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Original Article

Mizoribine requires individual dosing due to variation of bioavailability

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Abstract ***Background***: Mizoribine (MZR) is an immunosuppressant used for the treatment of glomerular diseases, but there are few reports on the pharmacokinetics of MZR in children.

Methods: First, we performed a pharmacokinetic study on nine childhood-onset glomerular disease patients. The MZR dosages ranged from 1.8 to 14.5 mg/kg/dose. Pharmacokinetic parameters were analyzed using 38 MZR concentration-time curves. Second, nine patients who were newly treated with MZR were enrolled to validate the findings obtained from prior investigation.

Results: In the prior study, peak serum MZR concentration (C_{max}) was dose-dependent in each patient. Although proportionality between dosage and C_{max} was observed in each patient, the regression coefficient was in a wide range from 0.075 to 1.04 and was specific to each patient. This variability was likely caused by individual variation of bioavailability. When the optimal time-point to monitor C_{max} was investigated, the time-to-reach peak serum MZR concentration (T_{max}) was similar among all the patients, which was from 2.5 to 3.5 h after administration of MZR. T_{max} was most frequently observed at 3 h and the serum MZR concentration ratio relative to C_{max} at 3 h was also highest (0.93 ± 0.07). In the following study, it was validated that monitoring C_3 is reproducible and reliable after adjusting the dosage of MZR to obtain target serum concentration.

Conclusion: Individual dosing is required to optimize C_{max} in childhood-onset glomerular disease patients. The safe dosage of MZR for each patient could be predicted by evaluating the serum MZR concentration 3 h after administration.

Key words bioavailability, glomerular disease, linearity, mizoribine, pharmacokinetics.

Mizoribine (MZR), 4-carbamoyl-1-β-D-ribofuranosyl-imidazolium-5-olate, is an orally administered immunosuppressant. It was isolated from a culture medium of the mold *Eupenicilium brefeldianum* and developed in Japan.[1] The clinical use of MZR as an immunosuppressant for renal transplantation has been increasing because of its lower toxicity and better tolerance for it by patients than other immunosuppressants. Recently, the efficacy and safety of MZR for treating nephrotic syndrome,[2–8] IgA nephropathy,[9–12] and systemic lupus erythematosus[13,14] have been reported. However, there are some studies showing conflicting results, that is, MZR does not show a significant efficacy.[3,15] Because of its relatively low efficacy, particularly in children compared with adults, the clinical use of MZR is not as widespread as that of cyclophosphamide, mycophenolate mofetil, or cyclosporine. We consider that the major reason for its lower efficacy than other immunosuppressants is insufficient blood MZR concentration. Although the precise therapeutic concentration range of MZR is not clear yet, Sonda *et al*. reported that the appropriate blood MZR concentration for the effective inhibition of the human mixed lymphocyte reaction is in the range of 3.0–6.0 μg/mL.[16] According to the package insert, the C_{max} of MZR is 1.35 μg/mL when an adult MZR dose of 50 mg is administered to a male adult. If 150 mg of MZR, equivalent to the adult daily dosage, is administered to a 6-year-old child whose bodyweight is 20 kg, on the basis of the Augsberger equation and von Harnack table, the daily dosage should be 3.3 and 3.8 mg/kg, respectively. However, when dosages of 1.5–2.5 mg/kg were administered to patients in our preliminary study, the C_{max} of MZR was 0.67 μg/mL.[17] This C_{max} in children is much lower than that in adults who received an equivalent dosage of MZR and it seemed to be insufficient for MZR to show its efficacy. Because the pharmacokinetic parameters of MZR in children change during their growth, the bioavailability of MZR will also vary in each child.

Recent phase 1 single- and multiple-dosage studies of healthy male volunteers have shown that MZR does not increase the risk of any adverse event except for a transient elevation of serum uric acid concentration when the dosage is up to 12 mg/kg/day, and reports of these studies showed dose proportionality, time-to-reach peak serum concentration (T_{max}), and half-life ($T_{1/2}$).[18,19] However, these studies are population pharmacokinetics, not individual analyses. To the best of our knowledge, there are no pharmacokinetic studies of MZR focusing on the dose

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proportionality of serum MZR concentration in each subject and there are few reports on the pharmacokinetics of MZR in children.[6,20] The establishment of dose proportionality in pediatric patients should improve the efficacy and safety of MZR in clinical practice.

