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ORIGINAL ARTICLE

Treatment of tacrolimus or cyclosporine A in children with idiopathic nephrotic syndrome

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Abstract
Background Cyclosporine A (CsA) and tacrolimus (TAC) are often alternative treatment choices for patients with nephrotic syndrome.
Methods In this prospective study, the efficacy and safety of CsA and TAC in inducing and maintaining remission in 74 children with idiopathic nephrotic syndrome (INS) were evaluated.
Results In terms of short-term efficacy, TAC was more effective than CsA in children with steroid-resistant nephrotic syndrome (χ^2=13.75, P=0.001), although no significant difference in number of episodes of relapse were found in patients with complete remission between the two treatment groups (first year: χ^2=0.261, P=0.88; second year: χ^2=2.685, P=0.26). In patients with frequently relapsing or steroid-dependent nephrotic syndrome, no significant difference in short-term remission (χ^2=1.908, P=0.39) or in relapse frequency during follow-up (within first year: χ^2= 1.046, P=0.59; within second year: χ^2=0.587, P=0.75) were found between the two groups. There was a difference in the rate of adverse effects between the two treatment groups [nephrotoxicity: 4/24 (CsA) vs .0/50 (TAC), P= 0.002; hirsutism: 8/24 (CsA) vs. 0/50 (TAC), P<0.001].
Conclusions In our pediatric patient cohort, the treatment of steroid-resistant nephrotic syndrome with tacrolimus was associated with higher efficacy and lower renal toxicity in comparison to CsA, although no favorable outcome in relapse rate during long-term follow-up was seen. On the other hand, tacrolimus was not always the better choice to replace CsA in the treatment of severe frequently relapsing or steroid-dependent nephrotic syndrome.

Keywords Idiopathic nephrotic syndrome · Therapy · Cyclosporine A · Tacrolimus

Introduction

Many children with idiopathic nephrotic syndrome (INS) initially respond to steroid therapy, but patients with frequent relapses (frequently relapsing nephrotic syndrome, FRNS), steroid dependency (steroid-dependent nephrotic syndrome, SDNS), or resistance to steroid therapy (steroid-resistant nephrotic syndrome, SRNS) require alternative treatments [1].

For children with FRNS, SDNS, or SRNS [2], cyclophosphamide [3], levamisole [4], or mycophenolic acid [5] are regarded as first-choice options. As a calcineurin inhibitor (CNI), cyclosporine A (CsA) is usually effective and is often used after cytotoxic treatment, but long-term treatment is necessary, raising concerns for the potential health risk of various side effects, such as nephrotoxicity, due to the continued exposure to the drug [6]. Tacrolimus (FK506; TAC), a new type of CNI, has recently been used in clinical practice as a substitute for CsA, and it has been suggested that TAC has stronger immunosuppressive effects and significantly reduced side effects than CsA [7, 8].

In the prospective study reported here, we evaluated the efficacy and safety of CsA and TAC in inducing and maintaining remission in 74 children with INS.

Wenjing Wang and Yonghui Xia contributed equally to this work.

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Materials and methods

Patients

Between October 2007 and June 2011, 74 patients with INS provided consent to be included in the study. The inclusion criteria were: (1) SRNS: prednisone (or its equivalent) at 1.5–2 mg/kg per day resulted in non-response when treatment was continued for more than 4 weeks; (2) FRNS: relapse occurred more than three times within 1 year, or at least twice within 6 months; (3) SDNS: recurrence of nephrotic syndrome when the dose of corticosteroids is reduced, or within 14 days after the discontinuation of therapy; (4) normal initial blood glucose level (3.9–6.1 mmol/L) and normal renal [estimated glomerular filtration rate (eGFR) of approx. 90–120 mL/min] and liver function test results (alanine transaminase 8–40 U/L, serum total bilirubin 5.5–19 μmol/L, serum direct bilirubin 1.7–6.8 μmol/L, total protein 60–80 g/L, albumin 35–55 g/L). The exclusion criteria were: (1) known secondary causes of nephrotic syndrome, such as Henoch–Schönlein purpura nephritis, systemic lupus erythematosus, hepatitis B virus-associated nephritis; (2) active infectious disease; (3) immunocomplex-mediated glomeruronephritis, such as membranous nephropathy and membranoproliferative glomerulonephritis. The study was approved by the ethics committee of The Children's Hospital of Zhejiang University School of Medicine.

