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ORIGINAL ARTICLE

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High-dose mizoribine therapy for childhood-onset frequently relapsing steroid-dependent nephrotic syndrome with cyclosporin nephrotoxicity

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Abstract Cyclosporin A (CsA) is an effective treatment for frequently relapsing steroid-dependent nephrotic syndrome (FR-SDNS), but its use can be complicated by renal toxicity and a high incidence of relapses after withdrawal. We report 9 adolescent patients with childhood-onset FR-SDNS who had been treated with long-term CsA that resulted in moderate-to-severe CsA nephropathy (CsAN). They were treated with high-dose (mean: 10.1 mg/kg per day) mizoribine (MZR) in an attempt to allow weaning of CsA and/or steroid therapy, and reduce the frequency of relapses. Seven out of 9 patients were weaned off CsA by 1-year follow-up, although in the remaining 2 patients, MZR did not show any beneficial effects. Overall, this high-dose MZR therapy results in significant steroid sparing and reduction in relapse rates in our patients. Our experience shows that high-dose MZR therapy in patients with FR-SDNS who are also CsA-dependent appears to be effective in reducing CsA exposure as well as in decreasing the frequency of relapses.

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Introduction

According to the report from the International Study of Kidney Disease in Children (ISKDC), 78.1% of 471 children with primary nephrotic syndrome (NS) responded to corticosteroid therapy, and of these, 91.8% had minimal change histology [1]. About 25% of the children with minimal change nephrotic syndrome (MCNS) have a single relapse, and 50% experience frequent relapses or become corticosteroid-dependent with a clinical course stretched many years [2]. These children are difficult in clinical practice because of the need for repeated moderate-to-high-dose steroid exposure and/or long-term cumulative steroid exposure, therefore, immunosuppressive agents are often used for their steroid-sparing effects.

The recent systemic review of randomized controlled trials shows cyclophosphamide, chlorambucil, cyclosporine, and levamisole reduce the risk of relapse in children with steroid-sensitive nephrotic syndrome compared with prednisone alone [3]. Among them, treatment with cyclosporin A (CsA) significantly decreases relapse rates and steroid requirements [3], but the dose and duration of therapy are important for side effects and complications. The most important problems are nephrotoxicity and hypertension [4]. Furthermore, most patients experience a relapse when CsA is discontinued [5], in some of whom, the control of NS is subsequently more difficult [4].

For these patients, mycophenolate mofetil (MMF), an immunosuppressive agent used for the prevention of acute rejection of renal allografts [6], has been reported to be beneficial [7, 8]. Since MMF is not currently approved for use in patients with nephrotic syndrome in Japan, we have used mizoribine (MZR) (4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate), another inhibitor of inosine monophosphate (IMP) dehydrogenase, which is the key enzyme in de novo synthesis of purine nucleotides [9], for

Table 1 Summary of clinical backgrounds of 9 patients treated with high-dose mizoribine (*NS* nephrotic syndrome, *GFR* glomerular filtration rate, *CsA* cyclosporin A, *MZR* mizoribine)

Patient No.	Age, gender	Age at onset of NS	Height (SD)	GFR by Schwartz's formula (ml/min/1.73m^2)	Duration of CsA therapy (months)	Trough level of CsA pre-MZR therapy (ng/ml)
1	12 years, 9 months, M	1 year, 10 months	0.53	117.0	98	46.4
2	16 years, 1 months, M	3 years, 8 months	−1.21	138.1	41	42.8
3	17 years, 1 month, M	2 years, 5 months	−4.95	155.6	133	128.5
4	17 years, 2 months, M	2 years, 0 months	−2.46	142.1	93	81.8
5	18 years, 9 months, M	2 years, 8 months	−1.04	110.8	108	158.2
6	19 years, 0 months, M	4 years, 3 months	−1.84	112.1	46	68.1
7	19 years, 1 month, M	1 years, 10 months	−3.91	115.5	165	80.7
8	19 years, 11 months, F	1 year, 11 months	−3.72	95.8	86	86.6
9	20 years, 9 months, F	7 years, 11 months	−3.58	77.0	206	113.5
Mean	17 years, 9 months	3 years, 2 months	−2.46	118.2	108.4	89.6
SD	2 years, 4 months	1 year, 11 months	1.73	24.2	53.2	37.8