The aim of this study was to elucidate the appropriate MZR dosage and to avoid unexpectedly high or low serum MZR concentrations in children. We investigated individual dose proportionality, i.e. linearity for MZR dosage and serum MZR concentration in each patient, and speculated the optimal blood collection time to estimate C_{max} of MZR in childhood-onset glomerular disease patients with normal renal function. We also investigated the cause of interindividual variability of the pharmacokinetic parameters of MZR. Our findings indicate that we should optimize the dosage of MZR on the basis of the monitored serum MZR concentration in an individual pediatric patient.

Methods

Subjects

In order to perform two different investigations, two groups were enrolled in this study.

First, nine Japanese patients (patients A–I, three boys and six girls) who had been admitted to Showa University Hospital between 2001 and 2011 were enrolled. At least two pharmacokinetic curves were obtained from all patients to analyze pharmacokinetic parameters of MZR. Of these nine patients, two had minimal-change nephrotic syndrome, three had lupus nephritis, two had immunoglobulin (Ig)A nephropathy, and two had Henoch–Schönlein purpura nephritis. These patients underwent physical examination, and their medical histories were obtained. They showed normal renal function, and none of these patients had a history of gastrointestinal tract operation. The age at disease onset was 7.2 ± 3.2 years (median, 8; range, 2–11). Treatment with MZR was started at the age of 7.7 ± 3.6 years (median, 8; range, 2–13). A pharmacokinetic study of MZR was performed at the age of 9.8 ± 4.3 years (median, 9.5; range, 2–19). The clinical characteristics of the nine patients are shown in Table 1.

Second, nine Japanese patients (patients 1–9, six boys and three girls) were enrolled to confirm our prospective prediction of C_{max} using the MZR concentration indicated hours after MZR administration. They were newly treated with MZR between 2009 and 2011. We examined the serum MZR concentration, and subsequently the dose of MZR was adjusted to achieve a target serum MZR concentration (around 3 μg/mL). Although no pharmacokinetic curve was obtained from them, spot serum concentration of MZR was measured mainly when they attended out patient department for regular check-up. Of these nine patients, seven had frequently relapsing steroid-dependent nephrotic syndrome (FRNS), one had biopsy-proven lupus nephritis, and one had biopsy-proven Henoch–Schönlein purpura nephritis. The age at disease onset was 5.1 ± 3.3 years (median, 5; range, 1–12). Treatment with MZR was started at the age of 6.6 ± 2.9 years (median, 6; range, 2–12). They showed normal renal function, and none of these patients had a history of gastrointestinal tract operation.

Table 1 Clinical characteristics and pharmacokinetic parameters of MZR in patients