Treatment protocol

This was a prospective uncontrolled open-label study performed in a single center. Patients with INS were divided into two groups. The selection of CsA or TAC was decided upon by the patients and/or their parents, with due consideration given to their economic situation (TAC is more expensive than CsA, and some of the patients did not have health insurance, especially those from rural areas), anxiety about side effects (hirsutism, nephrotoxicity, hepatic injury, etc.), and the convenience for follow-up (CsA concentrations can be monitored in some local hospitals in small cities, but TAC concentrations cannot). After July 2009, none of the patients in the study selected CsA treatment for INS; thus, all cases in the CsA group were recruited from October 2007 to July 2009.

In the CsA group, between October 2007 and July 2009, we initiated CsA treatment at 3–4 mg/kg/day, divided into two doses over 12-h intervals, in 24 patients. The dose was adjusted according to each patient's trough blood level, with a target of 100–150 ng/mL. The overall final dose of CsA was 2.72±0.59 mg/kg/day.

In the TAC group, from November 2008 to June 2011 we initially administered TAC to 50 patients at 50–150 μg/kg/day, divided into two doses over 12-h intervals, and subsequently adjusted the dose according to each patient's trough blood level, with a target of 5–12 ng/mL. The overall final dose of tacrolimus was 86.9±27.6 μg/kg/day for these patients.

Both groups of patients received with CsA or TAC for at least 24 months, unless the patient exited the study because of treatment failure or severe side effects, such as irreversible nephrotoxicity. The treatment duration was extended to more than 2 years in some patients with complete remission, but relapses did occur during the tapering off.

Lower levels were also acceptable in both groups if patients were in remission. However, for some patients with lower levels before remission, we administered additional ketoconazole [9] or diltiazem [10] to elevate their blood CsA or TAC concentration and to reduce medical expenses.

For each patient in the study, we administered 1 mg/kg/day prednisone (maximum daily dose 60 mg) at the beginning of CsA or tacrolimus therapy and maintained this dose for 4 weeks. We then administered 1 mg/kg every other day until week 8, followed by a gradual tapering off. The minimal maintenance dose of prednisone was 2.5 mg per alternate day.

Measurement of serum tacrolimus or CsA

Enzyme-linked immunosorbent assays (ELISA) were used to measure serum TAC or CsA levels according to the respective manufacturer's instructions. Serum levels of TAC or CsA were measured at the beginning of the fourth day. Whole blood TAC or CsA levels were regularly checked (every 4 weeks after achieving target level) for all patients.

Follow-up

Patients included in this study were followed up every 2 weeks during the first 4 weeks of treatment and then every month for the duration of the 2-year study. Each patient's clinical status and systolic and diastolic blood pressure were recorded. Serum creatinine, electrolytes, albumin, cholesterol, triglyceride, liver function, glucose, and 24-h urinary protein excretion were routinely monitored. eGFR was calculated using the Schwartz formula [11].

Definitions

Complete remission was defined as the disappearance of clinical symptoms and negative test measurements for urine protein on three consecutive occasions, or proteinuria of <4 mg/h per m^2 body surface area (BSA).

Partial remission was diagnosed if proteinuria was reduced to 4.1–40 mg/h per m^2 BSA, accompanied by the resolution of edema and an increase in serum albumin concentration to >35 g/L within 6 months after the plasma concentration of TAC or CsA reached effective plasma concentrations.

Non-responsiveness was diagnosed if there was no improvement in clinical symptoms or signs 6 months after the plasma concentration of TAC or CsA reached effective plasma concentrations, the urinary protein remained as +++ or greater, or proteinuria was >40 mg/h per m^2 BSA and the serum albumin concentration was <35 g/L.

FRNS was defined as steroid-sensitive nephrotic syndrome (SSNS) with two or more relapses within 6 months or more than three relapses within a 12-month period. SDNS was defined as SSNS with two or more consecutive relapses during tapering or within 14 days of stopping steroids.

Nephrotoxicity was defined as an increase in baseline serum creatinine of >25%, which included patients with acute reversible derangement of renal function or those with chronic irreversible worsening independent of CNI dose decrease or withdrawal. Gastrointestinal symptoms were defined as patients presenting with nausea, vomiting, diarrhea, abdominal pain, or abdominal discomfort. Psychiatric symptoms were defined as tremor, headache, paresthesia, insomnia, anxiety, or sleepiness.

Repeat renal biopsy

Our policy for repeat renal biopsy was when patients presented with an increase in baseline serum creatinine >25% for more than 2 weeks, independent of CNI dose decrease or withdrawal.

Statistical analysis

All continuous data were analyzed with Student's *t* test and categorical data were analyzed with the chi-square test. SPSS ver. 16.0 (SPSS, Chicago, IL) was used for statistical analyses. A *P* value of <0.05 indicated a statistically significant difference.

