Nephrol Dial Transplant (2009) 24: 3334–3342
doi: 10.1093/ndt/gfp280
Advance Access publication 13 June 2009

Safety and tolerability of sirolimus treatment in patients with autosomal dominant polycystic kidney disease

Andreas L. Serra[1,2], Andreas D. Kistler[1], Diane Poster[1], Fabienne Krauer[1], Oliver Senn[3], Shagun Raina[2], Ivana Pavik[2], Katharina Rentsch[4], Axel Regeniter[5], Dominik Weishaupt[6] and Rudolf P. Wüthrich[1,2]

[1]Division of Nephrology, University Hospital Zürich, [2]Institute of Physiology and Zürich Center for Integrative Human Physiology, [3]Institute of General Practice and for Health Services Research, University of Zürich, [4]Institute of Clinical Chemistry, University Hospital Zurich, [5]Laboratory Medicine, University Hospital Basel and [6]Division of Diagnostic Radiology, University Hospital Zürich, Switzerland

Correspondence and offprint requests to: Rudolf P. Wüthrich; E-mail: rudolf.wuethrich@usz.ch

Abstract

Background. We initiated a randomized controlled clinical trial to assess the effect of sirolimus on disease progression in patients affected by autosomal dominant polycystic kidney disease (ADPKD). Here we report the preliminary safety results of the first 6 months of treatment.

Method. A total of 25 patients were randomized to sirolimus 2 mg/day and 25 patients to no treatment except standard care. Treatment adherence was monitored electronically. At baseline and at Month 6, laboratory parameters were analysed and the urinary protein profile in 24-h urine collections was determined.

Results. Both treatment groups were well balanced for age, sex and renal function. In 94.1 ± 11.4% of the study days, patients in the sirolimus group were exposed to the drug when assuming a therapeutic efficacy duration of 30 h. At Month 6, the mean sirolimus dose and trough level were 1.28 ± 0.71 mg/day and 3.8 ± 1.9 μg/l, respectively. Glomerular (albumin, transferrin, IgG) and tubular (retinol-binding protein, α_1-microglobulin) protein excretion remained unchanged. Glomerular filtration rate also did not change significantly. Haematological parameters were similar in both groups, except for a mild reduction of the mean corpuscular volume of erythrocytes in patients receiving sirolimus. Lipid levels were similar in both groups. Adverse events were transient and mild, and no grade 3 or 4 events occurred. The incidence of infections was similar in the sirolimus group (80%) and the standard group (88%). The most common gastrointestinal adverse events were mucositis (72% in the sirolimus group versus 16% in the standard group, $P = 0.0001$) and diarrhoea (36% in the sirolimus versus 20% in the standard group, $P = 0.345$).

Conclusion. Treatment of ADPKD patients with sirolimus with a dose of 1–2 mg/day is safe and does not cause proteinuria or impairment of GFR. Treatment adherence was excellent. (ClinicalTrials.gov number, NCT00346918.)

Keywords: autosomal dominant polycystic kidney disease (ADPKD); safety; sirolimus; treatment adherence; urinary protein profile

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) represents the most frequent potentially lethal monogenic hereditary disease of mankind [1]. The estimated number of cases in Europe and in the United States amounts to 700 000 and 300 000, respectively. The relentless development and growth of innumerable cysts lead to progressive destruction of the normal renal parenchyma and massive enlargement of the kidneys. Subsequently, the glomerular filtration rate decreases in an accelerated mode, and end-stage renal disease with the need for dialysis and/or transplantation ensues. Data from the consortium for radiologic imaging studies of polycystic kidney disease (CRISP) have shown that the rate of kidney volume growth is an excellent predictor of renal functional decline [2]. Therefore, kidney volume growth can be used as a surrogate marker of disease progression [3]. Despite decades of intense basic and clinical research, effective treatment that alters the course of ADPKD has not been established.

Sirolimus, also termed rapamycin, is an immunosuppressant that binds to FK-binding protein-12 (FKBP-12) and inhibits the activation of mTOR, a key regulatory kinase of growth and proliferation [4]. We and others have previously shown that the mTOR inhibitors sirolimus and everolimus effectively reduce cyst growth and loss of renal function in an experimental animal model for polycystic kidney disease (PKD) [5–7]. Additional studies have shown that sirolimus is also effective in various mouse models of PKD, including dominant and recessive forms [8]. Of interest, analyses of ADPKD patients that received a renal allograft revealed that cystic kidney and liver volumes regressed under immunosuppression with sirolimus [8,9].

Based on these encouraging studies, we have initiated a randomized controlled clinical trial as a proof-of-concept study to examine the effect of sirolimus on cyst volume growth in young patients with documented ADPKD and normal GFR [10]. Here we report the preliminary safety and tolerability results of this clinical trial, with particular emphasis on the effect of sirolimus on proteinuria and GFR.

Methods

Patient population and study design

The SUISSE ADPKD study represents an ongoing single-centre, prospective, open-label, randomized controlled clinical trial to assess the efficacy of sirolimus (Rapamune®, Wyeth AG, Zug, Switzerland) to decrease renal volume enlargement in patients with ADPKD. The study involves 100 ADPKD patients aged 18–40 years with an estimated creatinine clearance >70 ml/min. Prior to randomization, polycystic kidneys are visualized by MRI without contrast media within an interval of 6 months, and kidney and cyst volumes are measured by volumetry. The volumetry method has been previously validated and showed an excellent reliability [3]. Patients with documented total kidney volume (TKV) enlargement of >2% are randomized at a one-to-one ratio of sirolimus 2 mg/day or standard care for 18 months. At the discretion of the treating physician, the starting dose was reduced to 1 mg in case of anticipated sirolimus-associated toxicity. An independent biostatistics unit generated the randomization list, using a permuted block design with a random block size of 4 or 6 to guarantee a balanced allocation. Patients in the standard arm had visits at Months 3 and 6, and patients in the sirolimus arm had four extra visits at Weeks 2 and 4 and Months 1 and 2 after randomization to allow for blood level monitoring and dose adjustments. The sirolimus dose was reduced or withheld when the trough level exceeded 10 μg/l, or when elevated liver enzymes (>2-fold above normal values), thrombopenia (<100 000/mm^3) and leukopenia (<3000/mm^3) occurred. Standard care included blood pressure control and symptomatic treatment of flank pain, cyst bleedings and cyst infections.