Patient	Sex	Diagnosis	Age at onset	Age MZR started	Combined immunosuppressant	BUN (mg/dL)	Cr (mg/dL)	No. of analyzed pharmacokinetic curve	Dosage of MZR (mg/kg/dose)	C_{trough} (μg/mL)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (μg · h/mL)	Vd_{ss}/F (L/kg)	Vd_{ss} (L/kg)	fu (%)
A	Female	LN/WHO Vb	11	13	PSL	5.0–17.6	0.4–0.6	6	1.8–3.7	0.10–0.34	1.5–4.0	0.67–2.19	1.14–3.69	3.25–24.36	0.89–2.73	0.49–0.72	33.7–65.6
B	Male	IgAN	9	9	PSL	13.3–21.6	0.4–0.6	4	1.8–4.0	ND–0.13	2.0–3.0	0.65–2.40	0.90–1.77	2.74–11.91	1.68–2.42	1.00–1.02	41.4–50.5
C	Male	HSPN/ISKDC III	7	8	PSL	11.9–17.6	0.3–0.5	3	1.9–3.0	0.11–0.33	2.0–3.0	0.55–1.82	2.18–2.40	3.51–12.05	1.25–1.79	0.85–1.06	35.4–85.1
D	Male	MCNS	2	2	CsA, PSL	7.4–11.1	0.3–0.31	4	1.9–5.6	ND	4.0	0.61–2.67	1.06–1.80	2.88–16.91	1.32–6.81	0.76	50.1
E	Female	LN/WHO IV G (A)	11	12	PSL	12.1–14.1	0.4–0.5	5	3.5–7.1	ND–0.13	2.0–4.3	1.45–3.93	1.39–2.11	9.01–20.65	1.39–1.93	0.60–1.06	44.1–59.0
F	Female	IgAN	8	8	PSL	5.7–8.6	0.2–0.4	6	1.8–2.4	ND–0.24	2.2–4.0	0.71–1.17	0.85–2.74	4.88–8.84	1.36–2.02	0.62–1.54	37.6–84.3
G	Female	LN/WHO IV G (A)	8	8	PSL	9.5–15.4	0.3–0.5	6	2.7–13.4	ND–0.06	3.0–3.3	0.65–3.00	2.24–4.60	3.94–20.01	2.45–3.64	0.70–1.10	22.0–38.1
H	Female	MCNS	3	3	PSL	8.5–9.7	0.2	2	5.1–9.5	ND	2.0	0.74–1.07	0.89–0.94	3.62–5.18	5.14–5.98	0.89–1.03	17.2–17.4
I	Female	HSPN/ISKDC III	6	6	PSL	15.7–18.0	0.37–0.43	2	11.7–14.5	ND	1.0–2.0	1.87–2.85	2.26–2.99	11.88–15.77	3.94–4.80	0.71–0.76	15.9–17.9
Average			7.2 ± 3.2 ($n = 9$)	7.7 ± 3.6 ($n = 9$)				4.2 ± 1.6 ($n = 9$)	4.4 ± 3.3 ($n = 38$)	0.078 ± 0.10 ($n = 38$)	2.8 ± 0.9 ($n = 38$)	1.67 ± 1.04 ($n = 38$)	2.2 ± 1.5 ($n = 38$)	9.67 ± 5.85 ($n = 38$)	2.41 ± 1.41 ($n = 38$)	0.86 ± 0.23 ($n = 32$)	42.4 ± 20.8 ($n = 24$)

The range of value is shown for each patient. Average was shown as mean ± SD. AUC, area under the serum MZR concentration-time curve; BUN, blood urea nitrogen; C_{max}, peak serum concentration; Cr, serum creatinine; CsA, cyclosporine; C_{trough}, trough concentration; fu, rate of urinary excretion; HSPN, Henoch-Schönlein purpura nephritis; IgAN, immunoglobulin A nephropathy; ISKDC, International Study of Kidney Disease in Children; LN, lupus nephritis; MCNS, minimal-change nephrotic syndrome; MZR, mizoribine; ND, not detected; PSL, prednisolone; $T_{1/2}$, half-life; T_{max}, time-to-reach peak serum concentration; Vd_{ss}, absolute volume of distribution; Vd_{ss}/F, apparent volume of distribution in steady state following oral administration; WHO, World Health Organization.

Informed consent was obtained from either one or both of the parents of each child before enrollment in the study. This study was approved by the ethics committee of Showa University School of Medicine.

MZR therapy

MZR (Bredinin, Asahi Kasei Pharma, Tokyo) was administered orally to all patients in a single dose, or two or three equally divided daily doses. The dose of MZR was adjusted to obtain the effective but non-toxic C_{max}.

