Pediatr Nephrol (2010) 25:539–544
DOI 10.1007/s00467-009-1377-5

BRIEF REPORT

Single infusion of rituximab for persistent steroid-dependent minimal-change nephrotic syndrome after long-term cyclosporine

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Received: 27 August 2009 / Revised: 23 October 2009 / Accepted: 26 October 2009 / Published online: 5 January 2010

Abstract Rituximab (RTX) has been successfully used as a rescue therapy in children with steroid-dependent nephrotic syndrome (SDNS). However, little is known regarding maintenance therapy after a successful response to RTX in such patients. The efficacy and safety of a single RTX infusion (375 mg/m^2) were assessed in ten patients who had persistent SDNS associated with minimal-change disease (MCD) despite the long-term use of cyclosporine (CsA). The mean follow-up after RTX infusion was 17 months. Applying RTX resulted in a significant reduction in the mean prednisolone (PSL) dose from 0.39±0.18 to 0.15±0.14 mg/kg per day. The mean 12-month relapse rates significantly decreased from 4.1±1.7 to 0.6±0.6. All but one patient who had continued CsA as maintenance therapy after a single RTX infusion were able to withdraw from PSL without any relapses during the study period, whereas the remaining five patients who discontinued CsA experienced relapses after CD19 cells re-emerged, leading to the reintroduction of CsA or an additional RTX infusion. Infusion reactions occurred in five of ten patients. These data indicate that a single RTX infusion may improve response to CsA in patients with persistent SDNS due to the phenomenon of secondary resistance to CsA.

Keywords Rituximab · Steroid-dependent nephrotic syndrome · Minimal-change disease · Cyclosporine

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Introduction

Most children with idiopathic nephrotic syndrome (NS) have minimal-change disease (MCD) and respond to steroids. However, up to 50% of patients with MCD develop frequently relapsing and/or steroid-dependent nephrotic syndrome (SDNS) and often require treatment with immunosuppressive agents, such as cyclophosphamide (CPM), cyclosporine (CsA), mizoribine (MZR), and mycophenolate mofetil (MMF) [1]. The long-term prognosis is generally good, but >25% of these patients require prolonged treatment with steroids and/or CsA in adulthood [2] and develop severe drug-induced toxicity such as growth retardation, cataracts, osteoporosis, hypertension, and nephrotoxicity. Furthermore, reduced efficacy of CsA due to drug tolerance from its long-term use was reported in some children with intractable NS [3].

In the last 5 years, the anti-CD20 monoclonal antibody rituximab (RTX) has been successfully used as a rescue therapy in patients with severe SDNS [4–6]. Although two multicenter studies have been reported in the literature [7, 8], there is no consensus on the optimal number of RTX infusions or maintenance therapy after successful response to the biological agent, which is due to a short observation period (both less than 1 year), heterogeneous series, and discordant treatment among the enrolled hospitals. The aims of this report are to describe the long-term clinical course of ten children with persistent SDNS due to MCD in a single center over a mean of 17 months with emphasis on their pre- and post-RTX use of immunosuppressants.

Patients and methods

Patients

Ten children (five boys, five girls) with persistent SDNS and biopsy-proven MCD despite the use of multiple immunosuppressive agents, including CsA, who were treated with a single RTX infusion at Saitama Children′s Medical Center between July 2007 and April 2008 were enrolled in this study. This included two patients (patients 11 and 12) whose clinical course during the 6 months before and after RTX infusion was already published in this journal [8]. Patients with steroid-resistant nephrotic syndrome (SRNS) were excluded from this study. The definitions and criteria for NS, remission, relapse, frequent relapse, steroid dependency, and steroid resistance were those of the International Study of Kidney Disease in Children. All patients and their parents gave informed consent, and the study was approved by the institutional review board of Saitama Children′s Medical Center.