The study was approved by the institutional review board, conducted according to Good Clinical Practice guidelines and the Declaration of Helsinki and controlled by external monitoring. All participants gave written informed consent. Details of the study design have been reported elsewhere [10]. Briefly, from a local prospective ADPKD cohort, we selected 100 patients with documented TKV progression for inclusion (i.e. randomization) in the trial. Approximately 80% of patients considered for randomization had ≥2% TKV progression within an interval of 6 months (data not shown). According to a predefined interim analysis plan, the first 50 patients that had completed 6 months of sirolimus treatment ($n = 25$) or standard care ($n = 25$) were subjected to a safety and tolerability analysis. The entire study is powered to enrol a total of 100 patients and to continue until there is a mean 18-month follow-up. Here we report on the first 50 randomized patients, focusing particularly on the safety and tolerability of sirolimus treatment.

Study procedures

A detailed medical history was obtained from all patients, including ADPKD-related symptoms, previous hospitalizations and medication. Blood pressures were measured twice 5 min apart in each arm in the sitting position after a rest of 5 min, using an oscillometric blood pressure monitor (Boso-Medicus, Jungingen, Germany) at each visit. The lower of the two consecutive measurements in the arm with the higher blood pressure was used for analysis. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two or more study visits, or treatment with an antihypertensive drug.

Adherence to the prescribed study drug was assessed during the entire treatment period by an electronic system that monitors the date and time of the medication bottle opening (MEMS®, Aardex Ltd, Zug, Switzerland). This electronic system reliably assesses medication adherence as a period with a lack of medication bottle opening, which was considered to represent an episode of non-adherence [11,12]. The percentage of time when the patient was exposed to the drug action was calculated based on two different estimates of duration of therapeutic efficacy of sirolimus, namely 24 h plus 6 h (6-h forgetfulness period) and 24 h plus 24 h (24-h forgetfulness period).

Laboratory analyses

Blood was obtained for the determination of creatinine (IDMS-traceable modified Jaffé method), lipids, liver enzymes and haematologic parameters including erythrocyte indices. Trough levels of sirolimus were determined by liquid chromatography—mass spectrometry in samples of whole blood.

Twenty-four-hour urine samples were collected at baseline and at Month 6. Aliquots of these urine samples were centrifuged at 1500 *g* for 5 min, and the supernatants were stored at −80°C prior to analysis. Albumin, transferrin and immunoglobulin G (IgG) were analysed as urinary markers of glomerular damage, and retinol-binding protein (RBP) and α_1-microglobulin as urinary markers of tubular damage. Measurement of urinary total protein (benzethonium chloride method, Roche Ltd, Basel, Switzerland) and serum creatinine (enzymatic method, Wako Pure Chemical Industries Ltd, Osaka, Japan) were performed on a Hitachi 917 analyser, while urinary marker proteins were measured on a Beckman Coulter nephelometry system (Beckman Coulter, Brea, USA) using antibodies against albumin, transferrin, IgG and α_1-microglobulin (Beckman Coulter) and RBP (Dako, Glostrup, Denmark). The interassay coefficient of variation was <5% for these assays. The lower limit of quantification of the assays was 0.04 g/l for total protein, 2.0 mg/l for albumin, 0.61 mg/l for transferrin, 3.0 mg/l for IgG, 0.328 mg/l for RBP and 4.0 mg/l for α_1-microglobulin [13]. The concentration values in urine samples below the lower limit of quantification were set to zero for all calculations.

Safety assessment

Safety was determined on the basis of the occurrence of adverse events, findings on physical examination, and laboratory evaluations. Adverse events affecting ≥5% in either group were described and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 [14]. A patient with multiple occurrences of an adverse event was counted once in the corresponding category and a patient with multiple adverse events within a primary system organ class was also counted once for that class. Primary system organ classes and preferred terms were sorted by the frequency of adverse events in the total group. Safety and tolerability analyses included all randomized patients who received at least one dose of sirolimus and underwent at least one safety assessment.

Statistics

The results were expressed as means ± standard deviation or number of patients (percent). For comparisons between groups, means of continuous data were compared using Student's *t*-test or the Wilcoxon rank-sum test as appropriate and categorical data using Fisher's exact test. All *P*-values were two sided for the comparison between the groups or between baseline and follow-up values, and those <0.05 were considered statistically significant.

Results

Characteristics of the patients

Here we report on 50 ADPKD patients who were enrolled from March 2006 to March 2007 and have completed the first 6 months of the treatment. A total of 25 patients were randomly assigned to receive sirolimus and 25 patients to receive no treatment except standard care. Table 1 shows that both treatment groups were well balanced at baseline for age, sex, body mass index (BMI) and blood pressure. Approximately 70% of patients in each group had hypertension, mostly treated with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB). None of the patients were lost to follow-up.