the treatment of child-onset frequently relapsing steroid-dependent nephrotic syndrome (FR-SDNS) for patients who had been treated with long-term CsA and developed moderate-to-severe CsA nephropathy (CsAN).

Patients and methods

Nine patients (Table 1), aged 13–20 years (mean: 17.8 years), with child-onset FR-SDNS were enrolled in the study. All of them were treated at Saitama Children's Medical Center (Division of Nephrology), a major referral center for patients in the northern part of the Tokyo Metropolitan area. Patients were considered to have frequently relapsing NS if they had 2 or more documented relapses within a 6-month period of initial response, or 4 or more relapses within any 12-month period. FR-SDNS was defined as complete remission [urinary protein excretion of <4 mg/h per m^2, or reagent strip (Albustix) with negative or trace protein for at least 3 consecutive days] after an initial 4-to-8-week course of daily steroids, but relapse (urinary protein excretion of >40 mg/h per m^2 or reagent strip of 2+ or more protein for 3 consecutive days, having previously been in remission) immediately after weaning to alternate-day steroid dosing, and demonstration of significant steroid toxicity. The patients with SDNS at relapse were treated with prednisolone (PSL) at a dose of 60 mg/m^2 per day in 3 divided doses. CsA (Sandimmune and/or Neoral, oral formulation, Novartis) treatment was started at a dose of 3 mg/kg per day administered orally in 2 divided doses, and subsequently the dose of CsA was adjusted to maintain whole-blood 12-h trough levels between 60 ng/ml and 80 ng/ml, measured by a monoclonal radioimmunoassay. The steroids were then reduced and changed to alternate-day dosing, slowly tapered, and discontinued after 4–8 months.

All renal biopsies were performed percutaneously under ultrasound guidance by using a 16-gauge biopsy needle (Bard, Covington, GA, USA). Biopsies were examined by light microscopy (hematoxylin and eosin and periodic acid-Schiff stains), immunofluorescence microscopy, and electron microscopy. Specimens were examined by renal pathologists.

Tubular, interstitial, and arteriolar lesions were evaluated in order to assess CsAN. CsA nephrotoxicity was defined as tubular atrophy with accompanying interstitial fibrosis.

Mild toxicity was defined as scattered foci of atrophic tubules within discrete areas of interstitial fibrosis; moderate toxicity as several areas of tubular atrophy within areas of interstitial fibrosis; and severe toxicity as confluent or extensive areas of interstitial fibrosis with atrophic and/or collapsed tubules (striped fibrosis).

With the permission of the Institutional Ethics Board, MZR at higher doses than those for regular use was offered as a treatment option for CsA-dependent and/or steroid-dependent patients who had failed previous attempts at withdrawal from CsA or steroids. Prior to initiating treatment, signed informed consent for high-dose MZR treatment was obtained from the patient and/or parents. All patients and families were counseled regarding the unproven efficacy and unknown long-term side effects of MZR therapy, and patients were offered the option of alternative management with continuation of steroids with or without CsA.

The therapy with high-dose MZR was started from April 2003. MZR was begun at an initial dose of 3–4 mg/kg per day administered orally before every morning meal, and, subsequently, the dose of MZR was adjusted to achieve a C2 (peak blood level, 2 h after drug administration) around 3 mcg/ml (with the approval of the IRB). The target concentration of MZR in serum was set according to the report by Tanaka et al. [10], and the samples were measured by Asahi Kasei Pharma using high-performance liquid chromatography (HPLC).

