Mod Rheumatol (2007) 17:206–212
DOI 10.1007/s10165-007-0567-x

ORIGINAL ARTICLE

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Mizoribine therapy for patients with lupus nephritis: the association between peak mizoribine concentration and clinical efficacy

Received: September 11, 2006 / Accepted: February 5, 2007

Abstract The clinical efficacy of mizoribine (MZR; 4-carbamoyl-1-b-D-ribofuranosylimidazolium) in patients with lupus nephritis was investigated. Thirteen Japanese patients with biopsy-proved lupus nephritis were enrolled in this study. A change in global assessments score, total protein (TP) of serum, serum creatinine, creatinine clearance (Ccr), proteinuria, titers of serum anti-ds DNA antibody, C3, C4, and hemolytic complement activity (CH50) were examined. Following MZR treatment, the level of urinary protein decreased ($P < 0.05$), whereas the level of Ccr increased ($P < 0.05$). Moreover, the level of TP significantly increased from 5.5 g/dl to 6.3 g/dl ($P < 0.01$) and the level of C3 increased significantly ($P < 0.01$). However, there was no change in the levels of both C4 and CH50. The titer of anti-ds DNA antibody significantly decreased ($P < 0.05$). The dosage of prednisolone could be tapered from 24.8 mg to 14.9 mg daily during the period. The clinical effects associated with MZR concentration in the blood revealed that there was a significant correlation between the peak MZR blood concentration of more than 0.66 μg/ml and clinical improvement ($P = 0.021$). Our results suggest that an optimal MZR blood concentration was important for the treatment of lupus nephritis.

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Key words Lupus nephritis · Mizoribine · Systemic lupus erythematosus

Introduction

Mizoribine (MZR; 4-carbamoyl-1-b-D-ribofuranosylimidazolium) is a nucleoside of the imidazole class, which was isolated from the culture medium of the mold *Eupenicillium brefeldianum* M-2166 in Japan.[1] MZR blocks the purine biosynthesis pathway.[2,3] Because MZR was found to inhibit both humoral and cellular immunities by selectively inhibiting the proliferation of lymphocytes, it was developed as a new immunosuppressive agent.[4] The clinical efficacy of MZR was first approved in renal transplant recipients.[5] Controlled trials conducted in Japan demonstrated that this drug prolonged graft survival without myelosuppression or hepatotoxity.[6] Because of its safety and good tolerance in comparison with other immunosuppressants, MZR has been used in Japan for the prevention of renal transplant recipients,[7] rheumatoid arthritis (RA),[8] nephrotic syndrome,[9] immunoglobulin A (IgA) nephropathy,[10] and systemic lupus erythematosus (SLE).[11] Meanwhile, partly because of the mild efficiency, its substantial clinical use compared with cyclophosphamide or cyclosporine remains relatively rare.

Recent studies indicated that the peak blood levels of MZR were important for the clinical efficacy of this drug.[11,12] According to preliminary reports, the peak blood levels of MZR usually showed less than 2.0 μg/ml with regular use: oral administration of 2.5–4.0 mg/kg per daily dosages. On the other hand, in vitro studies revealed that an appropriate effective concentration of MZR to inhibit human mixed-lymphocyte reaction required peak blood levels ranging from 3.0 to 6.0 μg/ml.[13,14] MZR oral pulse therapy was recently reported to be of benefit to a proportion of patients with disease flare-up of lupus nephritis. For pulse therapy, the concentration of peak blood levels of MZR was approximately 2.5–4.8 μg/ml. The pulse therapy revealed satisfactory curative effects without severe

adverse effects.[11,12] In contrast, the correlation between the peak blood levels of MZR regular use and the clinical efficacy remained to be investigated. We therefore evaluated the efficacy and safety of MZR and the concentration of peak blood levels of MZR in patients with lupus nephritis.