Table 1. Characteristics of the patients at baseline[a]

Characteristic	Sirolimus $N = 25$	Standard $N = 25$	P-value
Age (years)	29 ± 6	29 ± 6	0.964
Gender, No. of male patients (%)	14 (56)	20 (80)	0.069
BMI (kg/m^2)	24 ± 4	25 ± 4	0.284
Systolic blood pressure (mmHg)	131 ± 16	131 ± 15	0.985
Diastolic blood pressure (mmHg)	87 ± 11	83 ± 10	0.122
Hypertension, No. (%)	17 (68)	17 (68)	1.000
Antihypertensive treatment, No. (%)			
All	10 (40)	12 (48)	0.776
ACEi and/or ARB therapy	8 (32)	11 (44)	0.561
Diuretics	2 (8)	5 (20)	0.417
Others	3 (12)	3 (12)	1.000
Symptoms and complications of ADPKD, No. (%)			
History of flank pain	6 (24)	15 (60)	0.021
History of macrohaematuria	6 (24)	6 (24)	1.000
History of cyst infections	1 (4)	3 (12)	0.609
History of intracranial bleeding	0 (0)	1 (4)	1.000
≥2 complications of ADPKD	3 (12)	4 (16)	1.000
Family history of intracranial bleeding	3 (12)	4 (16)	1.000

[a]Table shows either the mean ± standard deviation or the number of patients (percent). BMI denotes body mass index, ACEi angiotensin-converting enzyme inhibitor and ARB angiotensin receptor blocker.

Study drug adherence

The patients in the sirolimus group were exposed to the drug in 94.1 ± 11.4% of the study days if the duration of therapeutic efficacy was assumed to be 30 h, or in 96.6 ± 9.7% of the days assuming a therapeutic drug action of 48 h. The percentage of days with correct dosing was 93.0 ± 12.1%. A total of 17 patients received the correct sirolimus dose in >95% of the study days assuming 30 h of sirolimus therapeutic duration. The number of patients with correct dosing at >95% of the study days amounted to 22 patients when the therapeutic efficacy duration of sirolimus was set to 48 h.

Sirolimus dose and whole blood trough levels

Figure 1 shows the sirolimus dose and sirolimus whole blood trough levels. The starting dose was reduced to 1 mg/day in nine patients, mostly females (eight females, one male), due to anticipated more severe side effects in young female ADPKD patients. After adjustment of the sirolimus dose in the first 2 months, the sirolimus dose, dose per body weight and whole blood trough levels remained constant and after 6 months amounted to 1.28 ± 0.71 mg/day, 0.018 ± 0.01 mg/kg/day and 3.8 ± 1.9 μg/l, respectively.

Effect of sirolimus on renal function and urinary protein excretion

Renal function assessed by measured and estimated creatinine clearance as well as serum creatinine was similar in the sirolimus and standard groups at baseline and at Month 6 (Table 2). Thus, sirolimus did not adversely affect GFR.

Since it is well known that sirolimus can cause proteinuria in patients with preexisting renal disease, we examined in detail the urinary protein profile in 24 h-urine collections (Table 2). The median urinary excretion of total protein was low, and similar in both groups at baseline (78.0 mg/day in the sirolimus group versus 65.0 mg/day in the standard group, $P = 0.077$) and at Month 6 (120 mg/day in the sirolimus group versus 86.3 mg/day in the standard group, $P = 0.087$). The median urinary excretion of albumin was low in both groups at baseline (13.7 mg/day in the sirolimus group versus 9.0 mg/day in the standard group, $P = 0.450$) and remained low in response to sirolimus treatment at Month 6 (36.0 mg/day in the sirolimus group versus 18.9 mg/day in the standard group, $P = 0.349$, Figure 2). The number of patients developing microalbuminuria (as defined by urinary albumin excretion >30 mg/24 h) during the 6-month interval was similar in each group (+3 patients in the sirolimus group versus +2 patients in the standard group), and none of the patients had macroalbuminuria (defined by urinary albumin excretion >300 mg/24 h) at any time point. Furthermore, the urinary excretion of transferrin did not change significantly during the 6-month interval. The changes in the urinary protein excretion that occurred within the 6-month interval were similar in each group (sirolimus versus standard mean changes of albumin +8.4 mg/24 h, $P = 0.543$; transferrin +0.18 mg/mmol, $P = 0.791$; and total protein −1.8 mg/24 h, $P = 0.938$). IgG was detectable in four and two urine samples of the control group and in two and seven urine samples of the sirolimus group at baseline and at Month 6, respectively.

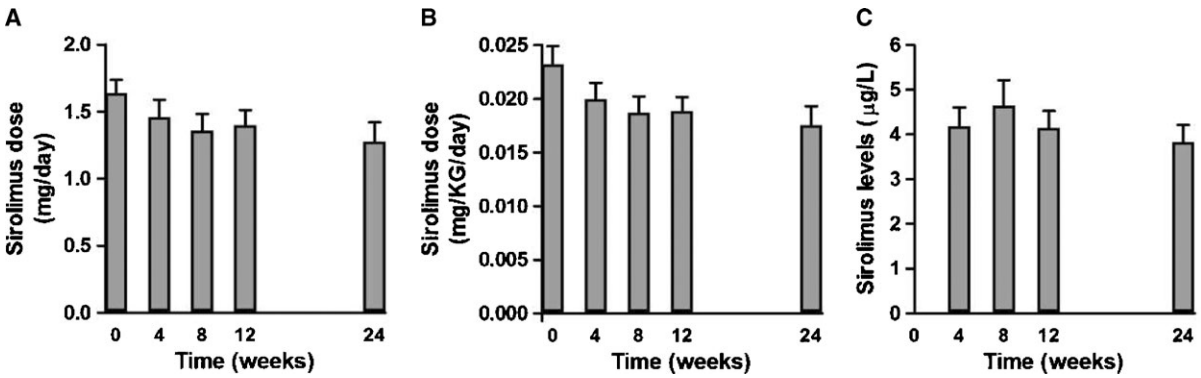

Fig. 1. Mean sirolimus dose (**A**), sirolimus dose per body weight (**B**) and sirolimus whole blood trough levels (**C**) at baseline and at follow-up visits.

