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BRIEF REPORT

Positive role of rituximab in switching from cyclosporine to mycophenolate mofetil for children with high-dose steroid-dependent nephrotic syndrome

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Abstract
Background Recent randomized studies indicate that mycophenolate mofetil (MMF) is inferior to cyclosporine (CsA) in preventing relapses of nephrotic syndrome (NS). During the last decade, rituximab (RTX) has emerged as a rescue therapy in patients with complicated, frequently relapsing, or steroid-dependent NS.
Case-Diagnosis/Treatment After introducing RTX in our single center, we analyzed 26 patients with steroid-dependent NS who had relapses while receiving long-term CsA and who were subsequently switched to MMF. MMF was adjusted to maintain a targeted predose mycophenolic acid (MPA) level of 2–5 μg/ml. Moreover, for patients who required MMF and high-dose prednisolone (PSL) to maintain remission, a single infusion of RTX (375 mg/m^2) was added. The primary endpoint was the probability of achieving PSL-free remission for >1 year. At a mean follow-up of 28.8±9.9 months, 11 of 26 patients (42 %) required RTX treatment, and 22 of those patients (85 %) achieved PSL-free sustained remission. The mean predose MPA levels for patients who achieved PSL-free sustained remission were significantly higher compared with those for patients who did not (3.1 μg/ml vs. 1.7 μg/ml, $p<0.05$).
Conclusions After RTX introduction, most patients were able to switch from CsA to MMF and achieve sustained PSL-free remission.

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Introduction

Efficacy and safety of the initial 2-year cyclosporine A (CsA) treatment is well established for children with frequently relapsing or steroid-dependent nephrotic syndrome (NS) [1, 2]. In addition, previous studies have suggested that long-term CsA use (>2 years) is a significant risk factor for chronic nephrotoxicity development [3, 4]. However, in clinical practice, its long-term use becomes inevitable, because most patients relapse after its discontinuation and consequently develop chronic CsA nephrotoxicity and secondary CsA resistance [3–5].

Mycophenolate mofetil (MMF), which strongly inhibits both T- and B-lymphocyte proliferation, has been used as a steroid- and CsA-sparing agent for such NS patients. Although we have demonstrated the efficacy and safety of MMF therapy based on predose mycophenolic acid (MPA) levels in children with steroid- and CsA-dependent NS, one fourth of these patients fail treatment and require reintroduction of CsA [6, 7]. Furthermore, randomized trials, including a crossover study, also indicate that MMF is less effective than CsA in maintaining NS remission [8, 9].

The anti-CD20 monoclonal antibody, rituximab (RTX), has emerged as rescue therapy for patients with complicated, frequently relapsing, or steroid-dependent NS [10, 11]. However, several studies suggest that RTX monotherapy efficacy for NS relapse prevention is transient, with most patients relapsing with the re-emergence of B cells. Therefore, some pediatric nephrologists recommend regular and repeated

treatment with RTX, with the aim of inducing a long-lasting B-cell depletion period [12]. However, life-threatening complications, such as lung fibrosis and myocarditis, have been described [13, 14].

We hypothesized that the addition of a single RTX infusion as a rescue therapy, in the setting of continuing relapses despite MMF use, may play a positive role in treating high-dose steroid-dependent NS while avoiding serious adverse events associated with repeated administration of RTX. After introducing RTX in our single center, we analyzed 26 patients with complicated steroid-dependent NS who had relapses during CsA therapy and who were subsequently switched to MMF.

Patients and methods

Patients

We enrolled 26 patients (16 boys and 10 girls; mean age, 12.1 ±4.0 years) with complicated steroid-dependent NS who had experienced relapses during CsA therapy and who were subsequently switched from CsA to MMF at Saitama Children's Medical Center between March 2008 and October 2012. Patients who had received MMF treatment prior to RTX introduction in our hospital (before 2008) were excluded.

In this study, the definitions and criteria used for NS, remission, relapse, frequent relapse, and steroid dependency and resistance were in accordance with the International Study of Kidney Disease in Children. All patients and their parents provided written informed consent, and the off-label use of RTX and MMF was approved by the institutional review board of Saitama Children's Medical Center.