Patients and methods

Patients

Thirteen Japanese patients with biopsy-proved lupus nephritis between 1993 and 1996 at the Niigata University Hospital were enrolled in this study. All patients had long-term histories (2–10 years) of SLE and had been treated with corticosteroids combined with several cytotoxic agents and anti-platelet agents. All patients received corticosteroids before the combination with MZR, and the other immunosuppressive agents were not used with MZR at the same period. Corticosteroids had been started in all the cases from the diagnosis of SLE. Unsatisfactory therapeutic effects from corticosteroids, such as elevation of the titer of anti-ds DNA antibody, depression of the level CH50 and depression of creatinine clearance (Ccr) and proteinuria, led us to try MZR in addition to corticosteroids. Renal biopsies were performed before MZR treatment. Informed consent was obtained from the patients before entry in this study.

Study design

Mizoribine (Bredinin; Asahi-Kasei, Tokyo, Japan), 75–150 mg in three divided daily dosages, was orally administered every day. The corticosteroid dosages remained unchanged or were decreased on a gradual basis throughout the study according to the individual clinical status. No other additional treatment such as plasma exchange or intravenous immunoglobulin therapy was performed. All patients were seen at least every month and were evaluated at each visit for symptoms and routine laboratory data. We estimated clinical disease activity by means of adverse effects, through symptoms and routine laboratory data. A change in total protein (TP), creatinine (Cr), Ccr, proteinuria, titers of serum anti-ds DNA antibody, C3, C4, and CH50 was observed. The clinical effects were evaluated for 3–12 months (6.7 ± 3.3) after MZR had been started. The peak serum levels of MZR were measured once in 2 months (3 h after drug administration) after MZR had been started. The concentration of MZR was measured by high-performance liquid chromatography during therapy. The earlier pilot study indicated that 3 h after drug administration peak serum levels of MZR were shown.[15–17]

Assessment of clinical effect

The clinical findings of lupus nephritis were determined using global assessment systems of Honma et al.[18] The summary of this assessment and scoring systems is shown in Table 1.

Table 1. Assessment of clinical findings

The following endpoints should be evaluated.

(1) *Effect on the quantitative analysis of urinary protein*
D_p is calculated using the following formula:
$D_p = (P_0 - P_t)/P_0 \times 100$
where
P_0 is the baseline 24-h urinary protein
P_t is the 24-h urinary protein at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Markedly improved ($D_p \geq 75\%$)
2: Improved ($75\% > D_p \geq 50\%$)
3: Slightly improved ($50\% > D_p \geq 25\%$)
4: Unchanged ($25\% > D_p \geq -25\%$)
5: Slightly worsened ($-25\% > D_p \geq -50\%$)
6: Worsened ($-50\% > D_p \geq -75\%$)
7: Markedly worsened ($-75\% > D_p$)

(2) *Effect on the qualitative analysis of urinary protein*
D_p is calculated using the following formula:
$D_p = (P_0 - P_t)/P_0 \times 100$
where
P_0 is the baseline 24-h urinary protein
P_t is the 24-h urinary protein at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Markedly improved (the degree of positive result improved by three grades or more)
2: Improved (the degree of positive result improved by two grades)
3: Slightly improved (the degree of positive result improved by one grade)
4: Unchanged (unchanged)
5: Slightly worsened (the degree of positive result worsened by one grade)
6: Worsened (the degree of positive result worsened by two grades)
7: Markedly worsened (the degree of positive result worsened by three grades or more)
8: Normally unchanged (negative for both P_0 and P_t)

Table 1. *Continued*

(3) *Effect on renal function*
D_{ccr} is calculated using the following formula:
$D_{ccr} = (Ccr_t - Ccr_0)/Ccr_0 \times 100$
where
Ccr_0 is the baseline creatinine clearance
Ccr_t is the creatinine clearance at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Improved ($D_{ccr} \geq 25\%$)
2: Slightly improved ($25\% > D_{ccr} \geq 10\%$)
3: Unchanged ($10\% > D_{ccr} \geq -10\%$
4: Slightly worsened ($-10\% > D_{ccr} \geq -25\%$)
5: Worsened ($-25\% > D_{ccr}$)
6: Normally unchanged (both Ccr_0 and Ccr_t are ≥80 ml/min, irrespective of D_{ccr} values)

