesized that it would retard kidney volume growth in humans [31,32].

The activation of mTOR is linked to tubular cell proliferation in animal models and humans, and mTOR inhibitors impede cell proliferation and cyst growth in polycystic kidney disease models. Previous studies found that the mTOR pathway is upregulated in ADPKD, possibly due to loss of tonic inhibition on mTOR from a tuberin-polycystin-1 complex [33]. To date, several experimental studies in rodent models of PKD have revealed that inhibition of the mTOR pathway results in reduction of kidney size, prevents the loss of kidney function, and lowers cyst volume [14,15]

However, two clinical trials using different mTOR inhibitors in ADPKD patients published simultaneously in 2010 reported no significant benefits of either drug on TKV or GFR.

In early-stage ADPKD patients (n=100, eGFR >70 ml min-1 $1.73m^{-2}$),18 months of sirolimus (target dose 2mg day-1) with achieved steady-state blood concentrations from 4.1–4.9 mg/l had no impact on TKV or eGFR compared with the control group [34]. In later stage ADPKD patients (n=433, eGFR 20–89 ml min^{-1} 1.73 m^{-2}), 2 years of everolimus (2.5 mg twice daily) with a mean trough concentration of 5.3 mg l^{-1}) significantly reduced the annual increase in TKV by 35% in the first year. However, this effect was not sustained over the second year [35]. In addition, there was an overall increase in eGFR decline (–5.42 ml min^{-1}) in the treatment group compared with the placebo control group (–3.22 ml min^{-1}, P = 0.004) during the first year of study.

A third study (n=55,eGFR 40–80 ml min^{-1} 1.73 m^{-2}) demonstrated that a higher Rapamycin dose (through blood concentration ~6–8 mg l^{-1}) among PKD1 patients was associated with therapeutic benefit with respect to changes in TKV and eGFR [36]. Consistent with these data, a recent study in PKD1 mice has clearly demonstrated dose dependent effects of sirolimus on mTOR signaling. These results suggest that conventional doses in humans (blood concentrations ~3 mg/l) are ineffective in slowing cystogenesis [37,38].

Among the different studies about ADPKD and Rapamycin we mention the SUISSE study, where 100 patients with ADPKD were randomized into Rapamycin treatment and control groups with no demonstrated differences in kidney volume growth or eGFR following 18 months of treatment [39].

Hypertension and proteinuria are the major treatable risk factors for the progression of CKD in ADPKD patients, our cohort had a normal eGFR at baseline and we controlled proteinuria and hypertension with the use of enalapril and losartan [10,40,41]

At the end of our study only 3 patients had the lowest significant proteinuria. It is possible, however, that these patients will develop more symptoms in the future considering the limited time of this study and the fact that we were not able to reduce kidney enlargement.

One explanation for the lack of efficacy for Rapamycin is having provided an insufficient drug dose compared with animal models for PKD. Some publications pointed out that long-term. Treatment with conventional doses is insufficient to inhibit mTOR activity in renal cystic tissue [42,43]. It has been suggested that the PKD1 animal model has a different response to the treatment when compared with humans [44]. It also is possible that the 24-month treatment duration was not long enough to assess the efficacy of Rapamycin over kidney

and cyst growth [45,46]. Finally, we had little power to to detect an effect of the drug over the TKV and TCV due to our small sample size.

In relation to AEs, two patients had iron-deficiency anemia likely caused by mechanisms including some interference with primitive erythroid cell proliferation. This was corrected with ferrous sulfate and folic acid. Erythropoietin was not necessary in any case [47,48]. An additional AE was acute diarrhea, which was found in 4 patients treated with Rapamycin. These patients demonstrated a rapid response to the temporary dose reduction and a dietary change [49].

In agreement with the SIRENA study, we were not able to halt or slow down kidney enlargement associated with ADPK. However, wedid not find an increase in proteinuria related to treatment with the mTOR inhibitor [50]. Finally, the presence of oral sores related to Rapamycin treatment (dose-dependent drug reaction) was found in 4 patients, but resolved in all 4 following local treatment with sucralfate [51].

In summary, we were not able to demonstrate the efficacy of Rapamycin treatment for slowing either kidney volume or cyst volume growth in our pediatric cohort. In contrast, BP remained normal and there were no significant changes in creatinine clearance and proteinuria following treatment. Only minor, controllable adverse effects were detected. Further studies in larger pediatric cohorts are needed to establish better conclusions.

The authors declare that they have no conflict of interest

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