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BRIEF REPORT

Single daily high-dose mizoribine therapy for children with steroid-dependent nephrotic syndrome prior to cyclosporine administration

Shuichiro Fujinaga · Daishi Hirano · Naoto Nishizaki · Tomonosuke Someya · Yoshiyuki Ohtomo · Yoshikazu Ohtsuka · Toshiaki Shimizu · Kazunari Kaneko

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Abstract Although cyclosporine (CsA) therapy is effective in the management of children with steroid-dependent nephrotic syndrome (SDNS), a recent study has revealed that the use of CsA itself was a significant predictor of NS relapse in adulthood. The efficacy of single daily high-dose mizoribine (MZR) therapy was assessed in 10 children with SDNS (mean age, 6.2 years) who had never been treated with CsA previously. MZR was started at 5 mg/kg, administered as a single daily dose after breakfast, and the dose was adjusted to achieve 2-h post-dose MZR levels (C2) of approximately 3 μg/ml. In 9 of the 10 patients, treatment with a single daily dose of MZR (mean dose, 8.4 mg/kg/day) over a period of 22 months (median) resulted in significant reduction of the mean prednisolone dose from 0.39 to 0.15 mg/kg/day and the median 12-month relapse rate from 3.0 to 0.4 episodes/12 months. Although cyclophosphamide was initiated in one patient because of treatment failure, none of the 10 patients required treatment with CsA during the observation period (median, 33 months). These data indicate that single daily high-dose MZR therapy is possibly useful in treating children with SDNS and that it may also eliminate the need for CsA in some patients.

Keywords High-dose mizoribine · Steroid-dependent nephrotic syndrome · Cyclosporine

S. Fujinaga (✉) · D. Hirano · N. Nishizaki
Division of Nephrology, Saitama Children's Medical Center,
2100 Magome, Iwatsuki-ward,
Saitama-city, Saitama 339 8551, Japan
e-mail: f_shuich@d2.dion.ne.jp

T. Someya · Y. Ohtsuka · T. Shimizu
Department of Pediatrics,
Juntendo University School of Medicine,
Tokyo, Japan

Y. Ohtomo
Department of Pediatrics, Juntendo Nerima Hospital,
Tokyo, Japan

K. Kaneko
Department of Pediatrics, Kansai Medical University,
Osaka, Japan

Introduction

Idiopathic nephrotic syndrome (INS) of childhood is characterized by steroid responsiveness in >90% of cases. However, up to 50% of patients with INS show frequent relapses (FR) and/or steroid-dependent nephrotic syndrome (SDNS), and often require treatment with immunosuppressive agents [1]. Over the last 20 years, the use of cyclosporine (CsA) has been extended to the treatment of children with SDNS. It has thus been believed that these patients are consequently protected from steroid toxicity and that they show a significant improvement in their quality of life and psychosocial adaptation. However, compared with the results for INS relapse from surveys in the 1980s conducted in the pre-CsA era (5.5%, 13.5%, 19.2%), recent studies have indicated a higher INS relapse rate in adulthood (33%, 42.2%) despite the introduction of CsA therapy for treating children with SDNS [2–6]. Kyrieleis et al. have reported the adverse effects of immunosuppressive treatment, such as osteoporosis, hypertension, and cataracts in adult patients with childhood-onset INS who displayed active disease following long-term use of CsA [7].

We have previously reported in this journal the efficacy of high-dose mizoribine (MZR) therapy, which is a novel immunosuppressive agent developed in Japan, as an

alternative to CsA for treating adolescent patients with persistent SDNS following long-term use of CsA [8]. The aim of the present study was to investigate whether single daily high-dose MZR therapy is successful in eliminating the need for CsA therapy in treating children with SDNS.

Patients and methods

Patients

Ten patients (9 boys, 1 girl) who had SDNS before the administration of CsA and who were undergoing treatment at Saitama Children's Medical Center between October 2006 and June 2009 were enrolled in the present study. Cyclophosphamide (CPM) was discontinued for a period of at least 6 months before patients were considered eligible for enrollment in the study. Patients with steroid-resistant nephrotic syndrome (SRNS) were excluded.

In the present study, the definitions and criteria adopted for NS, remission, relapse, frequent relapse, steroid dependency, and steroid resistance were those elaborated in the International Study of Kidney Disease in Children. All patients and their parents provided written informed consent and the study was approved by the institutional review board of Saitama Children's Medical Center.