Routine blood examination including complete blood counts, serum levels of urea, creatinine, electrolytes, albumin, cholesterol, transaminases, amylase, and uric acid was performed 2 weeks after the initiation of therapy and then at monthly intervals.

Patients were administered MZR for approximately 4 weeks before attempting to wean CsA over the subsequent 6–12 months. Relapses were treated with daily PSL until remission, with tapering later. Any other drugs such as angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, nonsteroidal anti-inflammatory drugs and lipid-lowering agents were not used.

Numerical data were analyzed using paired t -test (two-tailed) using StatView (Abacus Concepts, Berkeley, CA, USA). Data are expressed as mean ± standard deviation (SD), with p value <0.05 considered significant.

Results

Patient characteristics are outlined in Table 1. The patients were diagnosed with NS at an average age of 38.0 months (range 22–95 months). All patients carried the diagnosis of FR-SDNS.

Each patient had been previously treated with one 12-week course of oral cyclophosphamide (dosage 168 mg/kg per course) prior to initiation of treatment with CsA. On initial biopsy, 7 patients were noted to have pathology consistent with MCNS, while 2 carried the diagnosis of IgM nephropathy (patients 1 and 3). All the patients had cushingoid features, and 5 patients (55.6%) had growth retardation (height SDs <–2). Before CsA treatment, 7 out of 9 patients had normal creatinine clearance, estimated using the Schwartz formula; mean, 118.2 ml/min/1.73m^2, range 77.0–155.6 ml/min/1.73m^2 [11]. All the patients had been treated with CsA (Sandimmune and/or Neoral)

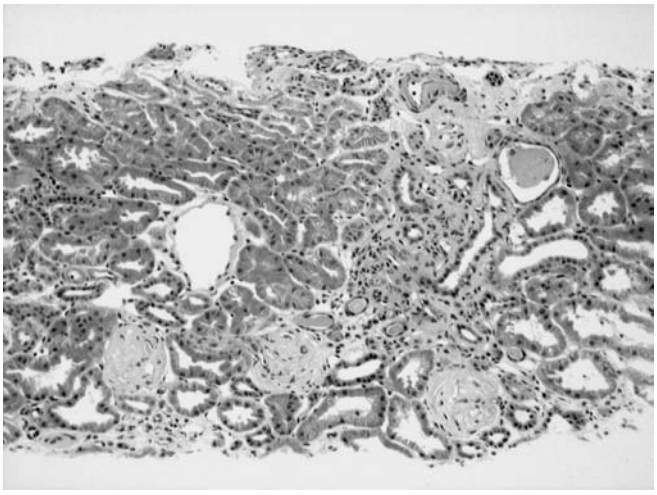

Fig. 1 Moderate chronic cyclosporin nephropathy in patient 8; striped interstitial fibrosis with tubular atrophy is noted among the glomeruli (HE × 10)

for an average of 108.4 months (range 46–206 months) and had failed prior attempts to wean CsA. These patients were offered high-dose MZR in an attempt to allow weaning of CsA. CsA trough levels prior to initiation of therapy with MZR had been maintained at approximately 60–80 ng/ml (radioimmunoassay). Before the initiation of MZR therapy, the mean CsA dose and the mean CsA blood trough levels were 4.09 mg/kg per day (range 3.13–5.30 mg/kg per day) and 89.6 ng/ml (range 42.8–158.2 ng/ml), respectively. All of them had surveillance biopsies consistent with moderate-to-severe CsA toxicity (interstitial fibrosis)(Fig.1, Patient 8).

