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Original Article

Pharmacokinetic study of mizoribine in child-onset glomerulonephritis

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Abstract ***Background***: Mizoribine (MZR) has been successfully used without serious adverse effects in patients with various types of glomerulonephritis, but there are only a few pharmacokinetic studies of MZR. The purpose of the present paper was to report the results of a pharmacokinetic study of MZR in child-onset glomerulonephritis.

Methods: Nine patients were enrolled. MZR was administered orally at 60–300 mg/day (3.0–8.4 mg/kg bodyweight/day) divided in one or two daily doses. Blood samples were collected 7–10 times before and after drug administration. Urine samples were also collected during the blood sampling periods. Twenty-three concentration curves of MZR were analyzed in the present study. Pharmacokinetic parameters for mizoribine were estimated using concentration–time profiles. The non-parametric Spearman correlation coefficient was calculated to determine significant associations between variables. $P < 0.05$ was considered significant.

Results: The obtained pharmacokinetic values at a dose of 3.36 ± 1.91 mg/kg bodyweight were as follows: time to peak serum MZR concentration, 2.94 ± 0.82 h; peak serum MZR concentration, 1.59 ± 1.16 μg/mL; half-life, 1.96 ± 0.92 h; area under the serum MZR concentration–time curve from time zero to infinity, 9.36 ± 6.58 μg h/mL; volume of the distribution of MZR at a steady state, 2.03 ± 0.80 L/kg; and rate of urinary excretion of MZR, 49.1 ± 18.7%.

Conclusions: The parameters estimated in the present study may be useful for the MZR treatment of patients with child-onset glomerulonephritis.

Key words children, glomerulonephritis, mizoribine, pharmacokinetics.

Mizoribine (MZR) is a novel immunosuppressive agent that was developed in Japan. The drug has been successfully used without serious adverse effects in the treatment of patients undergoing renal transplantation.[1] Furthermore, recent studies have demonstrated the efficacy and safety of MZR in the treatment of patients with nephrotic syndrome,[2,3] IgA nephropathy,[4] and systemic lupus erythematosus.[5,6] But, because of its relatively low efficacy, particularly in children, the clinical use of MZR is not as widespread as that of cyclophosphamide, mycophenolate mofitil or cyclosporine. According to preliminary reports, the peak serum MZR concentration (C_{max}) of MZR is usually low with regular use. Therefore, we carried out a pharmacokinetic study of MZR in child-onset glomerulonephritis. To our knowledge there are few reports on the pharmacokinetic study of MZR in patients with child-onset glomerulonephritis.

Methods

Nine Japanese patients (five boys, four girls) were enrolled in the study. Of these nine patients, three had lupus nephritis, three had minimal-change nephrotic syndrome, two had IgA nephropathy, and one had focal segmental sclerosis. These patients were normal on physical examination in terms of renal function. No patient had a past history of gastrointestinal tract operation.

Mizoribine was administered orally to all patients in the range of 60–300 mg/day (3.0–8.4 mg/kg/day) in one or two divided daily doses. Blood samples were taken 7–10 times before and after drug administration. Urine was collected during the sampling period.

The serum and urine MZR concentrations were determined on high-performance liquid chromatography.[7] The detection limit was 0.05 μg/mL. C_{max} and time to C_{max} (T_{max}) were determined from the measured values. The slope of the terminal elimination phase (k_{el}) was obtained by least-squares linear regression analysis. The half-life ($t_{½}$) 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/k_{el} (C_t, the final concentration point). The volume of the distribution of MZR at a steady state (Vd_{ss}) was predicted using mean residence time (MRT) and clearance (Cl) according to Vd_{ss} = MRT Cl.

The rate of urinary excretion of MZR was calculated as the ratio of the amount of MZR eliminated into urine to the dose of MZR during the sampling period.