(4) *Effect on serum creatinine*
D_c is calculated using the following formula:
$D_c = (C_0 - C_t)/C_0 \times 100$
where
C_0 is the baseline serum creatinine
C_t is the serum creatinine at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Improved ($D_c \geq 25\%$)
2: Slightly improved ($25\% > D_c \geq 10\%$)
3 Unchanged ($10\% > D_c \geq -10\%$)
4: Slightly worsened ($-10\% > D_c \geq -25\%$)
5: Worsened ($-25\% > D_c$)
6: Normally unchanged (both C_0 and C_t are normal range, irrespective of D_c values)

(5) *Effect on serum total protein*
D_t is calculated using the following formula:
$D_t = (T_t - T_0)/T_0 \times 100$
where
T_0 is the baseline serum total protein
T_t is the serum total protein at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Improved ($D_t \geq 20\%$)
2: Slightly improved ($20\% > D_t \geq 5\%$)
3: Unchanged ($5\% > D_t \geq -5\%$)
4: Slightly worsened ($-5\% > D_t \geq -20\%$)
5: Worsened ($-20\% > D_t$)
6: Normally unchanged (both T_0 and T_t are ≥6.5 g/dl, irrespective of D_t values)

(6) *Effect on serum complement (C3, C4, and CH50)*
D_{cm} is calculated using the following formula:
$D_{cm} = (Ccm_t - Ccm_0)/Ccm_0 \times 100$
where
Ccm_0 is the baseline serum complement
Ccm_t is the serum complement at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Markedly improved ($D_{cm} \geq 75\%$)
2: Improved ($75\% > D_{cm} \geq 50\%$)
3: Slightly improved ($50\% > D_{cm} \geq 25\%$)
4: Unchanged ($25\% > D_{cm} \geq -25\%$)
5: Slightly worsened ($-25\% > D_{cm} \geq -50\%$)
6: Worsened ($-50\% > D_{cm} \geq -75\%$)
7: Markedly worsened ($-75\% > D_{cm}$)
8: Normally unchanged (both Ccm_0 and Ccm_t are ≥60 mg/dl for C3, ≥15 mg/dl for C4, and ≥30 U/ml for CH50, irrespective of D_{cm} values

(7) *Effect on anti-DNA antibody titer*
D_{aDNA} is calculated using the following formula:
$D_{aDNA} = (aDNA_0 - aDNA_t)/aDNA_0 \times 100$
where
$aDNA_0$ is the baseline anti-DNA antibody titer
$aDNA_t$ is the anti-DNA antibody titer at the time of assessment
Then, clinical assessment should be made according to the following criteria:
1: Markedly improved ($D_{aDNA} \geq 75\%$)
2: Improved ($75\% > D_{aDNA} \geq 50\%$)
3: Slightly improved ($50\% > D_{aDNA} \geq 25\%$)
4: Unchanged ($25\% > D_{aDNA} \geq -25\%$)
5: Slightly worsened ($-25\% > D_{aDNA} \geq -50\%$)
6: Worsened ($-50\% > D_{aDNA} \geq -75\%$)
7: Markedly worsened ($-75\% > D_{aDNA}$)
8: Normally unchanged (both $aDNA_0$ and $aDNA_t$ are ≤010 IU/ml, irrespective of D_{aDNA} values)