The precise laboratory data of the patients treated with MZR was summarized in Table 2. The average age of patients at time of initiation of therapy with MZR was 16 years, 7 months (range 12 years, 0 months to 19 years, 4 months). The mean MZR dose was 10.1 mg/kg per day (range 6.97–16.44 mg/kg per day). The clinical parameters before treatment and on MZR treatment are depicted in Table 3. The number of relapses in the 12 months prior to initiation of MZR therapy was compared with the number of relapses during the 12 months after starting MZR. The mean number of relapses in the 12 months preceding MZR therapy was 2.33 (±0.71), compared with 1.11 (±0.78) in the 12 months following initiation of MZR treatment (±SD, p =0.016, paired t -test). Because 2 patients (patients 5 and 7) had intractable relapses and progressed to steroid-resistant nephrotic syndrome (SRNS) while tapering CsA, they were considered to have MZR failure and discontinued MZR therapy. They remain on CsA and steroids. In the remaining 7 patients, with the mean number of relapses during the 12 months after starting MZR being 0.86 (±0.69), 6 were successfully converted from CsA to MZR, and there was a reduction in the dose of CsA required to remission in 1 patient (patient 2). The cumulative CsA dose (mg/kg per day) in the 12 months before treatment and on MZR therapy was 3.20±0.83 and 1.75±0.79 (mean ± SD, p =0.0091, paired t -test), respectively. The cumulative PSL dose (mg/kg per day) in the 12 months before treatment and on MZR therapy was 0.21±0.07 and 0.19±0.11 (mean ± SD, p =0.1185, paired t -test), respectively. Other clinical parameters such as the patients' body weight, the amount of urinary protein excretion and blood pressure were not statistically changed before treatment and on MZR treatment.

Two patients (patients 4 and 9) have remained relapse free with MZR, and the other 5 patients responded to a short course of steroids without the need to restart CsA therapy at relapse(s).

Only 1 patient experienced side effects of MZR. Patient 4 developed zoster while on the combination therapy of MZR with CsA and steroids. Treatment of acyclovir with discontinuation of CsA and reduction of MZR dose was well-tolerated and effective. No other significant complications were observed in the rest of the patients.

Table 2 Laboratory data pre-therapy and on mizoribine therapy (*CsA* cyclosporin A, *MZR* mizoribine, *PSL* prednisolone)

Patient No.	CsA dose before the start of MZR (mg/kg/day) <Cumulative CsA dose in the 12 months pre-MZR (mg/kg/day)>	CsA dose 12 months after MZR treatment (mg/kg/day) <Cumulative CsA dose in the 12 months post-MZR (mg/kg/day)>	PSL dose before the start of MZR (mg/kg/day) <Cumulative CsA dose in the 12 months pre-MZR (mg/kg/day)>	PSL dose 12 months after MZR therapy (mg/kg/day) <Cumulative CsA dose in the 12 months post-MZR (mg/kg/day)>	MZR dose (mg/kg/day)	MZR C2 level (ng/ml)	Relapse(s) in 1 year pre-therapy/on MZR
1	3.13 <2.73>	0 <2.13>	0.42 <0.22>	0.26 <0.41>	13.57	3.45	3/1
2	3.29 <1.89>	1.55 <1.30>	0.10 <0.14>	0.10 <0.08>	9.67	3.07	3/1
3	4.11 <3.57>	0 <0.99>	0.14 <0.19>	0.28 <0.20>	16.44	3.09	2/1
4	4.24 <3.39>	0 <1.55>	0.64 <0.07>	0.16 <0.12>	7.42	3.07	2/0
5	5.30	4.05	0.32	0.28	9.21	3.03	2/2
6	3.98<4.45>	0 <2.98>	0 <0.002>	0.20 <0.20>	6.97	3.26	2/1
7	4.25	3.64	0	0.61	7.28	2.41	1/2
8	4.13 <2.69>	0 <2.43>	0.37 <0.11>	0.22 <0.19>	11.80	3.08	3/2
9	4.37 <3.66>	0 <0.85>	0.15 <0.12>	0.12 <0.12>	8.75	3.26	3/0

Table 3 Changes in clinical parameters pre-therapy and on mizoribine therapy (*CsA* cyclosporin A, *MZR* mizoribine, *SDNS* steroid-dependent nephrotic syndrome)