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Table 1 Patient clinical characteristics

Patient	Sex	Diagnosis		Age at onset	Height (cm)	Body-weight (kg)	BUN (mg/dL)	Cr (mg/dL)	CCr (mL/min/1.73m^2)	Age MZR started	Age pharmacokinetics of MZR studied	Dose (mg/day)	Dose per bodyweight (mg/kg)	Combined immunosuppressant	Effect of MZR therapy
		Clinical diagnosis	Renal biopsy												
1	Female	LN	WHO Vb	11	145.0	49.8	6.1	0.4	219.7	13	15	250 divided in two doses	2.5	PSL	Maintenance of clinical remission
					147.3	40.6	17.6	0.6	104.4		19	300 divided in two doses	3.7		
2	Male	NS	FSGS	6	114.2	20.6	7.9	0.3	178.4	6	6	80 divided in two doses	1.9	PSL	(–)
3	Male	AGN	IgAN	9	137.4	27.9	19.5	0.4	175.6	9	10	100 divided in two doses	1.8	PSL	Decrease in proteinuria severity
					137.4	31.0	13.3	0.4	143.9			120 divided in two doses	1.9		
					142.2	34.4	15.1	0.4	186.2			150 divided in two doses	2.2		
4	Male	NS	MCNS	9	148.2	33.4	18.0	0.5	–	11	11	100 divided in two doses	1.5	CsA, PSL	Decrease in proteinuria severity
5	Male	NS	MCNS	2	92.2	15.8	8.7	0.3	–	2	2	60 divided in two doses	1.9	CsA, PSL	Reduction of PSL
6	Female	LN	WHO IV G (A)	11	142.0	28.2	12.1	0.4	363.3	12	14	200 divided in two doses	3.5	PSL	Maintenance of clinical remission
									136.3			200 one dose	7.1		
			WHO III (C)		142.8	31.5	14.1	0.5	193.2		15	200 one dose	6.3		
					142.8	31.9	12.2	0.4	163.5			200 one dose	6.3		
7	Female	NS	IgAN	8	121.8	20.6	7.4	0.2	171.4	8	8	100 divided in two doses	2.4	PSL	Decrease in proteinuria severity
					122.0	23.0	5.7	0.3	139.2			100 divided in two doses	2.2		Reduction of PSL
8	Male	NS	MCNS	13	168.6	52.4	10.2	0.7	61.2	18	18	200 divided in two doses	1.9	CsA, PSL	Withdrawal of CsA
9	Female	LN	WHO IV G (A)	8	133.5	28.2	9.5	0.3	–	8	9	150 divided in two doses	2.7	PSL	Maintenance of clinical remission
					133.0	33.4	12.6	0.4	127.2			150 one dose	4.5		
					133.3	35.6	10.9	0.4	–			300 one dose	8.4		

AGN, acute glomerulonephritis; BUN, blood urea nitrogen; CCr, creatinine clearance; Cr, creatinine; CsA, cyclosporine; FSGS, focal segmental glomerulosclerosis; IgAN, immunoglobulin A nephropathy; LN, lupus nephritis; MCNS, minimal-change nephrotic syndrome; MZR, mizoribine; NS, nephrotic syndrome; PSL, prednisolone; WHO, World Health Organization.

Table 2 Obtained pharmacokinetic values

Dose (mg/kg)	T_{max} (h)	C_{max} ((μg/mL)	$t_{½}$ (h)	$Auc_{0-\infty}$ (μg h/mL)	Vd_{ss} (L/kg)	Rate of urinary excretion of MZR (%)
3.36 ± 1.91	2.94 ± 0.82	1.59 ± 1.16	1.96 ± 0.92	9.36 ± 6.58	2.03 ± 0.80	49.1 ± 18.7

$Auc_{0-\infty}$, area under the serum MZR concentration–time curve from time zero to infinity; C_{max}, peak serum MZR concentration; $t_{½}$, half-life; MZR, mizoribine; T_{max}, time to peak serum MZR concentration; Vd_{ss}, volume of the distribution of MZR at a steady state.

All data are presented as mean ± SD. The non-parametric Spearman correlation coefficient was calculated to test for significant associations between variables. $P < 0.05$ was considered significant.

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

Results

The clinical characteristics of the nine patients are shown in Table 1. The patient population consisted of five boys and four girls with an average age of 11.3 ± 5.1 years. The total number of MZR concentration curves analyzed was 23.

Pharmacokinetic parameters for MZR were estimated using concentration–time profiles. The obtained pharmacokinetic values are summarized in Table 2. Significant correlation coefficients were found between oral dose (corrected for bodyweight) and the C_{max} of MZR (Spearmean's rank correlation coefficient (rs) = 0.78, $P = 0.0002$), and oral dose and the $AUC_{0-\infty}$ of MZR (rs = 0.73, $P = 0.0006$). Significant linear relationships were found between oral dose and the C_{max} of MZR ($R = 0.82$; Fig. 1a), and oral dose and the $AUC_{0-\infty}$ of MZR ($R = 0.79$; Fig. 1b). No significant correlation coefficient was observed between age and Vd_{ss}.