D, depth

Table 2. Clinical characteristics of the 13 patients with lupus nephritis

Case	Age	Sex	Symptom	Renal biopsy (WHO)	U–P (g/day)	S–Cr (mg/dl)	CH50 (U/ml)	Anti-ds DNA ab (index)	MZR dosage (mg/day)	Peak MZR concentration (mg/ml)	Duration 1 (years)	PSL1 (mg/day)	Duration 2 (months)	PSL2 (mg/day)	Clinical effect	Adverse effect
1	29	W	None	II	2.2	0.7	23	30.8	150	0.95	10	13	13	17.5	Improved	Leukopenia
2	22	W	None	IV	2	1.3	27	4.7	150	1.55	6	22.1	2	35	Improved	None
3	51	W	None	V	2.6	0.6	41	16.1	150	0.67	6	15.3	3	30	Improved	None
4	34	W	None	V	4.5	0.9	8	2	150	0.39	10	14.1	9	17.5	Improved	None
5	55	W	None	I	0.2	0.6	16	2	150	1.08	6	17.4	3	30	Improved	None
6	40	W	None	II	0	0.6	24	8	150	0.66	9	13.4	6	20	Improved	None
7	46	W	None	V	5	0.7	30	7	150	0.65	3	20.5	11	17.5	Unchanged	None
8	31	W	None	V	2	1	9.7	66.6	150	0.79	7	15.7	14	15	Unchanged	None
9	16	W	Facial rash	IV	2.7	0.7	30	37	75	0.33	2	29.7	27	15	Unchanged	None
10	36	W	None	V	3.8	0.5	47	7.1	150	0.65	3	22.6	2	30	Unchanged	None
11	23	W	None	V	5.4	0.5	36	35	150	0.72	3	23.1	5	22.5	Improved	None
12	46	W	None	V	0	0.7	14	33	150	0.44	3	23.4	18	12.5	Improved	None
13	25	W	None	IV	5	0.9	31	1.7	150	0.73	7	16.2	8	17.5	Improved	None

MZR, mizoribine; PSL, prednisolone; Duration 1, between the SLE onset and MZR initiation; Duration 2, between renal biopsy and MZR initiation; PSL1, average prednisolone dose from the SLE onset to MZR initiation; PSL2, prednisolone dosage at the time of MZR initiation; W, woman

Statistical analysis

Data are expressed as mean ± SD throughout this article and were analyzed using the Wilcoxon *U* test and Fisher's exact test to compare the appropriate effective concentration of MZR in patients. A probability level of less than 0.05 was considered to be statistically significant.

Results

Patient characteristics

The clinical characteristics of the 13 patients with lupus nephritis are shown in Table 2. Only one patient appeared to have facial rash, but others showed no symptoms at the time of MZR initiation. All patients were women with a median age of 34.9 years (range 16–55 years). All patients except for one had normal blood pressure and normal Cr levels. These patients had mean urine protein excretion, serum anti-ds DNA antibody titer (normal less than 12 IU/ml by enzyme-linked immunosorbent assay), and CH50 (normal 28–53 U/ml) values of 2.7 ± 2.8 g/day, 19.3 ± 18.4 IU/ml, and 25.9 ± 26.1 U/ml, respectively. These patients were treated with MZR of 150 mg daily, except for one case.

Effects of MZR in lupus nephritis

Following MZR treatment, urinary protein decreased significantly from 2.8 g/day to 1.8 g/day ($P < 0.05$); Ccr increased significantly from 65.3 ml/min to 89.4 ml/min ($P < 0.05$); and the level of TP also increased significantly from 5.5 g/dl to 6.3 g/dl ($P < 0.01$; Fig. 1a, b, c). The level of serum Cr decreased from 0.75 mg/dl to 0.69 mg/dl, but it was not statistically significant.

Serological effect of MZR treatment

Following MZR treatment, C3 was significantly increased from 43.5 mg/dl to 52.8 mg/dl ($P < 0.01$; Fig. 2a). C4 was increased from 13.9 mg/dl to 14.5 mg/dl and CH50 was 25.9 U/ml to 26.3 U/ml, but they were not statistically significant. The titer of anti-ds DNA antibody was significantly decreased from 19.3 IU/ml to 7.1 IU/ml ($P < 0.05$; Fig. 2b). The dosage of prednisolone could be tapered from 24.8 mg to 14.9 mg daily during the period ($P < 0.01$; Fig. 3).