Pharmacokinetic analyses

To analyze pharmacokinetic parameters of MZR, blood samples were collected almost hourly 7–10 times, including predosing, from each patient. Urine collection was started after administration and pooled until the next administration. Serum and urine MZR concentrations were determined by Asahi Kasei Pharma by high-performance liquid chromatography (HPLC) or an enzymatic method for measuring MZR 5′-monophosphate concentration in serum.[21,22] C_{max} and T_{max} were determined from the measured values. The slope of the terminal elimination phase (kel) was obtained by least-squares linear regression analysis. $T_{1/2}$ was calculated as ln 2/slope. The area under the serum MZR concentration-time curve ($AUC_{0\text{-}t}$) was calculated using the trapezoidal method. $AUC_{0\text{-}\infty}$ was estimated as $AUC_{0\text{-}t}$ plus C_t/kel (C_t, the final concentration point). Oral clearance was calculated as dose/$AUC_{0\text{-}\infty}$.The apparent volume of distribution of MZR in a steady state following oral administration (Vd_{ss}/F) was predicted using mean residence time (MRT) and oral clearance (CL/*F*) according to Vd_{ss}/F = MRT·CL/*F*. *F* stands for bioavailability, which is the fraction of extravascularly administered dosage that reaches the systemic circulation. The rate of urinary excretion (fu) of MZR was calculated as the ratio of the amount of MZR eliminated into urine to the dosage of MZR for 24 h. To calculate absolute Vd_{ss}, Vd_{ss}/F was multiplied by the fu of MZR. To determine the optimal time for monitoring C_{max}, analyzed serum MZR concentration was adjusted to C_{max} in each concentration-time curve. The lower limits of quantification (LLOQ) by HPLC and the enzymatic method for both serum and urine were 0.05 and 0.08 μg/mL, respectively. All concentrations below LLOQ were treated as 0.001 in pharmacokinetic analysis.

Statistical analyses

Data are presented as mean ± SD, unless stated otherwise. The non-parametric Spearman's correlation coefficient was calculated to test for significant associations between variables. $P < 0.05$ was considered significant.

Results

MZR dosage was 4.4 ± 3.3 mg/kg/dose (median, 2.85 mg/kg/dose; range, 1.8–14.5 mg/kg/dose). The total numbers of obtained data points and analyzed MZR concentration-time curves were 305 and 38, respectively. The averages of pharmacokinetic parameters in all the patients are shown in Table 1.

The trough concentration (C_{trough}) was ≤0.34 μg/mL in all patients, and a marked accumulation was not observed. Hyperuricemia and herpes zoster were observed in patient A and alopecia was observed in patient F after the administration of MZR as transient adverse effects, no significant long-term adverse effects, such as bone marrow suppression or liver dysfunction, were observed.

The pharmacokinetic profiles of each patient are shown in Figure 1. At higher MZR dosages, C_{max} tended to increase in each patient. However, the actual C_{max} varied at a similar dosage of MZR in intra-patient and inter-patient evaluations. The series of blood samples at the same dosage were collected on the same day from each patient, when the patients were administered MZR twice a day. MZR is administered before breakfast and after supper in our department, and a higher C_{max} was observed when MZR was administered before breakfast in patient A, whereas a higher C_{max} was observed when MZR was administered after supper in patients E, F, and G.

The correlation was significant between MZR dosage and C_{max} in all the patients ($y = 0.17\ x + 0.90$, $r = 0.67$, $P < 0.0001$) as expected. With regard to the correlation between MZR dosage and C_{max} in each patient, the regression coefficient of each regression line was in the range from 0.075 to 1.04 and it was conspicuously unique in each patient (Fig. 2). Figure 3a shows the distributions of age and fu of each patient and the correlation between them was statistically significant ($y = 1.94,\ x + 22.7$, $r = 0.43$, $P < 0.05$). The fu of patient H and I was about 17%, that of patient G was about 25%; those of patients A, B, and E were about 50–55%, and as for patients C and F, a large variation in fu was observed in each patient. These findings suggest fu is peculiar to each patient. Figure 3b shows the distributions of age and Vd_{ss} obtained from 32 data points. The correlation between age and Vd_{ss} was statistically significant ($y = -0.024,\ x + 1.12$, $r = -0.40$, $P < 0.05$).

In order to determine the optimal time to collect blood samples for C_{max} monitoring, we analyzed 38 concentration-time curves of the nine patients. The distribution of T_{max} was similar among these patients, as shown in Figure 1. The average T_{max} of each patient and all 38 curves was approximately 3 h and T_{max} was also most frequently observed at 3 h. It should be noted, however, that the ranges of T_{max} values of patients A (1.5–4.0 h) and E (2.0–4.3 h) were relatively wider than those of other patients. Although blood collection time could not be controlled strictly, at least one sample was obtained between 2.5 and 3.5 h after MZR administration for all 38 analyzed concentration-time curves. When the ratio of serum concentration of MZR to observed C_{max} was calculated for each concentration-time curve, the highest ratio (0.93 ± 0.07; range, 0.78–1.00) was observed 3 h after MZR administration (Table 2). In each patient, linear correlation was shown between dosage and concentration from 2.5 to 3.5 h after MZR administration, and the correlation between dosage and concentration from 2.5 to 3.5 h was statistically significant in all patients except patients H and I, whose data were insufficient. Taken together, these findings suggest that 3 h after MZR administration is the optimal time to monitor C_{max}.