Therapeutic protocol

MMF (Cellcept, oral formulation, Chugai Co., Tokyo, Japan) was administered after patients achieved complete remission with prednisolone (PSL). As per the therapeutic protocol, MMF was introduced at an initial dose of 250 mg/12 h and adjusted to maintain a target predose MPA level of 2–5 μg/ml (maximum 1 g twice daily), in accordance with previously reported findings [7]. MPA was measured in plasma by Special Reference Laboratory (SRL Inc) using the enzyme-mediated immune technique. All pharmacokinetic monitoring of MPA were performed during remission of NS.

After starting MMF therapy, CsA dosage was gradually tapered by 20–50 mg every 4 weeks. Relapses were treated with PSL 2 mg/kg per day until proteinuria was undetectable for >3 consecutive days. PSL was administered on alternate days thereafter, and the dose was tapered off within 6 months by decreasing the dose at a rate of 5–10 mg every 2–4 weeks. Treatment failure was defined as any condition requiring high-dose PSL administration (>0.5 mg/kg or >15 mg) on alternate days to maintain remission, despite MMF use. When patients developed treatment failure, they additionally received a single RTX infusion (Zenyaku Co., Tokyo, Japan). According to the therapeutic protocol reported previously, RTX was intravenously administered in a single dose of 375 mg/m^2 (maximum, 500 mg) during a proteinuria-free period [10]. Premedication comprised diphenhydramine and acetaminophen administered 30 min before RTX infusion. PSL was tapered off and then withdrawn within 6 month after RTX administration. In addition, the primary endpoint was the probability of achieving >1 year of PSL-free sustained remission. For patients who achieved such remission, MMF was gradually tapered off.

To assess treatment outcome and detect potential drug toxicity, clinical and laboratory assessments were performed prior to and 1–2 weeks after MMF and RTX infusion, after which these tests were performed every 1–3 months. Laboratory assessments included the detection of CD19+ and CD20+ lymphocytes by flow cytometry and complete blood count, as well as levels for MPA, immunoglobulins, urea, creatinine, electrolytes, albumin, cholesterol, transaminases, bilirubin, amylase, uric acid, and C-reactive protein.

Statistical analysis

Data are expressed as means±standard deviations (SD). Numerical data were analyzed using the Mann–Whitney *U* test or the Wilcoxon signed-rank test. Fisher's exact test was used to evaluate the association between categorical variables. The level of statistical significance was set at $p<0.05$.

Results

Average patient age at NS diagnosis was 7.0±4.0 (range, 1.3–13.6) years. Patients developed steroid-dependent ($n=23$) or steroid-resistant ($n=3$) NS and were prescribed CsA at a mean age of 8.3±4.1 (range, 1.6–13.7) years for a mean of 46.5±27.2 (range, 26–133) months. Although three patients with initial steroid-resistant NS (two with minimal change disease and one with focal segmental glomerulosclerosis) achieved complete remission after CsA therapy initiation, they subsequently developed steroid-dependent NS. Prior to CsA therapy, renal biopsies were performed in all patients. Furthermore, histological diagnoses were minimal change disease (24 patients) and focal segmental glomerulosclerosis (two patients). During CsA therapy, all 26 patients experienced relapses (median of three relapses; range, 1–18).

Before MMF therapy, posttreatment renal biopsies were performed in all patients who received CsA therapy for >24 months. Biopsies showed evidence of CsA