Therapeutic protocol

According to the therapeutic protocol previously reported in this journal [8], RTX was administered intravenously in a single dose of 375 mg/m^2 (maximum 500 mg) during a proteinuria-free period. Premedication consisted of diphenhydramine and acetaminophen administered 30 min before RTX infusion. Repeated renal biopsies were performed prior to RTX infusion in nine of ten patients who had been treated with CsA for >2 years. CsA-induced nephrotoxicity (CsAN) was assessed by the presence of typical CsA-associated arteriolopathy (CAA) with or without characteristic striped tubulointerstitial lesions (STIL). CAA and STIL were defined when the ring-like nodular hyaline deposits in the outer wall of the afferent arteriole and tubular atrophy accompanying interstitial fibrosis were observed, respectively [9]. After RTX treatment, CsA was discontinued in patients who had CsAN on renal biopsy, whereas maintenance therapy with twice-daily CsA (2-h postdose levels of 400–600 ng/ml) was continued in patients who did not have CsAN or had been treated with CsA for <2 years [10]. The use of MMF, maintaining predose mycophenolic acid (MPA) levels at 2–5 μg/ml (maximum 1 g twice daily), was also continued during the study period [11]. The initial treatment of NS consisted of PSL 2 mg/kg per day in divided doses for 4 weeks, followed by taper to 1.3 mg/kg every other day for 4 weeks. NS relapse was treated with PSL at 2 mg/kg per day until proteinuria had disappeared for 3 consecutive days. Thereafter, PSL was switched to alternate days, and the dose was tapered gradually by 5–10 mg every 2–4 weeks. Treatment failure was defined as a condition requiring a high dose of PSL >0.5 mg/kg on alternate days to maintain remission despite the introduction of RTX. To assess treatment outcome and detect potential drug toxicity, clinical and laboratory assessments were performed before and 1 week after RTX infusion and then every 1–3 months. Laboratory assessments included CD19$^+$ lymphocytes by flow cytometry, complete blood counts, and serum levels of CsA, MPA, immunoglobulin, urea, creatinine, electrolytes, albumin, cholesterol, transaminase, bilirubin, amylase, and uric acid.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Numerical data were analyzed using the paired *t* test (two-tailed), Mann–Whitney *U* test, or Student′s *t* test. The level of statistical significance was set at $P<0.05$.

Results

Patients were diagnosed with NS at an average age of 5.6± 4.2 (range 1.1–13.5) years. They had developed SDNS and were prescribed CsA for an average of 44±22 (range 8–78) months. However, all patients continued to show steroid dependence despite the use of multiple immunosuppressive agents. Table 1 provides patient baseline characteristics. For 12 months before introducing RTX, the mean dose of PSL and mean number of relapses were 0.39±0.18 mg/kg per day and 4.1±1.7 episodes per year, respectively. Immunosuppressive agents used during the 12-month period before RTX were CsA in four; CsA and MMF in four; CsA, MMF, and CPM in one; and CsA and MZR in one.

Their age at the time of RTX treatment ranged from 3.9 to 18.8 (mean 11.1±4.5) years. After RTX infusion, five patients with severe CsA-induced nephrotoxicity discontinued CsA, whereas the remaining five continued the drug

Table 1 Baseline patient characteristics

Patient No.	Gender	Age at onset of NS (years)	Immunosuppressant before RTX	Number of relapses before RTX	At the moment of RTX infusion				
					Age (years)	Blood pressure (mmHg)	Weight (kg)	Obesity index (%)	Serum creatinine (mg/dL)
1	M	1.8	CsA, CPM, PSL	7	5.0	120/70	18.6	6.9	0.29
2	M	9.7	CsA, MZR, PSL	11	13.1	108/62	51.5	−5.2	0.41
3	F	9.9	CsA, PSL	8	13.6	120/70	49.4	−3.1	0.5
4	F	13.5	CsA, MMF, PSL	11	18.8	112/68	50.5	−2.5	0.38
5	M	4.1	CsA, CPM, MMF, PSL	16	14.9	128/84	50.5	3.6	0.56
6	F	3.9	CsA, CPM, MMF, PSL	10	10.4	100/50	41.2	4.3	0.45
7	M	6.8	CsA, MMF, PSL	4	11.5	120/80	29.4	−20.5	0.32
8	F	1.8	CsA, CPM, PSL	17	11.7	120/80	53.6	23.5	0.35
9	M	3.8	CsA, CPM, MMF, PSL	13	8.2	118/60	37.7	48.4	0.45
10	F	1.1	CsA, PSL	4	3.9	118/60	19.6	33.9	0.28