To validate that monitoring MZR concentration at 3 h after MZR administration (C_3) is reproducible and reliable after adjusting the dosage of MZR to obtain our target serum

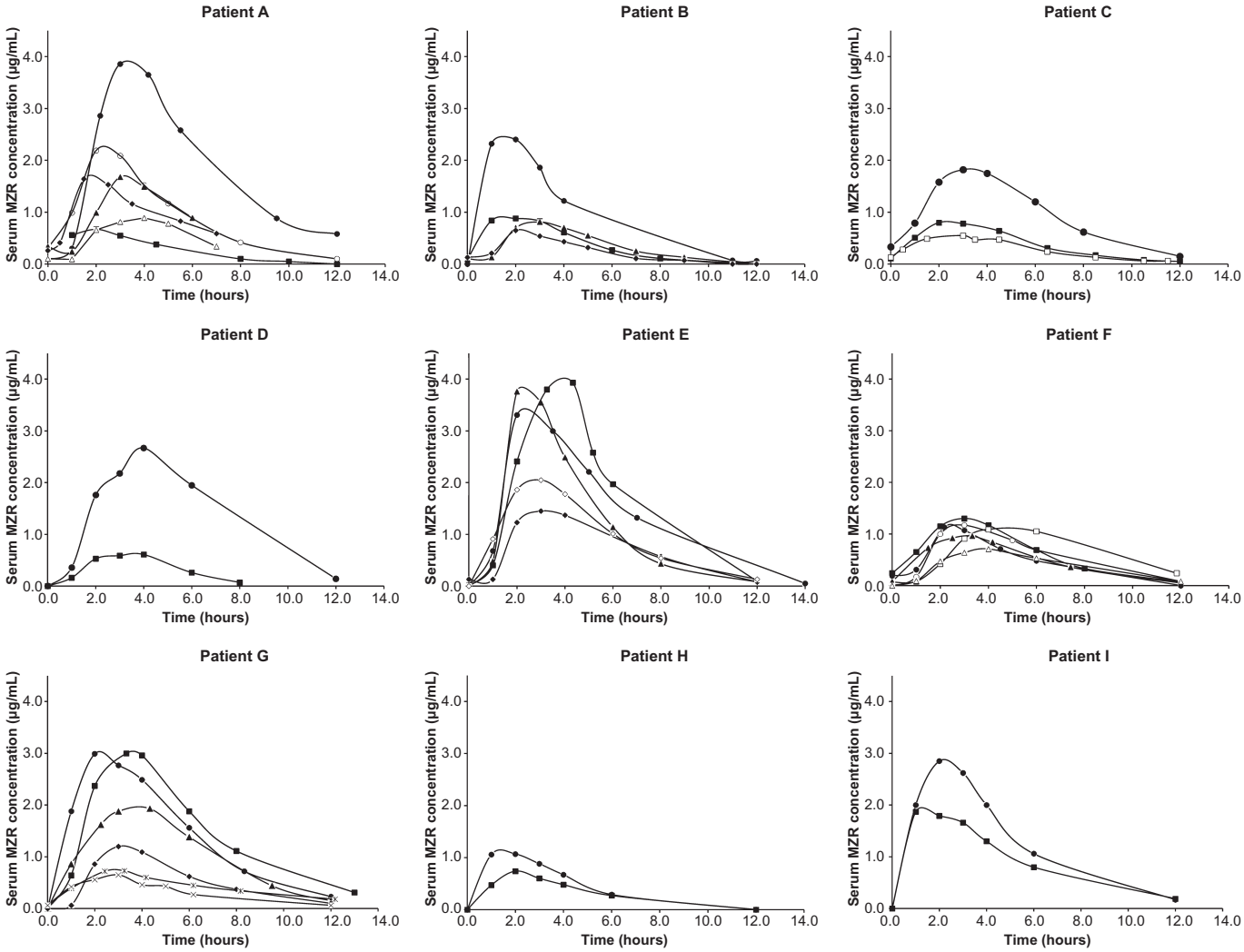

Fig. 1 Individual pharmacokinetic curves. All the patients were administered at least two different dosages of mizoribine (MZR). Patient A: ●, 3.7 mg/kg; ○, 3.7 mg/kg; ■, 2.5 mg/kg; ▲, 2.1 mg/kg; △, 2.1 mg/kg; ◆, 1.8 mg/kg. Patient B: ●, 4.0 mg/kg; ■, 2.2 mg/kg; ▲, 1.9 mg/kg; ◆, 1.8 mg/kg. Patient C: ●, 3.0 mg/kg; ■, 1.9 mg/kg; □, 1.9 mg/kg. Patient D: ●, 4.4 mg/kg; ■, 1.9 mg/kg. Patient E: ●, 7.1 mg/kg; ▲, 6.3 mg/kg; ■, 6.3 mg/kg; ◆, 3.5 mg/kg; ◇, 3.5 mg/kg. Patient F: ●, 2.4 mg/kg; ○, 2.4 mg/kg; ■, 2.2 mg/kg; □, 2.2 mg/kg; ▲, 1.8 mg/kg; △, 1.8 mg/kg. Patient G: ●, 13.4 mg/kg; ■, 8.4 mg/kg; ▲, 8.2 mg/kg; ◆, 4.5 mg/kg; ✶, 2.7 mg/kg; ×, 2.7 mg/kg. Patient H: ●, 9.5 mg/kg; ■, 5.1 mg/kg. Patient I: ●, 14.5 mg/kg; ■, 11.7 mg/kg.