Therapeutic protocol

Mizoribine (bredinin, oral formulation; Asahi Kasei, Tokyo, Japan) was administered when patients had achieved complete remission by steroid therapy. As per the therapeutic protocol followed, MZR therapy was started at a single daily dose of 5 mg/kg administered after breakfast. White blood cell (WBC) count was measured 2–4 weeks after the initiation of the therapy. If the WBC was greater than 4,000 cells/μl, the dosage of MZR was increased by 25–50% (maximum: 300 mg/day) and adjusted to achieve 2-h post-dose MZR levels (C2) of approximately 3 μg/ml (range: 2–5 μg/ml) in accordance with the findings reported in previous data [8]. MZR was tapered off after a 12-month period of steroid-free remission, except for one patient who initially discontinued MZR. The samples were evaluated by the Asahi Kasei Pharma Co. using high-performance liquid chromatography.

Relapses were treated with 2 mg/kg/day of prednisolone (PSL) until proteinuria disappeared, observed over 3 consecutive days. Thereafter, PSL was administered on alternate days, and the dose was tapered off within 6 months by reducing by 5–10 mg every 2–4 weeks. MZR therapy continued to be administered during the NS relapses. Treatment failure was defined as a condition requiring high-dose PSL (>0.5 mg/kg) administered on alternate days to maintain remission despite starting MZR therapy.

To assess treatment outcomes and detect potential drug toxicity, clinical and laboratory assessments were performed 2 weeks after the initiation of MZR therapy, and then every 1–3 months at the least. The laboratory assessments performed included MZR C2 levels, complete blood counts, and serum levels of urea, creatinine, electrolytes, albumin, cholesterol, transaminase, bilirubin, amylase, and uric acid.

Statistical analysis

Data were reported as means ± SD. Numerical data were analyzed using paired *t* tests (two-tailed), Mann–Whitney *U* test, or Student's *t* 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

Patient characteristics are shown in Table 1. The patients were diagnosed with NS at an average age of 3.2±1.3 years (range, 1.9–6.1). All patients had been diagnosed with SDNS. Among them, 4 patients with a high PSL threshold (i.e. at which the relapse had occurred) had been treated with CPM before the use of MZR; however, SDNS was not resolved. In spite of a high PSL threshold, one patient (no. 10) had been initially treated with MZR before the use of CPM because of family preference and subsequently showed treatment failure. Over a period of 12 months preceding initiation of MZR therapy, the mean dose of PSL administered was 0.4±0.16 mg/kg/day and the median number of relapses noted was 3.0 episodes/12 months. The age of the patients at the start of MZR therapy ranged from 3.4 to 11.3 years (mean, 6.2±2.6 years). No patients had received either angiotensin-converting enzyme inhibitors or angiotensin 2 receptor blockers.

In 9 of the 10 patients (90%), treatment with single daily high-dose MZR therapy (mean dose, 8.4±6 mg/kg; mean C2 level, 3.1 μg/ml) over a period of 22 months (median; range, 10–30) resulted in a reduction in the mean minimum PSL dose from 0.78±0.32 to 0.31±0.22 mg/kg on alternate days ($p<0.01$), the median relapse rate from 3.0 (1–3) to 0.4 (0–1.2) episodes/12 months ($p<0.01$), and the mean obesity index from 8.0±12.5% to 0.2±9.0% ($p<0.05$; Fig. 1). The mean obesity index was defined by the formula: $(\text{measured weight} - \text{ideal body weight})/\text{ideal body weight} \times 100\%$ [9]. On the other hand, no statistical difference was found in the mean standard deviation (SD) score for height of the patients at final observation in comparison to that at the start of treatment

Table 1 Baseline characteristics of the patients

Patient number	Gender	Age at onset of NS (years)	Immunosuppressant before MZR	Number of relapses before MZR therapy	At the time of initiation of MZR therapy				Mean MZR C2 levels (μg/ml)
					Age (years)	Blood pressure (mmHg)	Weight (kg)	Obesity index (%)	
1	Male	6.1	CPM, PSL	8	11.3	114/74	51.6	19	2.5
2	Male	2.2	CPM, PSL	7	5.6	96/57	19.3	−0.5	4.1
3	Male	2.6	PSL	7	9.1	124/64	35.7	27	2.9
4	Male	4.3	PSL	4	5.9	103/64	21.5	9	3.9
5	Male	2.9	CPM, PSL	7	5.7	99/59	20.4	−1	3.8
6	Male	3.9	PSL	3	5.3	89/49	19.8	8.2	2.1
7	Male	3.4	PSL	7	8.3	93/49	21.6	−13	2
8	Male	2.3	CPM, PSL	4	4.1	115/73	16.1	3	3.7
9	Female	1.9	PSL	5	3.4	112/56	18.5	20	4
10[a]	Male	2.3	PSL	3	3.4	116/70	15.1	9	4.5