Table 2. Changes in renal function and urinary protein excretion from baseline to Month 6[a]

Characteristic	Units	Normal range	Baseline			Month 6		
			Sirolimus $N = 25$	Standard $N = 25$	P-value	Sirolimus $N = 25$	Standard $N = 25$	P-value
mCrCl (24-h urine collection)	ml/min	90–120	91.7 ± 42.7	92.7 ± 46.7	0.937	110.1 ± 31.8	110.0 ± 30.7	0.996
eCrCl (Cockcroft–Gault formula)	ml/min	90–120	105.1 ± 25.4	114.1 ± 23.0	0.195	109.2 ± 24.1	113.9 ± 21.7	0.474
Serum creatinine	μmol/l	62–106 men 44–80 women	90.5 ± 18.3	95.4 ± 18.1	0.345	87.3 ± 15.7	96.2 ± 17.5	0.065
Urinary excretion (24-h urine collection)								
Albumin, median (IQR)	mg/24 h	<20	13.7 (7.4–25.2)	9.0 (4.0–27.2)	0.450	36.0 (12.0–65.0)	18.9 (9.2–46.7)	0.349
Transferrin, median (IQR)	mg/24 h	<1.7	0.87 (0.00–3.00)	0.00 (0.00–1.84)	0.280	2.04 (1.26–3.86)	1.48 (0.00–2.63)	0.273
Total protein, median (IQR)	mg/24 h	<100	78.0 (60.0–123.0)	65.0 (42.0–92.0)	0.077	120.0 (84.0–175.0)	86.3 (73.9–114.0)	0.087
IgG levels above LLQ, No. (%)			2 (8)	4 (16)		7 (36)	2 (8)	0.138
RBP levels above LLQ, No. (%)			0 (0)	0 (0)		0 (0)	1 (4)	1.000
A1M levels above LLQ, No. (%)			2 (8)	3 (12)		4 (16)	6 (24)	0.715

[a]Table shows either the mean ± standard deviation or number of patients (percent), with the exception of urinary albumin, transferrin and total protein excretion values, which are given as medians with interquartile range because of skewed data distribution.

mCrCl, measured creatinine clearance; eCrCl, estimated creatinine clearance; CG, Cockcroft–Gault; IQR, interquartile range; IgG, immunoglobulin G; LLQ, lower limit of quantification; RBP, retinol-binding protein; A1M, α_1, microglobulin.

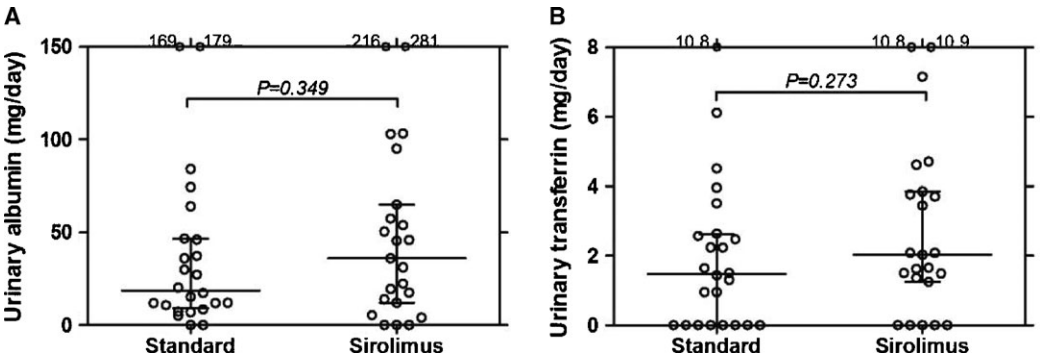

Fig. 2. The amount of albumin (**A**) and transferrin (**B**) in 24-h urine collections at Month 6. Lines represent the median and interquartile range.

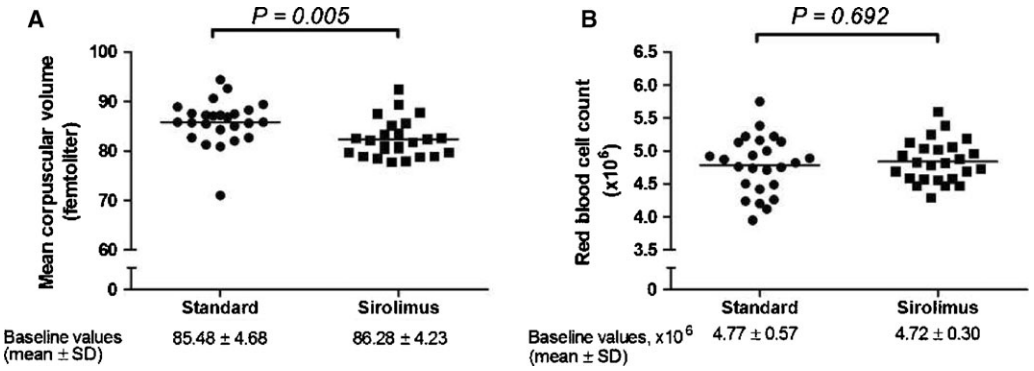

Fig. 3. Mean corpuscular volume of erythrocytes (**A**) and red blood cell count (**B**) at Month 6. The lines represents the mean. Baseline mean values ± standard deviations are shown below the *x*-axis.

Regarding the tubular proteins, RBP was detectable at low concentration only in one urine sample at Month 6 and was below the lower limit of quantification in all other samples. Likewise, α_1-microglobulin was only detectable in three and six urine samples of the standard group and in two and four urine samples of the sirolimus group at baseline and at Month 6, respectively. In urine samples of 10 patients of the standard group and of 9 patients of the sirolimus group, RBP or IgG or α_1-microglobulin was detectable at any time point of the study.

Taken together, the urinary excretion of markers for glomerular and tubular damage remained unchanged during the 6-month interval and was not adversely affected by sirolimus treatment.

Effect of sirolimus on clinical and laboratory parameters

Table 3 shows that the BMI and blood pressure were similar in the sirolimus and in the standard group at Month 6. We also analysed the influence of sirolimus on the antihypertensive treatment. At baseline, 40% patients in the sirolimus group and 48% patients in the standard group received an antihypertensive medication, mostly ACEi or ARB. The number of patients that started a new ACEi or ARB treatment during the 6-month treatment phase (30% of the sirolimus group versus 10% of the standard group) was also similar.