Table 2 Adjusted serum MZR concentration relative to peak serum concentration of MZR (C_{max})

Time after MZR administration (h)	Adjusted serum MZR concentration relative to C_{max}
1.0	0.40 ± 0.30 (*n*=35)
2.0	0.87 ± 0.16 (*n*=31)
3.0	0.93 ± 0.07 (*n*=32)
4.0	0.81 ± 0.15 (*n*=25)
5.0	0.67 ± 0.13 (*n*=7)
6.0	0.51 ± 0.17 (*n*=23)

Mean ± SD is shown for each time after MZR administration (h). C_{max}, peak serum concentration; MZR, mizoribine.

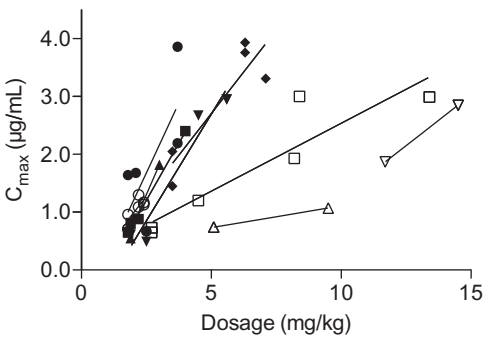

Fig. 2 Correlation between dosage and peak serum mizoribine concentration (C_{max}). The regression coefficient of each regression line ranged from 0.075 to 1.04 and it was conspicuously unique in each patient. ●, Patient A; ■, Patient B; ▲, Patient C; ▼, Patient D; ◆, Patient E; ⊖Patient F; ⊟, Patient G; △, Patient H, ▽, Patient I.