NS, nephrotic syndrome; CPM, cyclophosphamide; MZR, mizoribine; PSL, prednisolone

[a] This patient was considered to show treatment failure

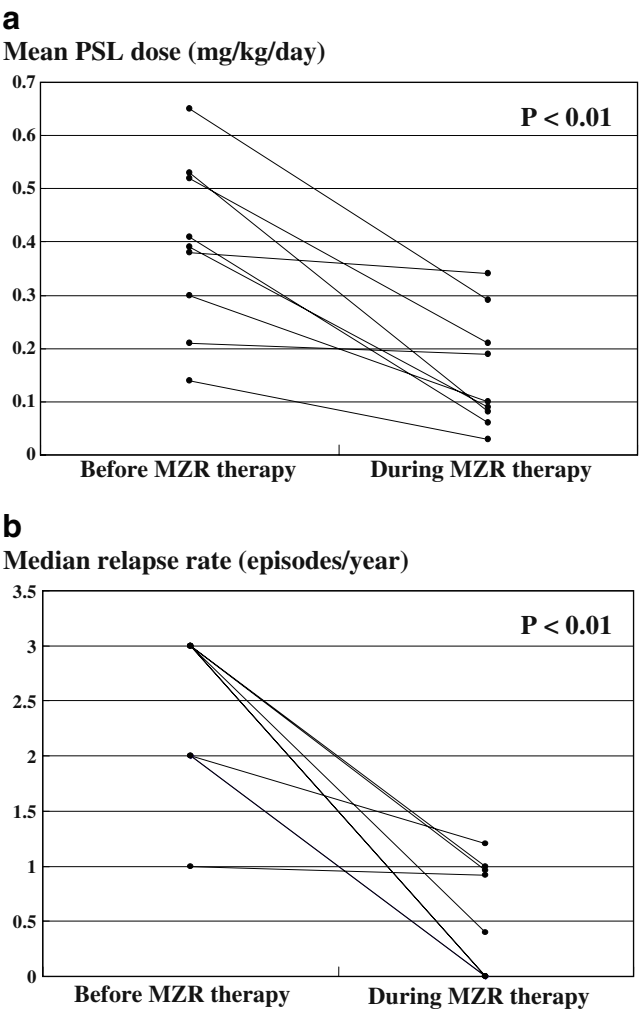

Fig. 1 **a** Comparison of mean prednisolone dose over a 12-month period before and during single daily high-dose mizoribine (MZR) therapy. **b** Comparison of median relapse rates over a 12-month period before and during single daily high-dose MZR therapy

(0.33 vs 0.27; p=0.73). Among the patients, 7 were successfully weaned off PSL while on MZR therapy. One patient was considered to show treatment failure despite the MZR C2 level being >3 μg/ml; therefore, CPM therapy was instituted for this patient. Of 5 patients who discontinued MZR after long-term administration (median, 23 months; range, 10–30), 2 showed INS relapse, leading to reintroduction of MZR in 1 (20%), and low-dose steroid therapy in the other (20%). Three patients did not have INS relapse after discontinuation of MZR. None of the 10 patients required treatment with CsA during the follow-up period (median, 33 months; range, 15–47) following treatment with MZR.

No significant side effects, such as leucopenia (<4,000/mm^3), hyperuricemia, or liver dysfunction were noted during the administration of MZR therapy.

Discussion

Previous data from the 1980s, before the use of CsA, suggested that the long-term prognosis of steroid-sensitive INS was excellent, and by the end of puberty >90% of patients show long-term remission with no further relapses [2]. However, two recent studies in the post-CsA era have revealed a higher INS relapse rate in adulthood (≥18 years) [5, 6]. First, Fakhouri et al. reported that 42% of patients with childhood minimal change NS (43 out of 102) suffered from at least one INS relapse in adulthood [5]. Second, Rüth et al. reported that 33% of NS patients who had minimal change during childhood (14 of 42) showed INS relapse in adulthood. In their study, linear regression model analysis revealed that only CsA therapy was a significant predictor of ongoing relapses [6]. Although it is possible that a higher INS relapse rate in adulthood of the two studies was simply due to cohorts consisting of more severely affected patients compared with the previous data before the use of CsA, the prevalence of patients with SDNS described in the studies of the pre-CsA era was similar to those published recently, because there was no difference in the rate of patients treated with alkylating agents (post-CsA group 50% [5], 57% [6] vs pre-CsA group 43% [4], >50% [2], 72% [3]), which have been used as first-line alternative drugs for childhood SDNS. Furthermore, Kemper et al. reported that severe SDNS recurred again in 14 of the 46 patients, despite long-term CsA therapy (median age, 5.1 years). They also found that half of the patients relapsed beyond the age of 18 years [10]. Based on the results of these recent studies, we inferred that the introduction of CsA might not be associated with improved long-term prognosis of steroid-sensitive INS; a worse relapsing disease course was encountered in some patients.