Sirolimus can cause haematological alterations in renal transplant recipients. The mean haemoglobin, mean corpuscular haemoglobin concentration (MCHC), and leukocyte and platelet counts did not differ significantly in the two groups of ADPKD patients (Table 3). The red blood cell count was unchanged, whereas, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH) were significantly lower in patients receiving sirolimus compared to patients in the standard group (Figure 3). Also the percentage of microcytic erythrocytes was higher in patients on sirolimus (1.3 $\pm$ 2.3% in the standard group versus 2.2 $\pm$ 1.3% in the sirolimus group, $P = 0.002$).

The mean aspartate aminotransferase (AST) value was significantly higher in patients receiving sirolimus at Month 6. However, all values of individual patients remained below the 2-fold upper limit of the normal range in either group. The mean alanine aminotransferase and gamma-glutamyltransferase values were similar in both groups.

Sirolimus is known to cause hyperlipidaemia. The mean cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels did not differ significantly among the groups (Figure 4). The ratio of LDL to HDL cholesterol was also similar among the groups. Although sirolimus treatment was associated with a tendency to higher values of triglyceride and cholesterol, the range of values and numbers of patients above the pre-defined cut-offs were similar in both groups at Month 6. One patient started a statin treatment in the sirolimus group and none in the standard group.

Table 3. Relevant clinical and laboratory data at Month 6[a].

Parameter	Sirolimus ($N = 25$)	Standard ($N = 25$)	P-value
BMI (kg/m^2)			
Mean ± SD	24 ± 4	25 ± 3	0.314
Range	18–30	17–35	
Systolic blood pressure (mmHg)			
Mean ± SD	126 ± 15	134 ± 12	0.054
Range	96–156	103–160	
Diastolic blood pressure (mmHg)			
Mean ± SD	83 ± 8	85 ± 9	0.505
Range	60–101	70–102	0.702
Antihypertensive treatment, No. (%)			
All	15 (60)	15 (60)	1.000
ACEi and/or ARB therapy	14 (56)	14 (56)	1.000
Diuretics	3 (12)	5 (20)	
Others	3 (12)	3 (12)	1.000
Haemoglobin (g/l)			
Mean ± SD	139.4 ± 8.49	144.2 ± 11.19	0.097
Range	118–154	128–166	
MCV (fl)			
Mean ± SD	82.40 ± 3.81	84.12 ± 4.47	**0.005**
Range	77.80–92.50	71.00–94.40	
MCH (pg/cell)			
Mean ± SD	28.88 ± 0.64	30.25 ± 1.91	**0.009**
Range	26.50–32.60	23.60–32.50	
MCHC (g/dl)			
Mean ± SD	35.04 ± 2.15	35.24 ± 1.34	0.465
Range	32.10–36.90	33.20–37.20	
Leukocyte count ($\times 10^3$/mm^3)			
Mean ± SD	6.03 ± 2.06	5.81 ± 1.64	0.673
Range	3.0–12.2	3.9–12.2	
Platelet count ($\times 10^3$/mm^3)			
Mean ± SD	253.7 ± 63.11	253.3 ± 53.70	0.981
Range	146.0–380.0	172.0–386.0	
AST (U/l)			
Mean ± SD	30.28 ± 8.54	24.92 ± 4.76	**0.010**
Range	19–53	17–36	
Number of patients >2 × ULN	0	0	
ALT (U/l)			
Mean ± SD	33.96 ± 25.79	26.36 ± 14.90	0.109
Range	9–114	12–71	
Number of patients >2 × ULN	1	0	
Cholesterol (mmol/l)			
Mean ± SD	4.94 ± 1.19	4.52 ± 0.84	0.147
Range	3.4–8.5	3.1–7.1	
Number of patients ≥6.2	4	1	
LDL cholesterol (mmol/l)			
Mean ± SD	2.89 ± 1.01	2.66 ± 0.65	0.357
Range	1.6–6.1	1.4–4.6	
Number of patients ≥4.1	3	1	
HDL cholesterol (mmol/l)			
Mean ± SD	1.40 ± 0.36	1.30 ± 0.36	0.331
Range	0.8–2.1	0.7–1.9	
Number of patients ≤1	4	5	
Triglyceride (mmol/l)			
Mean ± SD	1.43 ± 0.86	1.24 ± 0.56	0.345
Range	0.5–3.4	0.4–2.7	
Number of patients ≥4.5	0	0	

[a]BMI, body mass index; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ULN, upper limit of normal range.

Adverse events

Table 4 lists the adverse events. All patients reported at least one adverse event during the 6 months in both groups. No grade 3 or 4 events were reported. The incidence of any infection was similar in the sirolimus group (80%) and the standard group (88%). The most frequent infection was upper respiratory infection, and its incidence was also similar in the sirolimus group (64%) and in the standard group (80%). No clinically significant opportunistic infections were reported. Headache was more common among patients receiving sirolimus (48%) than among patients of the standard group (12%). The total number of patients with gastrointestinal adverse events was higher in the sirolimus group (84%) than in the standard group (32%). The most common gastrointestinal adverse events were mucositis (72% of the sirolimus group versus 16% of the standard group, $P = 0.0001$) and diarrhoea (36% of the sirolimus group versus 20% of the standard group, $P = 0.345$). A total of 48% patients in the sirolimus group had adverse events leading to sirolimus dose reduction, namely mucositis (16%), tooth extraction (8%), acne (4%), sirolimus trough level >10 μg/l (4%), infection (4%), leucopenia (4%) and surgery (4%).

Discussion

Sirolimus is an immunosuppressant drug with strong antiproliferative properties. It has been approved for the prevention of rejection after kidney and liver transplantation and is used in combination with other immunosuppressive drugs. The favourable effect of sirolimus in rodent models for ADPKD has prompted the initiation of clinical trials testing the efficacy of sirolimus in halting PKD progression. The potential risks of a sirolimus treatment have limited its use in transplantation and may hamper a potential therapeutic application in ADPKD patients. In this trial, however, we show that sirolimus at doses of 1–2 mg/day was well tolerated and safe in ADPKD patients.