Clinical effects associated with MZR concentration

The peak blood level of MZR was 0.74 ± 0.32 μg/ml. The dose-dependent efficiency of MZR on clinical improvement was investigated. Following MZR treatment, the rate of change of urinary protein, Ccr, TP, Cr, C3, C4, and CH50 was measured and listed according to the scoring system in Table 1. There was a significant correlation between peak MZR concentration levels in the blood and clinical efficacy.

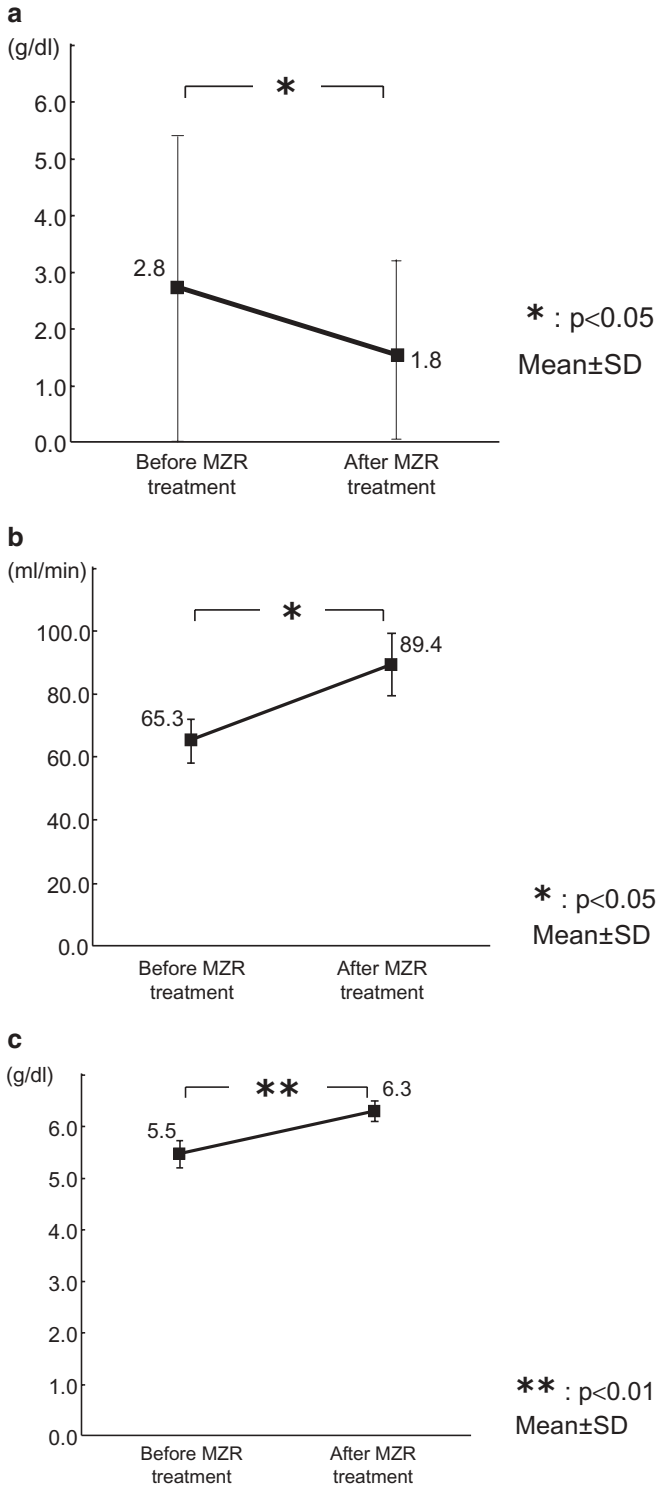

Fig. 1. **a** Changes in the amount of urinary protein. **b** Changes in the levels of creatinine clearance. **c** Changes in the levels of total protein