Patient No.	Relapse(s) in 1 year pre-therapy/on MZR	Body weight pre-therapy/ on MZR (kg)	Urinary protein (mg)/ creatine (mg) ratio pre-therapy/on MZR	Systolic blood pressure (mmHG) pre-therapy/on MZR	Diastolic blood pressure (mmHg) pre-therapy/on MZR
1	3/1	52.8/45.6	0.21/0.04	128/110	84/58
2	3/1	77.0/83.3	0.26/0.03	108/126	70/58
3	2/1	42.0/44.8	0.10/0.32	126/122	78/80
4	2/0	69.9/71.5	3.48/0.22	104/122	60/78
5*	2/2	-	-	-	-
6	2/1	49.7/51.6	0.69/0.59	134/122	76/78
7*	1/2	-	-	-	-
8	3/2	45.4/44.1	1.83/0.20	110/110	76/78
9	3/0	41.0/39.7	0.01/0.19	126/108	78/64
Mean	2.3/1.1	54.0/54.4	0.94/0.23	119.4/117.1	74.6/70.6

* Because patients 5 and 7 had intractable relapses and progressed to SDNS while tapering CsA, they were considered to have MZR failure and discontinued MZR therapy

Discussion

The present study is noteworthy because it is the first study that examines the efficacy of MZR in children with long-standing SDNS who had continued to experience relapses despite therapy with sufficient CsA. MZR and MMF act as immunosuppressants by inhibiting IMP dehydrogenase (IMPDH) by different mechanisms. MZR competitively inhibits IMPDH isoenzymes with Ki for type I isoenzyme 8.2 nM and for type II isoenzyme 3.9 nM, whereas, MMF inhibits those isoenzymes in an uncompetitive manner. Both drugs are mostly (more than 85–90%) eliminated into urine [12, 13]. MZR was originally isolated as an antibiotic agent with an activity against *Candida albicans*, and subsequently it was found to have strong immunosuppressive activity in various animal experimental models [14]. MZR is phosphorylated inside the cell to the corresponding 5'-monophosphates that inhibit the enzyme by binding at the substrate-binding domain of IMPDH. MMF (a prodrug of mycophenolic acid) does not require metabolic activation and binds at the cofactor (nicotinamide adenine dinucleotide: NAD) region of the catalytic site, mimicking the interaction of nicotinamide mononucleotide moiety of NAD with the protein. After phosphorylation, mizoribine 5'-monophosphate (MZ-5-P) inhibits GMP synthesis by inhibiting IMPDH as well as GMP synthetase [9]; both enzymes are required for synthesis of GMP from IMP in the de novo pathway.

Thus, MZ-5-P almost completely inhibits guanine nucleotide synthesis, which may explain one of the important immunosuppressive roles of MZR, inhibitory effects for T cell and B cell proliferation [15]. A series of in vitro studies showed that the inhibition of T cell proliferation by MZR was associated with a decrease in intracellular GTP and is reversible with GTP repletion using a highly purified T cell preparation [16]. By cell cycle analysis, MZR prevented cells from exiting G1 phase and entering S phase by blocking T cell proliferation via a guanine nucleotide-dependent mechanism [15]. In addition to its use after renal transplantation [17], recent studies from Japan have demonstrated the efficacy and safety of MZR in the treatment of childhood nephrotic syndrome [18, 19, 20]; however, more recent reports suggest that the use of MMF is likely to be more beneficial for a similar patient population [21, 22, 23]. This might be attributed to the dosage of MZR used for treatment, as was proposed by Honda [20].

As immunosuppressive therapy after renal transplantation, MZR had been commonly used in combination with CsA and methylprednisolone since the mid-1980s in Japan [24].