There have been reports of nephrotoxicity and proteinuria related to sirolimus use in solid organ transplant recipients. Studies have shown an increase in urinary protein excretion in patients converted from calcineurin inhibitor-based therapy to sirolimus therapy as well as with *de novo* use of sirolimus [15–17]. The origin of sirolimus-associated urinary protein excretion, i.e. glomerular versus tubular origin, is still a subject of investigations. However, the facts that proteinuria associated with the use of sirolimus is mainly composed of albumin and, ACEi effectively reduce sirolimus induced proteinuria support the hypothesis that mTOR inhibitors increase glomerular permeability [18]. In addition to albuminuria, glomerular proteinuria is characterized by increased excretion of transferrin and IgG, two glomerular markers with different molecular weights, which allow us to distinguish selective from unselective proteinuria [19]. We analysed in detail the urinary protein excretion in 24-h urine collections and found unchanged levels of albumin, transferrin and IgG. Furthermore, the urinary excretion of the low-molecular weight proteins α_1-microglobulin and RBP,

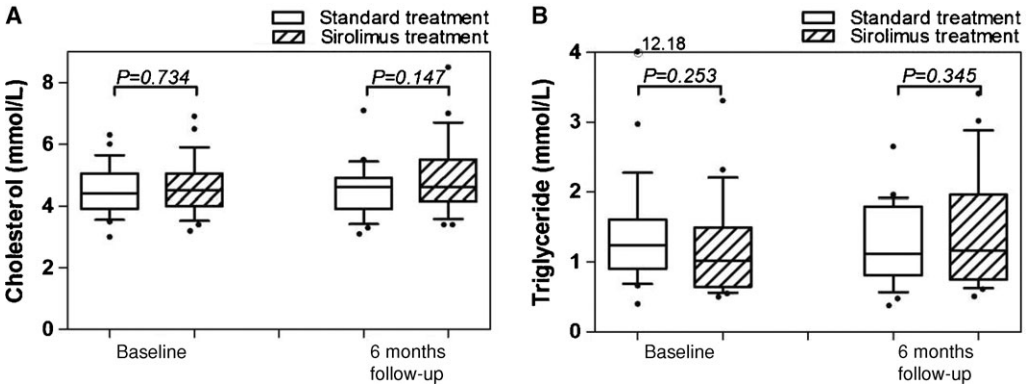

Fig. 4. Cholesterol (**A**) and triglyceride (**B**) levels were similar in both groups at baseline and at 6 months follow-up. The box indicates 50% of the observed data points between the 1st and 3rd quartiles. The line within the box represents the median. The whiskers show the data between the 10th and 90th percentiles.

characterizing tubular proteinuria [20], was unchanged by sirolimus treatment. Together, these data reveal that albuminuria is minimal in young patients affected by ADPKD. The mild albuminuria that is seen in some patients results from glomerular leaking. Sirolimus at a dosage between 1 and 2 mg/day did not significantly deteriorate glomerular proteinuria, or induce tubular proteinuria. Since a 6-month treatment with sirolimus also did not impair the GFR, sirolimus appears to have an excellent renal safety profile in ADPKD patients.

In two randomized, double-blind multicentre studies, the safety and efficacy of sirolimus compared with azathioprine (Study 1) [21] or placebo (Study 2) [22] in combination with cyclosporine and prednisone for the prevention of rejection after renal transplantation were examined. These studies compared the dose of 2 mg or 5 mg of sirolimus per day. For the dose of 2 mg/day, the following side effects compared to the control group were seen more frequently: hypercholesterolaemia, hypertriglyceridaemia, hypertension, thrombocytopenia, acne and skin rash. All side effects occurred in comedication with cyclosporine and prednisone. We found unchanged lipid levels, although there is abundant evidence that sirolimus causes an increase in serum triglyceride levels and in the levels of cholesterol, LDL and HDL [23]. In our current study, sirolimus treatment was associated with a tendency to higher values of cholesterol, LDL and triglyceride. These changes did not reach statistical significance, most probably due to type II error. Metabolic balance studies have also shown that the defect in lipid metabolism is largely dose dependent [24].

The leukocyte and platelet counts remained unchanged. However, we noted a mild but significant reduction of erythrocyte volume in patients treated with sirolimus. Several studies with sirolimus in differently combined immunosuppressive regimen as well as one study with the sirolimus derivate everolimus as a monotherapy have reported reduced MCV and microcytosis in solid organ transplant recipients [25–29]. The aetiology of the reduced MCV is not well understood. In these previous studies, patients received concomitant immunosuppressive medication to prevent organ rejection, iron supplementation and erythropoietin stimulation agents for the treatment of anaemia, and many patients had impaired kidney function, factors that may influence the morphological characteristic of erythrocytes. In our current study, we are able to ascribe this sirolimus-associated effect on MCV and MCH to the drug itself. These changes occurred at a low sirolimus trough level and were not accompanied by a decrease of leukocyte, platelet or red blood cell count, and are therefore not attributable to the well-known anti-proliferative effect of sirolimus on bone marrow colony-forming cells. Our finding of a disproportionately low MCV in comparison with unchanged red blood cell count suggests a defect in the globin synthesis. The globin protein synthesis is under tight control of eukaryotic initiation factor 2 (eIF2), and the phosphorylation of eIF2α prevents translation initiation and hence the synthesis of globin. As rapamycin causes an increase in phosphorylation of eIF2α, we speculate that the decrease in erythrocyte volume and haemoglobin content seen in our patient is due to reduced globin synthesis rather than an impaired red blood cell production [30,31].