M male, *F* female, *NS* nephrotic syndrome, *CsA* cyclosporine, *CPM* cyclophosphamide, *MZR* mizoribine, *MMF* mycophenolate mofetil, *PSL* prednisolone, *RTX* rituximab

as maintenance therapy during the observation period. In three patients, maintenance therapy with MMF was also continued. In ten patients, RTX infusion for a mean follow-up period of 17 (range 13–21) months), resulted in a reduction of the mean PSL dose from 0.39±0.18 to 0.15± 0.14 mg/kg per day (63% reduction, $p<0.01$), the mean 12-month relapse rates from 4.1±1.7 to 0.6±0.6 episodes (86% reduction, $p<0.01$), and the obesity index, which is defined by the formula: (measured weight – ideal body weight) / ideal body weight × 100% [12] from 8.9±20.5 to 1.9± 12.5% ($p<0.05$).

Complete depletion of $CD19^+$ lymphocytes ($<5/mm^3$) was achieved in all but one patient at 1 week after RTX treatment. The patient in whom RTX did not induce a complete CD19-lymphocyte depletion experienced a relapse of NS 2 days after RTX infusion. The mean duration from the single RTX infusion until CD19 count was detectable (>1% of total lymphocytes) was 5.1 (range 2–7) months. NS relapses were associated with the re-emergence of CD19 cells in eight of nine episodes.

Four of five patients who had continued CsA after a single RTX infusion (group A) were able to withdraw PSL without any NS relapses during the study period, whereas the remaining five patients who discontinued CsA (group B) experienced relapses again after peripheral CD19 cells re-emerged. Figure 1a, b shows the clinical course of group A and group B between the 12-month period before RTX and the follow-up period after RTX. Three patients (8, 9, and 10) who discontinued all immunosuppressive agents except for PSL after RTX treatment became treatment failures shortly after the re-emergence of CD19 cells, requiring the reintroduction of CsA in two and an additional single RTX infusion in one. Two patients (6 and 7) who had continued maintenance therapy with MMF experienced one NS relapse at 14 and 16 months after RTX treatment, respectively. Several clinical and laboratory parameters were compared between group A and group B. Although there were no significant differences between the two groups in terms of the period of re-emergence of CD19 cells after RTX treatment, the PSL dose and number of relapses before RTX treatment, the PSL dose and number of relapses after RTX in group A was significantly lower than in group B (Table 2).

Adverse reactions occurred in five of ten (50%) patients and included facial flushing (two patients) or malaise (three patients) associated with the infusions. However, adverse events requiring hospitalization, such as bacterial infection or hypogammaglobulinemia, did not occur in any patients during the study period.

Discussion

In 1974, Shalhoub proposed that steroid-sensitive NS associated with MCD is a disorder of T-cell function with release of a circulating factor inducing proteinuria [13]. Since then, this circulating permeability factor associated with activated T cells has been thought to play a key role in MCD pathogenesis. However, recently, accumulated data indicate a strong contribution of B-cell immunity in children with steroid-sensitive MCD [14, 15]. RTX is a chimeric monoclonal antibody directed against the CD20 antigen, which is a membranous protein found on B cells. It has been successfully used in patients with B-cell lymphoma autoimmune disease such as systemic lupus erythematosus [16]. In addition, several case reports have suggested