Results

Baseline patient characteristics

Clinical and biochemical characteristics of the 74 patients recruited in the study are summarized in Table 1. The baseline clinical and laboratory characteristics were similar between two groups. Several participants had previously failed to respond to treatment with intravenous cyclophosphamide (8 cases) and mycophenolate mofetil (MMF) (15 cases) (see Table 1).

Renal histology

Seventeen of the 24 patients (70.8%) in the CsA group and 42 of the 50 patients (84%) in the TAC group undertook an initial renal biopsy. The histological findings are listed in Table 2.

Only one patient undertook a second renal biopsy after 2 years of therapy with TAC, and the results indicated the presence of interstitial fibrosis and tubular atrophy in 8% of the area. This patient continued on TAC therapy and did not show an increase in baseline serum creatinine even though he received CNI for more than 2 years. The second biopsy was undertook upon request of his parents due to their concerns about the nephrotoxicity of CNI.

For biopsy-identified FSGS patients, mutation screening of the *NPHS2* gene was performed, but no significant mutations were found in these patients [12–14].

Table 1 The demographic and clinical characteristics of 74 children with refractory nephrotic syndrome treated with cyclosporine A or tacrolimus

Clinical/demographic characteristics	CsA (*n*=24)	TAC (*n*=50)	*P* value
Age at onset of disease (year)	7.6±4.5	8.3±4.8	0.62
Age at treatment (year)	7.7±5.0	8.6±5.8	0.59
24-h urinary protein excretion (g)	3.71±1.64	4.85±2.57	0.07
Serum albumin (g/L)	23.3±20.1	18.9±15.8	0.29
Serum cholesterol (mmol/L)	11.75±6.33	9.65±5.67	0.15
Serum triglycerides (mmol/L)	4.47±2.23	5.59±3.58	0.16
Serum creatinine (μmol/L)	47.3±33.2	54.6±34.6	0.39
eGFR (Schwartz formula) (ml/min)	128.6±42.2	120.7±47.5	0.52
Systolic blood pressure (mmHg)	93±66	98±67	0.78
Diastolic blood pressure (mmHg)	67±39	74±45	0.59
Gender (Male/female)	18/6	33/17	0.43
SRNS (*n*)	8	26	0.13
FRNS/SDNS (*n*)	16	24	
Patients (*n*) who received other steroid-sparing agents before CsA/TAC therapy			
Mycophenolate mofetil	6	9	0.49
Cyclophosphamide	4	4	0.28

CsA, Cyclosporine A; TAC tacrolimus; eGFR, estimated glomerular filtration rate; SRNS, steroid-resistant nephrotic syndrome; FRNS/SDNS, frequently relapsing/steroid-dependent nephrotic syndrome

Table 2 The results of renal biopsy in patients taking refractory nephrotic syndrome treated with CsA or TAC

Treatment	SRNS					FRNS/SDNS			
	n	MCD	FSGS	MsPGN	IgMN	*n*	MCD	FSGS	IgMN
CsA	8/8[a]	4	2	2	0	9/16[a]	6	1	2
TAC	23/26[b]	13	5	0	5	19/2[b]	10	3	6

MCD, Minimal change disease; FSGS, focal segmental glomerulosclerosis; IgMN, immunoglobulin M nephropathy; MsPGN, mesangial proliferative glomerulonephritis; SRNS, steroid-resistant nephrotic syndrome; TAC, tacrolimus; CsA, cyclosporine A; SDNS, steroid-dependent nephrotic syndrome; FRNS, frequently relapsing nephrotic syndrome

[a] Number of patients receiving renal biopsy/all patients in this group

[b] Three SRNS/TAC patients did not undertake renal biopsy; 5 FRNS/SDNS/TAC patients did not undertake renal biopsy, and 7 FRNS/SDNS/CsA patients did not undertake renal biopsy

Short-term response to therapy

We evaluated the short-term response to CsA or TAC therapy according to the outcomes of patients within a 6-month interval. The results are summarized in Table 3. TAC appeared to be better than CsA in inducing remission in the SRNS group (Wilcoxon Rank sum test $Z=-2.992$, $P=0.003$; chi-square test $\chi^2=13.75$, $P=0.001$). In FRNS/SDNS patients, no significant difference in outcome was found between CsA and TAC therapy.

Relapses during follow-up

The rate of relapses in SRNS and FRNS/SDNS patients with complete remission in the first and second year of follow-up in both groups is summarized in Table 4. No significant differences were found between the SRNS and FRNS/SDNS groups.