Adverse events were transient and mild and no serious adverse events occurred in our study. The incidence of infections was similar in both groups, whereas oral ulcers and headache occurred more frequently among patients receiving sirolimus compared to patients receiving standard care. Oral adverse events, usually involving superficial ulcerations of the gingival and buccal mucosa and tongue, named aphthous mouth ulcers, have been reported in patients receiving sirolimus. Aphthous mouth ulcers occurred in 10–19% of patients receiving sirolimus in phase III clinical trials [21,22] and in up to 32% of patients switched from a calcineurin inhibitor therapy to sirolimus [32]. Notably, the comedication with corticosteroids may decrease the risk of sirolimus-associated aphthous mouth ulcers. In a cohort of patients converted to sirolimus in the absence of corticosteroids, a high incidence (up to 60%) was reported for this complication [33]. We found a 72% incidence of aphthous mouth ulcers among ADPKD patients treated with sirolimus. Various hypotheses concerning the cause of these ulcers have been generated without clear evidence. The strong anti-proliferative properties of sirolimus might function as the primary trigger for the development of these ulcers, and the absence of corticosteroids may hamper their secondary healing. Additionally, the true incidence of this adverse event might be underestimated as these mucosal

Table 4. Adverse events in the safety population from Month 0 to Month 6[a]

Category	Number of patients (%)			
	Sirolimus ($N = 25$)	Standard ($N = 25$)	Total ($N = 50$)	*P*-value
Any category	25 (100)	25 (100)	25 (100)	1.000
Infection				
Total	20 (80)	22 (88)	42 (84)	0.702
Upper respiratory infection, sinusitis or bronchitis	16 (64)	20 (80)	36 (72)	0.345
Urinary tract infection or pyelonephritis	4 (16)	2 (8)	6 (12)	0.667
Pharyngitis	3 (12)	2 (8)	5 (10)	1.000
Perioral	2 (8)	0	2 (4)	0.490
Pain				
Total	18 (72)	19 (76)	37 (74)	1.000
Flank pain	7 (28)	11 (44)	18 (36)	0.377
Headache	12 (48)	3 (12)	15 (30)	**0.012**
Muscoloskeletal	7 (28)	8 (32	15 (30)	1.000
Abdominal	3 (12)	2 (8)	5 (10)	1.000
Genital	2 (8)	1 (4)	3 (6)	1.000
Gastrointestinal				
Total	21 (84)	8 (32)	29 (58)	**0.0004**
Aphthous ulcer or mucositis	18 (72)	4 (16)	22 (44)	**0.0001**
Diarrhoea	9 (36)	5 (20)	14 (28)	0.345
Teeth	2 (8)	0	2 (4)	0.490
Heartburn	2 (8)	1 (4)	3 (6)	1.000
Nausea	3 (12)	0	3 (6)	1.000
Skin related				
Total	13 (52)	10 (40)	23 (46)	0.571
Acne	13 (52)	7 (28)	20 (40)	0.148
Folliculitis	1 (4)	2 (8)	3 (6)	1.000
Pulmonary or upper respiratory				
Cough	6 (24)	1 (4)	7 (14)	0.098
Renal or genitourinary				
Macrohaematuria	1 (4)	4 (16)	5 (10)	0.349
Lymphatics				
Limb oedema	3 (12)	0	3 (6)	0.235
Neurologic				
Dizziness	3 (12)	0	3 (6)	0.235
Blood and bone marrow				
Leucopenia	2 (8)	0	2 (4)	0.490
Sexual or reproductive function				
Irregular menses	2 (8)	0	2 (4)	0.490

[a]Table shows number of patients (percent). Adverse events affecting ≥5% in either group were described and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

lesions can easily be mistaken for herpes simplex infection [34]. The incidence of headache was higher in patients receiving sirolimus. Thus far headaches were not known side effects of sirolimus treatment. Our finding has recently been validated by analysing pharmacovigilance data in other patient populations. As a consequence, the product information is being adapted on a worldwide basis to include this information.

A limitation of our study is the low number of patients and the short time of follow-up. Therefore, we cannot exclude the occurrence of rare adverse events or events occurring only after long-term treatment. However, based on experience in other patient populations treated with sirolimus, we anticipated that we could detect major side effects/adverse events in the sirolimus-treated patients with sufficient robustness in a relatively homogeneous ADPKD population in a 6-month time interval.

In conclusion, this short-term analysis reveals that sirolimus treatment is safe for ADPKD patients when the drug is used at a dosage between 1 and 2 mg/day. Treatment adherence was excellent, the renal safety profile was encouraging and the characteristic side effects of sirolimus were well manageable. The Data Safety Monitoring Board (DSMB) has therefore recommended continuation of the study. The upcoming efficacy data of this trial will establish whether sirolimus has a beneficial effect on the relentlessly progressive cyst volume growth in patients with ADPKD.

Acknowledgements. Funding for this study was provided by the Swiss National Science Foundation (No 310000-118166), by the Polycystic Kidney Disease Foundation (Kansas, MO, USA) and by an unrestricted research grant of Wyeth Europe. The authors thank Michael Pickering (BIOP, Basel, Switzerland), Paulus Kristanto (www.iadherence.org), Julia Hofmann, Ruth Russi and Marian Struker for the excellent technical assistance. We thank the following nephrologists for contributing patients: D. Aerne, J.A. Bleisch, A. Bock, W. Brunner, M. Cicvara, H.J. Gloor, Z. Glück, B.A. Gürtler, E. Grüter, H.M. Herb, D. Hertner, C. Jäger, H. Jungbluth, C. Kell, G. Keusch, M. Mayr, N. Höfliger, H.R. Räz, P. Ruedin, H. Saxenhofer, D. Schiesser, M. Schmid, C. Schönholzer, A.K. Schwarzkopf, M. Spöndlin, J. Steiger, C. Wahl.

Conflict of interest statement. None declared.