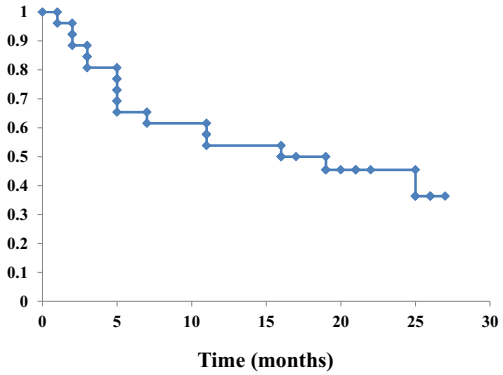

Fig. 1 Kaplan–Meier analysis showing proportion of patients with sustained remission among 26 patients switched from cyclosporine (CsA) to mycophenolate mofetil (MMF)

nephrotoxicity in 11 patients (42 %): three had arteriolopathy, and eight had striped tubulointerstitial lesions. Mean patient age at MMF initiation was 12.1±4.0 (range, 4.8–21.8) years, and mean follow-up period after MMF initiation was 28.8± 9.9 (range, 16–57) months. Furthermore, mean MMF dose required was 33.3±7.0 (range, 18.5–48.7) mg/kg per day to maintain a mean predose MPA level of 2.8±0.8 (range, 1.4–4.2) μg/ml. During the study period, 11 patients (42 %) did not experience NS relapse despite CsA withdrawal (Fig. 1). Eleven of 26 patients (42 %) received RTX treatment because of treatment failure. Twenty-two patients (85 %) achieved PSL-free sustained remission for >1 year, and 15 (58 %) were able to discontinue MMF. The mean relapse rate associated with MMF and RTX use (0.7±0.5/year) appeared to be lower than that associated with CsA use (1.0±0.9/year). However, this difference did not reach statistical significance (p=0.07). Table 1 shows the clinical characteristics of patients who achieved (group A) and who did not achieve (group B) PSL-free sustained remission . Mean predose MPA levels for group A were higher compared with those for group B (3.1 μg/ml vs. 1.7 μg/ml, p<0.05). The PSL-free sustained remission rate was significantly higher in patients with higher predose MPA levels (>2.0 μg/ml) compared with that in patients with lower levels (<2.0 μg/ml) (95 % vs. 40 %, p<0.05). A significant negative correlation was also observed between mean predose MPA levels and relapse rates (Spearman's correlation coefficient by rank test; rs=−0.44, p=0.029). The higher the predose MPA level, the lower was the relapse rate (Fig. 2). Table 2 summarize clinical characteristics of 11 patients who received RTX in group C (PSL-free remission period for >1 year) and group D (PSL-free remission period for ≤1 year). Although the mean predose MPA levels in group C appeared to be higher than that in group D, the difference did not reach significance. Median B-cell depletion period (defined by the time from RTX treatment until detection of CD19+ cell count >1 % of total lymphocytes) in group C appeared to be longer than that in group D. However, this difference did not reach statistical significance.

During MMF therapy, two patients experienced mild gastrointestinal symptoms, and two suffered from herpes simplex virus infection. RTX-associated adverse effects occurred in six of 11 patients; five had mild infusion reactions. One patient developed late-onset neutropenia 2 months after RTX administration and required granulocyte colony-stimulating factor (GCSF) treatment. However, no severe adverse effects, such as sepsis, progressive multifocal leukoencephalopathy, or interstitial pneumonia occurred in any patient during the study period.

Discussion

In this study of 26 patients with steroid-dependent NS who experienced relapse during treatment with CsA and who were

Table 1 Clinical characteristics of 26 patients in group A (prednisolone-free remission period for >1 year) and group B (prednisolone-free remission period for ≤1 year)

	Group A (N=22)	Group B (N=4)	P value
Gender (male/female)	13/9	3/1	0.50
Renal histology (MGA/FSGS)	20/2	4/0	0.71
Initial presentation (SDNS/SRNS)	19/3	4/0	0.59
Mean predose MPA levels (>2/≤2 μg/ml)	20/2	1/3	0.014
Administration of RTX (yes/no)	7/15	4/0	0.022
	Median (range)	Median (range)	
Age at onset of NS (months)	79.5 (16–163)	107 (33–138)	0.26
Duration of CsA (months)	39.5 (26–133)	30.5 (26–37)	0.09
Age at start of MMF (months)	159.5 (57–261)	160 (80–168)	0.80
Duration of MMF (months)	26 (16–57)	32 (20–40)	0.43
	Mean±SD	Mean±SD	
Relapse rate during CsA (/year/patient)	0.95±0.6	1.5±1.0	0.24
Mean MPA pre-dose levels (μg/ml)	3.1±0.82	1.7±0.75	0.025
Relapse rate during MMF (/year/patient)	0.2±0.48	2.2±0.61	0.011