In this setting, the initial dose of MZR was 2–3 mg/kg per day, divided into 2 doses. A similar dose (150 mg per day) was employed in a controlled trial in the treatment of adult patients with steroid-resistant nephrotic syndrome in Japan [25]. Based on the results, MZR was accordingly approved for use in pediatric patients with SRNS at a dose of 3–4 mg/kg/day (maximally 150 mg/day) divided into 2 doses. In a multicenter trial of MZR in children with FR-SDNS from Japan, in which MZR at a dose of 4 mg/kg per day was given orally, the relapse rate of NS was 1.85 episodes per 48 weeks [19], whereas, in another trial by Honda et al. using MZR at 5 mg/kg per day, the rate was 0.4 episodes per 24 weeks [20]. A breakthrough study was conducted by Tanaka H et al., who treated pediatric-onset patients with disease flare of lupus nephritis with orally administered MZR at a dose of 5–10 mg/kg per day (up to 500 mg) in 1 or 2 divided daily doses, twice a week (a new treatment regimen, namely "MZR oral pulse therapy"), which was beneficial without any serious adverse effects [10]. In line with previous reports, in which peak blood levels of MZR (2 h after drug administration) were less than 2.0 mcg/ml when the patients received 2.5–4.0 mg/kg per day in 2 or 3 divided daily doses [18, 26], the serum peak levels of MZR of patients treated by Tanaka et al. ranged from 2.47 to 4.80 mcg/ml (n =6) [10]. These might be almost equivalent to the doses at which MZR inhibits human mixed-lymphocyte reaction [23] as well as at which MZR affects the conformation of its binding proteins, 14–3-3 proteins, and enhances the interaction of glucocorticoid receptor and those proteins in vitro [27]. As a pilot study, we performed MZR oral pulse therapy for the treatment of FR-SDNS with CsAN

in 2 pediatric patients (9-year-old boys). However, an attempt to reduce CsA was unsuccessful with MZR 10 mg/kg per day in two divided daily doses twice a week (although serum peak blood levels of MZR were more than 3.0 mcg/ml in each case) (data not shown). These cases did not support the observation reported by Kawasaki et al. [28]. We assumed that a steady-state MZR level might be important for the treatment of those patient groups. We then started the current regimen for another nine patients with FR-SDNS, with MZR orally administered before every morning meal at doses achieving serum peak levels of MZR around 3 mcg/ml (the current study). In all patients, the trough levels of MZR were less than 0.05 mcg/ml. Six of 9 patients (66.7%) were successfully weaned off CsA with high-dose MZR treatment, and in an additional case, there was a reduction in the dose of CsA required to maintain remission. The remaining 2 patients were considered to have MZR failure. For them, we started MZR on the occasion of relapses. However, unlike CsA and steroids, MZR did not seem to have strong effects in inducing the nephrotic stage into remission, even with the high dose.

Only one apparent side effect was associated with the combination therapy of MZR with CsA and steroid (patient 4). The patient's zoster required both reduction of dosage of MZR for a short period and treatment with acyclovir. No other significant complications were observed in the rest of the patients.

We admit that an important limitation of this study is the absence of a control group, since we relied on the patients serving as their own controls. Although the decrease in relapse rates and CsA use during MZR therapy might be attributed to a spontaneous change in the course of the illness, we believe this is extremely unlikely.

All of the subjects experienced a prolonged duration of FR-SDNS, which previously was difficult to manage. We therefore believe that the improvement observed during MZR therapy was the direct result of that treatment. In this small, single-center, uncontrolled experience, high-dose MZR therapy in patients with FR-SDNS who require CsA appears to allow reduction of the CsA dose as well as CsA exposure. Given the lack of nephrotoxicity and adverse hemodynamic and metabolic effects, MZR represents a suitable alternative to the calcineurin-inhibitors as adjuvant for many patients, especially those with progressive renal insufficiency. Controlled, prospective trials are needed to confirm these findings and better define the optimal dose and duration of high dose MZR therapy. Also, additional studies are necessary to examine whether prolonged therapy at a lower dose of MZR is effective in maintaining remission in children with steroid dependence. Such an approach, if effective, would reduce the potential toxicity of MZR and the cost of treatment.

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