Discussion

Mizoribine is a newly developed immunosuppressive agent in Japan. Sonda *et al.* and Takada *et al.* reported the results of pharmacokinetic studies of MZR in renal transplant patients who were mainly adults.[1,8] Tanaka *et al.* and Yumura *et al.* reported the results of pharmacokinetic studies of MZR in patients with lupus nephritis.[9,10] The Yumura *et al.* study included only adult patients.[10] Although the Tanaka *et al.* study included pediatric patients, the majority were adolescents or adults.[9] Thus, there are few reports on the pharmacokinetic study of MZR in pediatric patients, particularly in patients with child-onset glomerulonephritis.

From the Sonda *et al.* data we found no correlation between peak serum MZR concentration and single MZR dose.[1] Compared with the studies of Tanaka *et al.*,[9] Takada *et al.*,[8] and Yumura *et al.*,[10] the present C_{max} is low (Fig. 2). It seems that a higher dose of MZR is required in children to obtain the same C_{max} as that obtained in other studies. In children the C_{max} of MZR is usually low with regular use. MZR is a water-soluble substance.[11] Furthermore, in children the extracellular fluid compartment is larger than in adults.[12] Therefore it seems reasonable that Vd_{ss} is higher in children than in adults, but no significant correlation was observed between age and Vd_{ss} in the present study. Although further analysis is required, the absence of a significant correlation could be caused by the small number of infant cases in the present study. The main elimination pathway of MZR from blood circulation is renal excretion. Eighty-five percent of an oral dose of MZR is excreted unchanged in the urine.[13] Because of its non-biotransformation and water solubility, the elimination rate of MZR is dependent on renal function.[8] Although the present patients had no renal dysfunction, the rate of the urinary excretion of MZR was 49.1 ± 18.7%. Intestinal

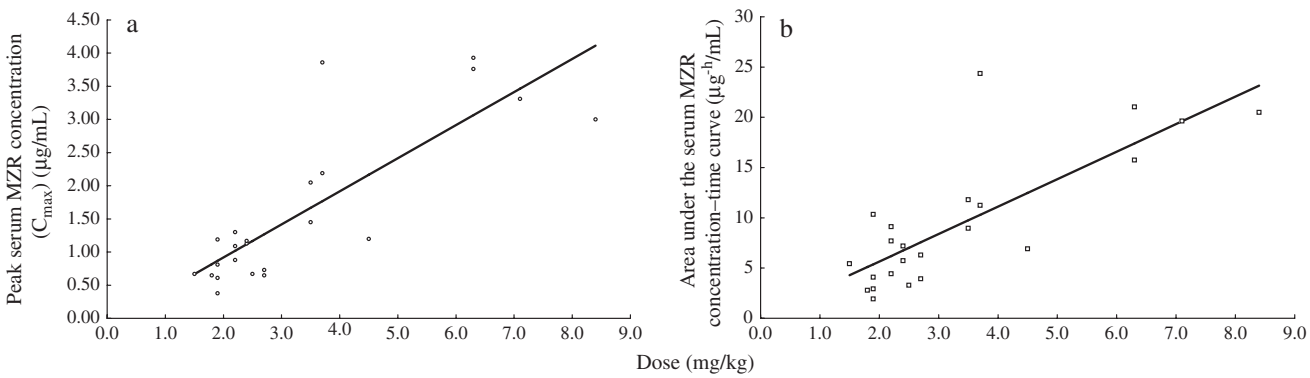

Fig. 1 Relationship between (a) mizoribine (MZR) dose and peak serum MZR concentration (C_{max}); and (b) MZR dose and area under the serum MZR concentration–time curve (AUC). (a) $y = 0.499x - 0.082$; $R = 0.82$; $R^2 = 0.67$; $P < 0.001$; (b) $y = 2.74x + 0.18$; $R = 0.79$; $R^2 = 0.63$; $P < 0.0001$.