References

1. Dalgaard OZ. Bilateral polycystic disease of the kidneys: a follow-up of two hundred and eighty-four patients and their families. *Acta Med Scand Suppl* 1957; 328: 1–255
2. Grantham JJ, Torres VE, Chapman AB *et al*. Volume progression in polycystic kidney disease. *N Engl J Med* 2006; 354: 2122–2130
3. Kistler AD, Poster D, Krauer F *et al*. Increases in kidney volume in autosomal dominant polycystic kidney disease can be detected within 6 months. *Kidney Int* 2009; 75: 235–241
4. Schmelzle T, Hall MN. TOR, a central controller of cell growth. *Cell* 2000; 103: 253–262
5. Wahl PR, Serra AL, Le Hir M *et al*. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). *Nephrol Dial Transplant* 2006; 21: 598–604
6. Wu M, Wahl PR, Le Hir M *et al*. Everolimus retards cyst growth and preserves kidney function in a rodent model for polycystic kidney disease. *Kidney Blood Press Res* 2007; 30: 253–259
7. Tao Y, Kim J, Schrier RW *et al*. Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol* 2005; 16: 46–51
8. Shillingford JM, Murcia NS, Larson CH *et al*. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci USA* 2006; 103: 5466–5471
9. Qian Q, Du H, King BF *et al*. Sirolimus reduces polycystic liver volume in ADPKD patients. *J Am Soc Nephrol* 2008; 19: 631–638
10. Serra AL, Kistler AD, Poster D *et al*. Clinical proof-of-concept trial to assess the therapeutic effect of sirolimus in patients with autosomal dominant polycystic kidney disease: SUISSE ADPKD study. *BMC Nephrol* 2007; 8: 13
11. Farmer KC. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. *Clin Ther* 1999; 21: 1074–90; discussion 3
12. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med* 2005; 353: 487–497
13. Regeniter A, Siede WH, Scholer A *et al*. Interpreting complex urinary patterns with MDI LabLink: a statistical evaluation. *Clin Chim Acta* 2000; 297: 261–273
14. Trotti A, Colevas AD, Setser A *et al*. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003; 13: 176–181
15. Senior PA, Paty BW, Cockfield SM *et al*. Proteinuria developing after clinical islet transplantation resolves with sirolimus withdrawal and increased tacrolimus dosing. *Am J Transplant* 2005; 5: 2318–2323
16. Butani L. Investigation of pediatric renal transplant recipients with heavy proteinuria after sirolimus rescue. *Transplantation* 2004; 78: 1362–1366
17. Saurina A, Campistol JM, Piera C *et al*. Conversion from calcineurin inhibitors to sirolimus in chronic allograft dysfunction: changes in glomerular haemodynamics and proteinuria. *Nephrol Dial Transplant* 2006; 21: 488–493
18. Letavernier E, Pe'raldi MN, Pariente A *et al*. Proteinuria following a switch from calcineurin inhibitors to sirolimus. *Transplantation* 2005; 80: 1198–1203
19. Maachi M, Fellahi S, Regeniter A *et al*. Patterns of proteinuria: urinary sodium dodecyl sulfate electrophoresis versus immunonephelometric protein marker measurement followed by interpretation with the knowledge-based system MDI-LabLink. *Clin Chem* 2004; 50: 1834–1837
20. D'Amico G, Bazzi C. Pathophysiology of proteinuria. *Kidney Int* 2003; 63: 809–825
21. Kahan BD. The Rapamune US Study Group. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. *Lancet* 2000; 356: 194–202
22. MacDonald AS. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 2001; 71: 271–280
23. Kasiske BL, de Mattos A, Flechner SM *et al*. Mammalian target of rapamycin inhibitor dyslipidemia in kidney transplant recipients. *Am J Transplant* 2008; 8: 1384–1392
24. Hoogeveen RC, Ballantyne CM, Pownall HJ *et al*. Effect of sirolimus on the metabolism of apoB100-containing lipoproteins in renal transplant patients. *Transplantation* 2001; 72: 1244–1250
25. Cahill BC, Somerville KT, Crompton JA *et al*. Early experience with sirolimus in lung transplant recipients with chronic allograft rejection. *J Heart Lung Transplant* 2003; 22: 169–176
26. Kim MJ, Mayr M, Pechula M *et al*. Marked erythrocyte microcytosis under primary immunosuppression with sirolimus. *Transpl Int* 2006; 19: 12–18
27. Masetti M, Rompianesi G, Montalti R *et al*. Effects of everolimus monotherapy on hematological parameters and iron homeostasis in de novo liver transplant recipients: preliminary results. *Transplant Proc* 2008; 40: 1947–1949
28. Sanchez Fructuoso A, Calvo N, Moreno MA *et al*. Study of anemia after late introduction of everolimus in the immunosuppressive treatment of renal transplant patients. *Transplant Proc* 2007; 39: 2242–2244
29. Maiorano A, Stallone G, Schena A *et al*. Sirolimus interferes with iron homeostasis in renal transplant recipients. *Transplantation* 2006; 82: 908–912
30. Cherkasova VA, Hinnebusch AG. Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. *Genes Dev* 2003; 17: 859–872
31. Kubota H, Obata T, Ota K *et al*. Rapamycin-induced translational derepression of GCN4 mRNA involves a novel mechanism for activation of the eIF2 alpha kinase GCN2. *J Biol Chem* 2003; 278: 20457–20460
32. Watson CJ, Firth J, Williams PF *et al*. A randomized controlled trial of late conversion from CNI-based to sirolimus-based immunosuppression following renal transplantation. *Am J Transplant* 2005; 5: 2496–2503
33. van Gelder T, ter Meulen CG, Hene R *et al*. Oral ulcers in kidney transplant recipients treated with sirolimus and mycophenolate mofetil. *Transplantation* 2003; 75: 788–791
34. Kuypers DR. Benefit-risk assessment of sirolimus in renal transplantation. *Drug Saf* 2005; 28: 153–181

Received for publication: 27.2.09; Accepted in revised form: 20.5.09