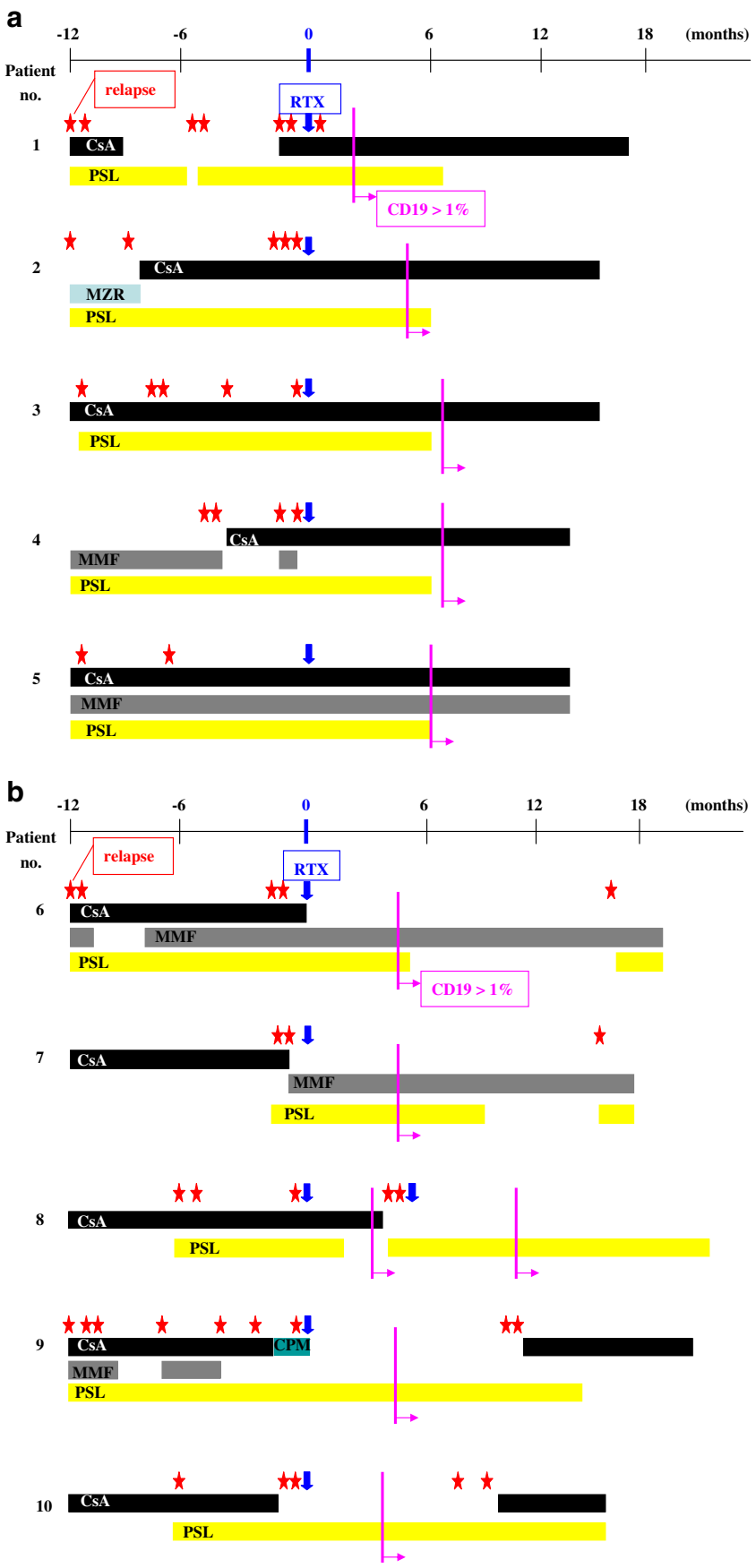

Fig. 1 **a** Clinical courses of group A (continuous cyclosporine group) between 12-month period before rituximab (RTX) and the follow-up period after RTX. *CsA* cyclosporine, *CPM* cyclophosphamide, *MZR* mizoribine, *MMF* mycophenolate mofetil, *PSL* prednisolone. **b** Clinical courses of group B (cyclosporine stop group) between 12-month period before RTX and the follow-up period after RTX

Table 2 Clinical characteristics of both group A (continuous cyclosporine group) and group B (cyclosporine stop group)

Clinical characteristics	Group A (*N*=5) Mean±SD	Group B (*N*=5) Mean±SD	*P* value
PSL dose before RTX (mg/kg/day)	0.40±0.13	0.38±0.23	0.75
Number of relapses before RTX (episodes/year)	4.4±1.5	3.8±1.9	0.60
Re-emergence of CD19 cells after RTX (months)	5.4±2.1	4.7±0.4	0.48
PSL dose after RTX (mg/kg/day)	0.07±0.02	0.22±0.16	0.01
Number of relapses after RTX (episodes/year)	0.14±0.31	1.0±0.37	0.02