Adverse effects

Table 5 summarizes the adverse effects reported in both groups. One patient in the TAC group was found to have hyperglycemia; his symptoms disappeared 3 months after insulin treatment, and TAC and low-dose prednisone therapy was continued. Nephrotoxicity was the most significant side effect seen in both groups. Of the four CsA-treated patients who suffered nephrotoxicity (3 with SRNS and 1 with FRNS), one patient with severe mesangial proliferative glomerulonephritis (MsPGN, *WT1* gene mutation was screened in this patient) showed persistent nephrotoxicity, while the other three patients showed reversible nephrotoxicity, with their serum creatinine levels returning to their previous concentrations 10–19 days after CsA therapy was stopped. None of the patients in the TAC group showed reversible or irreversible nephrotoxicity.

Discussion

In this prospective study we compared the efficacy and safety of TAC and CsA in children with idiopathic SRNS and FRNS/SDNS. We found that TAC was more efficient and safe than CsA in our SRNS patients, although many patients with complete remission in both groups still experienced relapse during the 1–2 years of follow-up. In FRNS/SDNS patients, there was no significant difference in short-term efficacy and number of relapses between the TAC and CsA groups.

At the end of 6 months of therapy, complete remission was seen in 84.6% (22/26) and 37.5% (3/8) of SRNS patients treated with TAC and CsA, respectively. This finding indicates that therapy with TAC is more efficient than CsA in inducing complete remission in children with SRNS. Similar response rates, ranging from 50 to 70%, have been reported in SRNS patients after therapy with TAC [15–17] or CsA [18, 19]. To the best of our knowledge, only one study has compared the treatment efficacy between TAC

Table 3 Short-term efficacy of children with primary nephrotic syndrome treated with CsA or TAC

Treatment	SRNS[a]				FRNS/SDNS[b]			
	n	Complete remission	Partial remission	No response	*n*	Complete remission	Partial remission	No response
CsA	8	3	1	4	16	14	2	0
TAC	26	22	4	0	24	22	1	1

[a] In SRNS patients, $\chi^2=13.75$, $P=0.001$;

[b] In FRNS/SDNS patients, $\chi^2=1.908$, $P=0.39$

CsA, cyclosporine A; TAC, tacrolimus; SRNS, steroid-resistant nephrotic syndrome; FRNS, frequently relapsing nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome

Table 4 Relapses within the first and second year of follow-up in patients with complete remission

Treatment	Follow-up	SRNS				FRNS/SDNS			
		n	No relapse	Relapses: <3 times/year	Relapses: >3 times/year	*n*	No relapse	Relapses: <3 times/year	Relapses: >3 times/year
CsA	First year	3	2	1	0	14	10	2	2
TAC		22	14	7	1	22	12	5	5
CsA	Second year	3	2	0	1	14	8	4	2
TAC		22	11	8	3	22	10	7	5

In SRNS patients during the first-year follow-up, χ^2 =0.261, P=0.88; during the second-year follow-up, χ^2 =2.685, P=0.26

In FRNS/SDNS patients during the first-year follow-up, χ^2 =1.046, P=0.59; during the second-year follow-up, χ^2 =0.587, P=0.75

SRNS, steroid-resistant nephrotic syndrome; FRNS, frequently relapsing nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; CsA, cyclosporine A; TAC, tacrolimus

and CsA in SRNS patients. Choudhry et al. [20] compared the efficacy and safety of TAC versus CsA in SRNS in a randomized controlled trial. After 6 months of therapy, these authors observed complete remission in 42.8% (9/21) and 50% (10/20) of patients treated with TAC or CsA, respectively, which led them to the conclusion that TAC or CsA in combination with low-dose steroids show similar efficacy in inducing remission in children with SRNS. Their results differ from those obtained in our study, although the reason for the discrepancy between these studies is unclear.

Severe FRNS/SDNS are common types of nephrotic syndrome observed in childhood. Sinha et al. [21] retrospectively compared the efficacy and complications of TAc and CsA in the management of severe FRNS/SDNS. They found no significant differences in rates of relapse per year, percentage changes in calculated GFR, and occurrence of renal toxicity between the two groups, leading them to conclude that the replacement of CsA by TAC did not lead to better management of severe SDNS.