In clinical practice, once CsA therapy is initiated for treating children with SDNS, its long-term use is inevitable to maintain remission in many cases because most patients show relapse when CsA is withdrawn or when CsA dosage is tapered. However, our previously reported data showed that children with SDNS undergoing treatment with CsA for longer than 36 months are at risk of developing drug-induced nephrotoxicity; this is especially true for patients younger than 5 years [11]. Furthermore, reduced CsA efficacy resulting from drug tolerance due to long-term CsA use was also reported in some of the children with intractable INS [12]. Therefore, early initiation of CsA therapy in young children with SDNS should be avoided as far as possible.

Mizoribine is a selective inhibitor of inosine monophosphate dehydrogenase in the de novo pathway, and therefore inhibits T-cell and B-cell proliferation. Although the MZR immunosuppressive mechanism is very similar to that of mycophenolate mofetil (reported to be effective in the treatment of various glomerular diseases including SDNS), MZR is still not widely used except in Japan because of its low efficacy. It has recently been reported that 14-3-3 proteins (MZR-binding proteins) interact with glucocorticoid receptors and provide a steroid-sparing effect. This effect via the 14-3-3 proteins is exerted when the blood MZR level reaches 2.6 μg/ml; the 14-3-3 protein peak concentration during standard MZR therapy (3 mg/kg daily in three divided doses) has been reported to be <1 μg/ml [13]. Our center previously administered high-dose MZR therapy maintaining C2 levels approximately 3 μg/ml only to patients with unresolved SDNS following CsA therapy. However, some patients developed a worse relapsing course after long-term use of CsA and subsequently continued to experience relapses, despite treatment with high-dose MZR [8]. Similarly, Hulton et al. reported that patients who had discontinued CsA subsequently tended to have a greater frequency of relapses and to require a high maintenance dose of steroids [14]. We therefore conducted the present study on single daily high-dose MZR daily therapy with C2 monitoring in children with SDNS before the administration of CsA.

Recently, a few uncontrolled clinical trials have reported a high-dose (6–10 mg/kg/day) intermittent pulse administration (2 days a week) of MZR emerging as an effective therapeutic option for management of patients with FR/SDNS [15–17]. Kawasaki et al. reported that 8 out of 11 patients (73%) with FRNS responded favorably to intermittent MZR pulse therapy (6 mg/kg/day) without displaying any adverse effects [15]. However, in 9 patients with more severe SDNS or CsA-dependent SRNS, the response rate to intermittent MZR therapy (10 mg/kg/day) decreased to 44% (4 out of 9) [16]. Similarly, Fujieda et al. demonstrated the efficacy of intermittent MZR pulse therapy, maintaining a peak blood level of about 3 μg/ml in 16 patients with FR/SDNS. Although their data showed that the

relapse rate was significantly reduced, the reduction in daily dose of PSL was not statistically significant [17]. In our study, 9 of the 10 patients (90%) were responders; treatment with single daily high-dose MZR therapy resulted in a significant reduction in the mean minimum PSL dose from 0.78 to 0.31 mg/kg on alternate days ($p<0.01$), the median relapse rate from 3.0 to 0.4 episodes/12 months ($p<0.01$), and the mean obesity index from 8.0 to 0.2% ($p<0.05$). Furthermore, no adverse effects such as bacterial infection were noted during the administration of single daily high-dose MZR and none of the patients required treatment with CsA during the follow-up period (median, 33 months).

In conclusion, these data indicate that single daily high-dose MZR therapy appears to be an effective alternative treatment for treating children with SDNS and that it may eliminate the need for CsA therapy in some patients. The elimination of CsA use could, in turn, improve the long-term prognosis of childhood-onset, steroid-sensitive INS. However, one limitation of the present study was the absence of a placebo control group, as in other recent studies. Therefore, larger randomized controlled studies need to be conducted to accurately determine the role of single daily high-dose MZR therapy in the management of patients with SDNS.

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