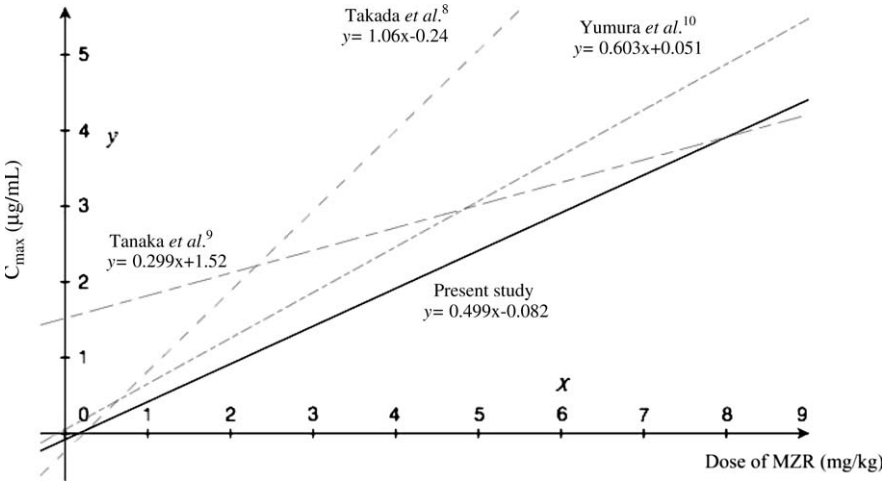

Fig. 2 Comparison of present results with those of other studies of relationship between mizoribine (MZR) dose and peak serum MZR concentration (C_{max}).

peristalsis or diet might be associated with the absorption of MZR.

In our preliminary report we found that MZR should be administered in a dose range of at least 3.0–5.0 mg/kg/day (1.5–2.5 mg/kg/dose) for patients with child-onset glomerulonephritis without renal dysfunction to obtain a peak serum MZR concentration of 0.67 μg/mL.[14] 14-3-3 proteins, that is, MZR-binding proteins, interact with glucocorticoid receptors after MZR administration to enhance the transcriptional activity of such receptors. At MZR concentrations 0.26–2.6 μg/mL, MZR enhances the induction of glucocorticoid receptor activity with dexamethasone without any cytotoxicity.[15] According to the type of nephritis, the optimal C_{max} of the drug is likely to change with treatment. But Sonda *et al.* reported that MZR had an inhibition of 50% on mixed-lymphocyte reaction at a concentration of approximately 1 μg/mL and the warning range of the trough level is ≥4 μg/mL.[1] Therefore we suggest that C_{max} should be increased to >1 μg/mL to obtain clinical efficacy and that the therapeutic range of MZR is 1–4 μg/mL.

Because MZR is used for various diseases, we cannot consistently define an absolute and effective MZR concentration; but there was only one patient who had to stop taking MZR. Tanaka *et al.* reported the efficacy of MZR oral pulse therapy for patients with a disease flare of lupus nephritis.[5] In their report, MZR in the dose range 5–10 mg/kg per day in one or two divided daily doses was orally administered twice a week for 3 months. They speculated that oral pulse therapy might achieve a sufficient C_{max} of MZR. We previously reported the efficacy of MZR at 300 mg divided into three daily doses in patient 1.[16] The efficacy of MZR seems to depend on the dose, but it is not clear which indicator is actually more reliable for assessing the efficacy of MZR with respect to C_{max} and AUC. If C_{max} is a sensitive predictor, the third hour (C_3) concentration after the ingestion of the drug should be used for follow-up monitoring because T_{max} was 2.94 ± 0.82 h in the present study.

Uemura *et al.* previously conducted a pharmacokinetic study of MZR in pediatric patients with chronic glomerulonephritis.[17] They reported that no significant correlation was shown between oral dose and the C_{max} of MZR, and oral dose of the $Auc_{0-\infty}$. Although they did not investigate Vd_{ss}, a significant difference in the dose of MZR between the present study and the Uemura *et al.* study was observed (3.36 ± 1.91 mg/kg bodyweight and 6.0 ± 2.1 mg/kg bodyweight, respectively; $P < 0.0001$, Mann–Whitney *U*-test). In the present study MZR was administered orally in the dose range 1.5–8.4 mg/kg bodyweight/dose. Uemura *et al.* studied the pharmacokinetics of only high-dose MZR.[17] The present study included patients treated with low-dose MZR. These differences seem to be responsible for the discrepancy between the two studies.

Although further analysis of the pharmacodynamics of MZR is required, the parameters obtained in the present study may be useful for MZR treatment of patients with child-onset glomerulonephritis.

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