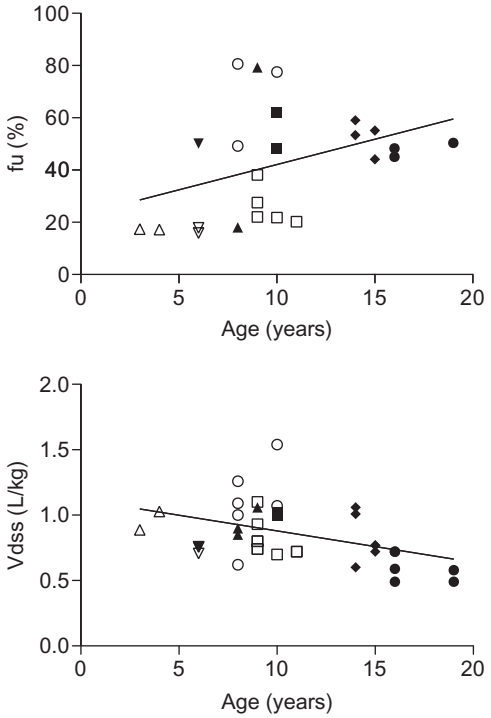

Fig. 3 (a) Correlation between age and the rate of urinary excretion of mizoribine (MZR) (fu). (b) Correlation between age and the volume of distribution of MZR in a steady state (Vd_{ss}). ●, Patient A; ■, Patient B; ▲, Patient C; ▼, Patient D; ◆, Patient E; ○ Patient F; □, Patient G; △, Patient H; ▽, Patient I.

concentration, we monitored C_3 in the newly diagnosed nine patients. The monitoring was performed mainly while the patients attended the outpatient department for regular check-up. As shown in Table 3, dose proportionality was confirmed in patients 1–5. It was also confirmed that actual C_3 values were consistent in patients who were given similar dosage of MZR at least two times (all patients except for patients 5 and 9), even though the discrepancy was observed between predicted values and actual ones (patients 3 and 8). However, the C_3 varied at a similar dosage of MZR in inter-patient evaluations. In particular, a few patients (patients 1 and 2) required more than 10 mg/kg/dose to reach 3 μg/mL of serum MZR concentration. No significant long-term adverse effects, such as bone marrow suppression or liver dysfunction, were observed.

Discussion

In this study, we showed the individual variation of pharmacokinetic parameters, dose proportionality, and optimal time to monitor C_{max} of MZR in patients with child-onset glomerular disease. Our findings suggest that the concentration at 3 h after MZR administration can be used as a substitute for C_{max} to optimize the dosage of MZR.

MZR is water-soluble and is eliminated from the kidneys after absorption in the gastrointestinal tract.[1,23] Because of its non-biotransformation and water solubility, fu should be dependent on renal function and we thus used fu as the index of bioavailability of MZR (F). Therefore, we calculated Vd_{ss} by multiplying Vd_{ss}/F by fu. The average Vd_{ss}/F was 2.41 L/kg in our present

Table 3 Mizoribine (MZR) concentration at 3 h after MZR administration (C_3) in patients

Patient No.	Sex	Diagnosis	Dosage	C_3 (μg/mL)	
			mg/day (mg/kg/dose)	Predicted value	Actual value
1	Male	FRNS	62.5 (3.3)	–	0.73
			150 (8.1)	1.79	1.73
			300 (12.7–14.0)	3.01	2.12, 2.71
2	Male	FRNS	25 (1.4)	–	0.36
			100 (5.6)	1.41	2.28
			125 (6.2)	2.52	1.34
			250 (12.5)	2.69	4.77
3	Male	FRNS	62.5 (2.2)	–	1.04
			125 (4.3)	2.06	1.82
			150 (5.0)	2.09	1.07, 1.64, 1.95
4	Male	HSPN	150 (4.5)	–	0.43
			150 (4.6)	0.44	0.84
			250 (7.8)	1.44	0.98
5	Female	FRNS	150 (7.1)	–	2.37
			200 (9.8)	3.16	1.68, 3.53
6	Female	FRNS	125 (5.2)	–	0.60
			150 (6.3)	0.72	2.84
			175 (6.4)	2.91	2.25
7	Female	LN	400 (8.2)	–	4.22
			400 (8.4)	4.32	4.31, 4.88
8	Female	FRNS	100 (4.1)	–	1.34
			150 (5.0)	1.65	2.11, 2.46, 2.80, 3.13, 1.63
9	Male	FRNS	150 (5.0)	–	2.75, 3.70

Predicted value was calculated using prior actual C_3 value and dose proportionality. Single value and/or multiple values are provided for actual C3 value. FRNS, frequently relapsing steroid-dependent nephrotic syndrome; HSPN, Henoch–Schönlein purpura nephritis; LN, lupus nephritis; MZR, mizoribine.