PSL prednisolone, *NS* nephrotic syndrome, *MGA* minor glomerular abnormality, *FSGS* focal segmental glomerulosclerosis, *SRNS* steroid-resistant NS, *SDNS* steroid-dependent NS, *MPA* mycophenolic acid, *RTX* rituximab, *CsA* cyclosporine, *MMF* mycophenolate mofetil, *SD* standard deviation

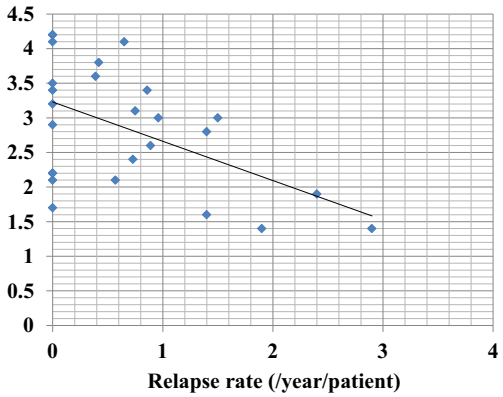

Fig. 2 Correlation between mean predose mycophenolic acid levels and relapse rate in 26 patients treated with mycophenolate mofetil (MMF). A significant negative correlation is shown between mean predose mycophenolic acid (MPA) levels and relapse rates ($r=-0.44$, $p=0.029$)

subsequently switched from CsA to MMF, we found that most patients (85 %) achieved PSL-free sustained remission for >1 year, although 11 patients (42 %) required additional RTX treatment because of treatment failure. In a prospective follow-up study of 49 Japanese children who received 2-year CsA treatment for frequently relapsing NS, most patients experienced NS relapse shortly after CsA discontinuation [15]. That study also revealed that three fourths of patients who experienced NS relapse during CsA therapy developed frequent relapses after its discontinuation, suggesting that the initial 2-year CsA treatment was clearly insufficient for those complicated patients. Therefore, establishing safe and effective immunosuppressive therapy following 2-year CsA administration is urgently desired, especially for patients who experience relapses during CsA therapy.

Although MMF has a favorable side-effect profile compared with CsA, studies indicate that MMF is inferior to CsA in preventing NS relapses [8, 9]. In a randomized controlled trial to compare MMF to CsA efficacy in 24 children with frequently relapsing NS, Dorresteijin et al. [8] reported that the relapse rate in the MMF group was higher compared with that in the CsA group (0.83/year vs. 0.08/year). Furthermore, in a randomized crossover study comparing the efficacy of a 1-year treatment with MMF or CsA in 60 children with frequently relapsing NS, Gellermann et al. [9] showed that time without relapse was significantly shorter with MMF compared with CsA during the first year (195 vs. 543 days). In contrast, our study demonstrated that the MMF relapse rate appeared lower compared with the CsA relapse rate (0.7/year vs. 1.0/year) and that 85 % achieved long-term PSL-free remission (>1 year) after switching from CsA to MMF.

The reason for explaining the better efficacy of MMF compared with CsA in this study could be the fact that MMF was administered after CsA and that the number of NS relapses spontaneously tends to decrease with time. Furthermore, we speculate that the beneficial effect of MMF may reflect carryover effects from previous CsA treatment or synergistic effects from the additional RTX treatment. Gellermann et al. similarly reported that prior CsA therapy was associated with higher MMF efficacy in the second treatment year. They also found that high predose MPA exposure (>3.5 μg/ml) provides therapeutic efficacy similar to CsA treatment. In our study, the sustained PSL-free remission rate was significantly higher in patients with higher predose MPA levels (>2.0 μg/ml) compared with that in patients with lower levels (≤2.0 μg/ml). The comparable efficacy in our patients with relatively low predose MPA exposure can be explained by the additional single RTX infusion. Ito et al. also reported that MMF therapy after a single RTX infusion for steroid-dependent NS significantly prolonged the relapse-free period compared with RTX monotherapy, although no pharmacokinetic monitoring was performed in their pilot study. In 11 patients who received RTX, we found that the sustained