PSL prednisolone, *RTX* rituximab

RTX may be effective in treating patients with MCD [6, 17]. Although RTX efficacy has been recently reported in a heterogeneous series of patients with SDNS/SRNS due to MCD or focal segmental glomerulosclerosis (FSGS) by two multicenter studies [7, 8], the follow-up period was relatively short (both <1 year), and the therapeutic protocol, such as the number of RTX infusions, timing of the introduction of RTX, or how to taper the dose of PSL, varied with each center. Guigonis et al. reported a therapeutic benefit from two to four infusions of 375 mg/m^2 RTX in 22 patients (MCD 16, FSGS three, unknown renal histology three) with steroid- or CsA-dependent NS [7]. However, deciding upon the number of RTX infusions was left to the clinician, and the median follow-up after RTX infusions was only 9.5 months. Similarly, in another multicenter study, the monitoring period for clinical parameters such as PSL dosage before and after RTX infusion was only 6 months, and the protocols for tapering PSL dose or discontinuation of immunosuppressive agents were not restricted [8]. Therefore, we analyzed the clinical information of ten patients with biopsy-proven MCD and persistent SDNS at a single center who were followed up for an average of 17 months after a single RTX infusion.

Although RTX efficacy as the sole therapy to prevent NS relapses in most patients was reported to be transient [8], there is little information on optimal maintenance therapy after RTX infusions [18]. In a previous study of a single RTX dose for refractory SDNS, six of eight patients (75%) who did not continue maintenance therapy with immunosuppressive agents experienced NS relapses after the re-emergence of CD19 cells and required additional RTX infusions [8]. On the other hand, Sharma et al. described a child who initially had SRNS due to FSGS who could benefit from the use of MMF in maintaining remission after an initial successful response to RTX [19]. In our study, five patients who discontinued CsA experienced relapses again after peripheral CD19 cells re-emerged, leading to the reintroduction of CsA or an additional single RTX infusion, whereas four of five patients who continued CsA as a maintenance therapy after RTX infusion were able to withdraw PSL without any NS relapses during the observation period; however, they previously required maintenance PSL dosage >0.5 mg/kg on alternate days during the 12 months preceding the RTX infusion. Our data indicate that patients can regain CsA efficacy to maintain NS remission after a single RTX infusion. This is the first report demonstrating a potential role for CsA in the long-term maintenance of remission despite the re-emergence of CD19 cells, even if patients had developed secondary resistance to CsA. Relapse of NS coincided with the re-emergence of CD19 cells (>1% of total lymphocytes) in eight of nine episodes. Because the re-emergence of CD19 cells after RTX treatment had a mean duration of 5.1 months in our study, a single RTX infusion every several months may be required to maintain NS remission if the patients discontinued CsA. However, further study is needed to determine the optimal interval for RTX infusion or the cutoff value of CD19 cells.

Despite the fact that CD19 cells were undetectable and CsA was continued, one patient showed a relapse 2 days after RTX infusion in our study (patient 1). Similarly, Kamei et al. reported that a patient suffered an NS relapse of 8 days after RTX infusion, although B-cell depletion was achieved [8]. Furthermore, in a series of patients with severe SDNS, it took >3 weeks after RTX infusions to achieve complete NS remission when RTX was administered during a nephrotic period, with no change in the immunosuppressive regimen [7]. In a report of five children with SRNS, four patients had complete remission and one had partial remission at a median interval of 4 (range 2–8) weeks after RTX treatment [20]. These results suggest that the duration required between RTX infusion and the onset of its effect was at least 2–4 weeks. Considering the time lag, we recommend avoiding rapid steroid reduction at least during the 4-week period after RTX treatment, even though B-cell depletion is achieved.

Mild reactions to RTX infusion occurred in half of our patients, but adverse effects requiring hospitalization, such as bacterial infection, were not seen in any of the patients. Although hypogammaglobulinemia associated with multiple RTX infusions was reported [7], serum immunoglobulin G levels did not decrease in our patients after a single RTX infusion.

In conclusion, data demonstrate that a single RTX infusion appears to be a disease-modifying therapy without serious side effects; the biological agent improved response

to CsA therapy and had significant steroid-sparing effects in children with refractory SDNS due to MCD who had developed secondary resistance to CsA. However, one limitation of this study is the absence of a placebo-controlled group. Therefore, a larger randomized controlled study is required to determine the role of RTX in treating severe SDNS. Furthermore, it must be considered that the use of this agent for NS patients is off-label at present.

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