study and was higher than that (0.83 L/kg) in healthy adult volunteers.[19] It was considered that Vd_{ss}/F is higher in children than in adults, because the extracellular fluid compartment in children is larger than that in adults.[24] However, the average Vd_{ss} was 0.86 ± 0.23 L/kg, which is similar to that of a water-soluble drug in adults. This finding is consistent with those of water-soluble drugs.[25] Our findings suggest that Vd_{ss} and/or fu are the reason why a higher dosage is necessary for children than for adults to achieve an equal blood MZR concentration. In our study, fu was shown to be peculiar to each individual and C_{max} or C_3 varied at a similar dosage of MZR in inter-patient evaluations, which suggest that an individualized dosage plan is necessary to maintain the appropriate concentration. Recently, Naito *et al*. have reported that MZR bioavailability is affected by concentrative nucleotide transporter 1 (CNT1) gene polymorphism in kidney transplant recipients.[26] Although we did not investigate CNT1 gene polymorphism in our patients, this polymorphism may be the reason for the individual variation of pharmacokinetic parameters of MZR in children.

An MZR concentration in the range of 3.0–6.0 μg/mL is required to inhibit the human mixed-lymphocyte reaction,[16] and MZR at 0.26–2.6 μg/mL enhances the induction of glucocorticoid receptor activity with dexamethasone without any cytotoxicity.[27] The efficacy of MZR oral pulse therapy, which is useful for increasing C_{max}, has been demonstrated in many studies.[4–6,13] The studies of healthy volunteers[18,19] and our study indicate that it is difficult to maintain a high C_{trough} in individuals without renal failure. From these observations, we speculate that C_{max} is more important than AUC or C_{trough} for obtaining sufficient efficacy when MZR is administered to patients without renal failure. Although the pharmacokinetic parameters showed individual variation, the dose proportionality to C_{max} shown in our study is useful for designing a dosage to obtain optimal C_{max}.

Because multipoint blood sampling is burdensome to children, it is required to infer the one-point blood collection time that surrogates C_{max} of MZR. Although many studies adopt the concentration 2 h after administration (C_2) as C_{max} to assess the efficacy of MZR,[5,13,28–31] there is no available evidence for the validity of adopting C_2 as C_{max}. On the other hand, the concentration 3 h after administration (C_3) was closest to the observed C_{max} in our present study. Hence, monitoring C_3 can be optimal for monitoring C_{max}. A recent study showing that one-point (C_3) sampling is promising for approximate C_{max} estimation[32] supports our current finding. Finally, we recommend an individualized MZR dosing using C_3 for adjusting dosage by adopting the correlation between C_3 and C_{trough}, for which we use 0.

It is important to remember that the population in our present study did not have a homogeneous disease and that the clinical efficacy of MZR was not investigated. The optimal C_{max} of MZR might vary depending on the type of nephritis or the stage of disease. Probably due to poor bioavailability of MZR, some patients required more than 10 mg/kg of MZR to obtain optimal C_{max} without significant adverse effects as previously reported.[5,7] Low-level C_{max} subsequent to poor bioavailability could be an indicator to switch MZR to the other immunosuppressants. Hence, monitoring C_{max} is important, even if the level of C_{max} is low and non-toxic. In our present study, the prediction of pharmacokinetic profiles was not our main purpose. A limitation of non-compartmental pharmacokinetic analysis is that it lacks the ability to predict blood concentration profiles, and no assumption was made on the basis of the shape of the concentration-time curve, whereas such an assumption is made when analysis is conducted by compartmental methods. Our present study revealed that the pharmacokinetic parameters of MZR largely varied among patients and were very unique to each patient, and a different dosage of MZR is required to reach an adequate serum MZR concentration in each patient. Therefore, population-based studies and randomized controlled trials are not suitable for determining MZR dosage for each patient.

In conclusion, the individualization of a dosage plan is required for MZR at least in childhood-onset glomerular disease patients and we should optimize the dosage of MZR on the basis of the monitored serum MZR concentration for each patient. From our findings, we recommend the optimization of safe dosing for an individual pediatric patient by evaluating serum MZR concentration 3 h after oral administration.

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