Table 2 Clinical characteristics of 11 patients who received rituximab in group C (prednisolone-free remission period for >1 year) and group D (prednisolone-free remission period for ≤1 year)

	Group C ($N=7$)	Group D ($N=4$)	P value
Gender (male/female)	3/4	3/1	0.35
Renal histology (MGA/FSGS)	7/0	4/0	0.67
Initial presentation (SDNS/SRNS)	6/1	4/0	0.64
Mean predose MPA levels (>2/≤2 μg/ml)	6/1	1/3	0.09
Duration of B-cell depletion (>6/≤6 months)	6/1	1/3	0.09
	Median (range)	Median (range)	
Age at onset of NS (months)	74 (48–132)	129 (33–138)	0.19
Age at start of MMF (months)	156 (57–193)	160 (80–168)	0.92
Duration of MMF (months)	33 (25–42)	32 (20–40)	0.92
Number of RTX	1 (1–2)	2 (1–2)	0.05
Duration after 1st RTX (months)	21 (10–29)	28 (16–33)	0.18
Duration of B-cell depletion (months)	7 (4–20)	5.3 (2.5-7)	0.11
	Mean±SD	Mean±SD	
Mean MPA predose levels (μg/ml)	2.8±0.8	1.9±0.8	0.11
Relapse rate during MMF (/year/patient)	1.0±0.3	2.2±0.6	0.008

PSL prednisolone, *NS* nephrotic syndrome, *MGA* minor glomerular abnormality, *FSGS* focal segmental glomerulosclerosis, *SRNS* steroid-resistant NS, *SDNS* steroid-dependent NS, *MPA* mycophenolic acid, *RTX* rituximab, *MMF* mycophenolate mofetil

PSL-free remission rate appeared to be higher in patients with longer B-cell depletion period (>6 months) compared with that in patients with the shorter period (≤6 months). We previously reported that the risk of early relapse during maintenance therapy with CsA or MMF after a single RTX infusion was positively associated with early recovery of CD19 cells [16]; however, the number of patients who received RTX was too small to draw definite conclusions on this point.

Although mild reactions to RTX infusion occurred in about half of our patients, severe adverse effects requiring hospitalization were not observed. However, long-term immunological complications after repeated RTX infusions, especially in children, remain unknown. Trujillo et al. reported that a 5-year-old boy with NS continues to have hypogammaglobulinemia and require gammaglobulin intravenously after 3 years of RTX administration [17]. As emphasized in a recent review, children with steroid-dependent NS are never cured by RTX but shift from CsA and PSL dependence to RTX dependence; these repeated RTX infusions could be hazardous [18]. Therefore, in our opinion, RTX use should be restricted to children who experience continuing relapses despite receiving optimal combinations of PSL and steroid-sparing agents, such as MMF.

Our study has several limitations. First, it was a single-center observational study. Second, the targeted predose MPA level was not achieved in all patients. Third, the number of patients in group B compared to that in group A (4 vs. 22) was too small to draw the robust conclusions. Fourth, although recent randomized study suggests a beneficial effect of a higher MPA area under the curve (AUC), this was not measured in our study. Fifth, correlation between mean predose MPA levels and relapse rates was a rather weak, which may be due to combination therapy such as steroid and RTX. A larger, multicenter randomized controlled study is required to determine optimal therapeutic monitoring of MPA levels and MMF dose adjustment.

In conclusion, our data suggest that MMF in combination with a single RTX infusion in the setting of treatment failure despite MMF use seems safe and effective in treating complicated steroid-dependent NS after the initial 2-year CsA therapy. With this therapeutic strategy, we postulate that both CsA nephrotoxicity and serious adverse events associated with repeated RTX infusions may be avoided in many NS patients.

Conflict of interest None.

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