Table 5 Reported side effects in children with refractory nephrotic syndrome treated with CsA or TAC

Adverse events	CsA (n=24)	TAC (n=50)	P
Nephrotoxicity	4	0	0.002
ALT/AST elevation	5	8	0.61
Gastrointestinal symptoms	5	11	0.91
Transient hypertension	3	12	0.23
Glucose intolerance and diabetes	0	1	0.37
Early-stage cataract	0	2	0.21
Hirsutism	8	0	<0.001
Psychiatric symptoms	0	2	0.21
Severe infections	9	15	0.52
Nutritional anemia	0	2	0.21

Values are given as the number of patients

ALT/AST, Alanine/aspartate transaminase; CsA, cyclosporine A; TAC, tacrolimus

In our study, we also observed no significant difference in short-term efficacy or relapse rate within the first- and second-year follow-ups between the two groups, indicating that TAC is not a better choice to replace CsA for the treatment of severe FRNS/SDNS. However, some side effects which are common in the CsA group (like hirsutism, nephrotoxicity) were rare in the TAC group. In our study, TAC appeared to be more effective than CsA in inducing remission in SRNS children, although no advantage of TAC over CsA was seen in terms of reducing the annual relapse rate. The reason for the different response to TAC in patients with SRNS versus those with SSNS is unknown. Individual patients showed a varied response from 1 year to the next, despite the targeted blood drug levels being maintained in both CsA and TAC patients. Relapse is a common and complex phenomenon in INS patients and involves many different factors. When targeted blood drug levels are sustained in patients, recurrence of proteinuria is not caused by varying levels of CsA or TAC, and so other reasons for relapse should be elucidated in individual patients with each episode of recurrence.

CsA and TAC can cause many adverse reactions, such as tubulointerstitial damage [22, 23], a problem that requires special attention from pediatric nephrologists. Fujinaga et al. [24] used CsA to treat children with nephrotic syndrome for more than 6 months and showed that CsA-related nephropathy occurred in 43% of children (13/30), which was manifested as cord-like fibrosis, tubular atrophy, and characteristic arterial disease [25]. In our study, we identified four patients in the CsA group with increases in baseline serum creatinine of >25%, which we defined as nephrotoxicity. At the same time, we found no patients with nephrotoxicity in the TAC group. Compared with the results from Choudhry et al.'s study [20], the incidence of nephrotoxicity in our study was much lower in both groups [CsA group 4/24 (our study) vs. 12/20; TAC group 0/50 (our study) vs. 8/21]. The reason for this significant difference in the incidence of nephrotoxicity between Choudhry et al.'s study and our study remains elusive because in both studies the desired levels for CsA (both 100–150 ng/mL) and TAC (5–12 vs. 5–8 ng/mL) were very similar. The initial dose of CsA

(3–5 mg/kg/day) and TAC (50–150 μg/kg/day) in our study are lower than the initial dose adopted in Choudhry et al.'s study [20] (CsA 5–6 mg/kg/day or TAC 0.1–0.2 mg/kg/day). For some patients who did not achieve the desired levels before remission, we supplemented the therapy with ketoconazole [9] or diltiazem [10] to increase their blood CsA or TAC concentration and to reduce their medical expenses. Thus, in our patient group, the overall final dose of TAC and CsA was 86.9±27.6 and 2.72± 0.59 mg/kg/day, respectively, which may partially explain the lower incidence of nephrotoxicity. Segarra et al. [26] reported that acute reversible nephrotoxicity is the main side effect (40%) of TAC therapy in cyclosporin-resistant or -dependent idiopathic focal glomerulosclerosis patients when started at a dose 0.15 mg/kg/day, whereas no new acute reversible nephrotoxicity was seen when a lower dose was used (0.08 mg/kg/day). Segarra et al.'s results are in accordance with our findings [26].

Podocin, encoded by the *NPHS2* gene, is an important molecule localized to glomerular podocytes and the slit diaphragm. Since the identification of the *NPHS2* gene [27], different groups have demonstrated that mutations in *NPHS2* represent a frequent cause of SRNS, occurring in 10–30% of the sporadic cases of SRNS [12–14]. However, in our study, no pathological mutations of the *NPHS2* gene were found in biopsy-proven FSGS subjects.

One limitation of our study is the lack of renal biopsy results for all patients, and very few patients had biopsy-proven assessment of calcineurin toxicity while on treatment. None of the subjects had formal assessment of GFR as performed in a number of other published studies. Also, the follow-up period was not prolonged, which would inevitably introduce some bias in the evaluation of the efficacy and safety of CsA or TAC in children with INS.

In summary, among our pediatric patients, the treatment of SRNS with TAC was associated with a higher efficacy and lower renal toxicity compared to CsA, although no favorable outcome in relapse rate during long-term follow-up was seen. On the other hand, TAC did not always appear to be the better choice to replace CsA in the treatment of severe FRNS/SDNS, although some side effects were observed less often in the TAC group. The chief limitations of this study are that it was not a randomized trial, the number of patients was small, and the duration of the follow-up was relatively short. We suggest future long-term randomized studies with larger sample sizes to assess the value of TAC in the treatment of SRNS and FRNS/SDNS.

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Conflict of interest statement None.

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