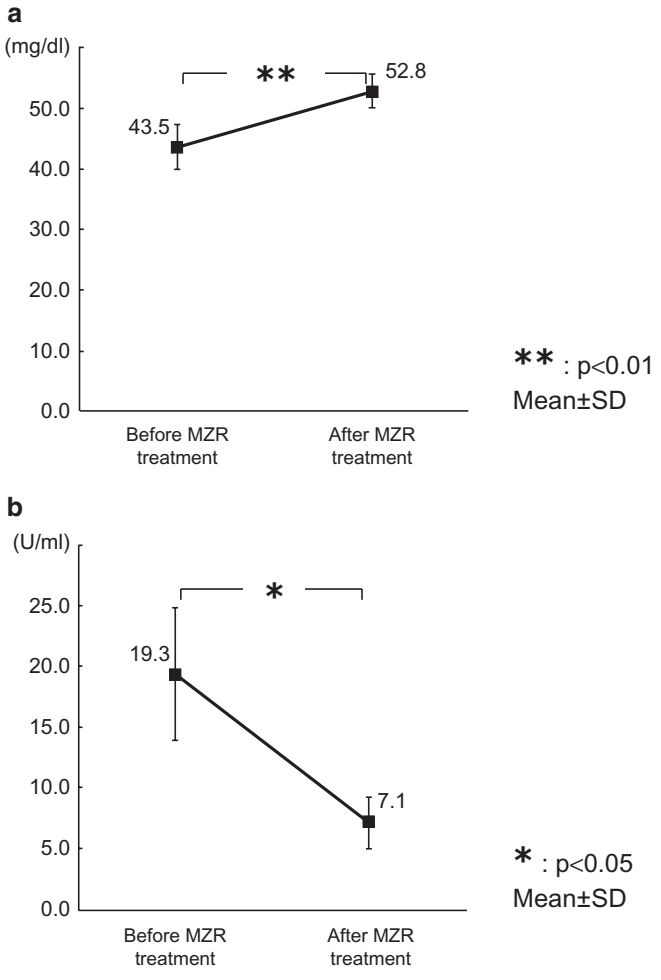

Fig. 2. **a** Changes in the levels of C3. **b** Changes in titer of anti-ds DNA antibody

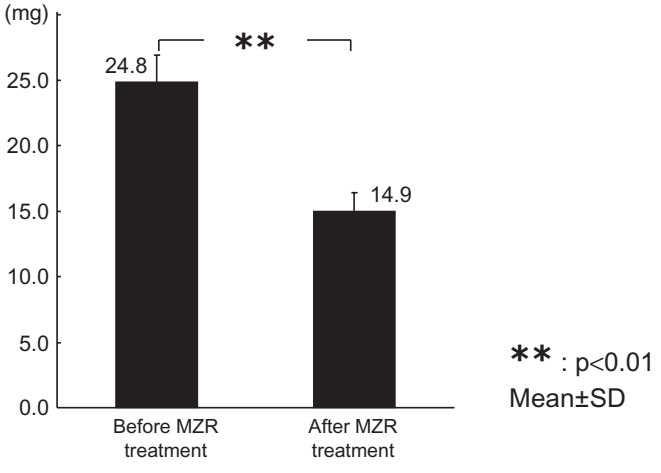

Fig. 3. Changes in the dose of prednisolone

High peak concentration of MZR was effective in patients with lupus nephritis ($P < 0.05$; Fig. 4a). Additionally, there was a significant correlation between the peak MZR concentration of more than 0.66 μg/ml and clinical improvement ($P = 0.021$; Fig. 4b). Table 3 shows the analysis of MZR concentration and clinical efficacy. We considered that the peak MZR concentration of more than 0.66 μg/ml showed high positive and negative predictive values. Additionally, a concentration of more than 0.66 μg/ml showed high sensitivity and high specificity. In this study, a concentration of more than 0.66 μg/ml maintained well-balanced relations with the improved and unchanged cases.

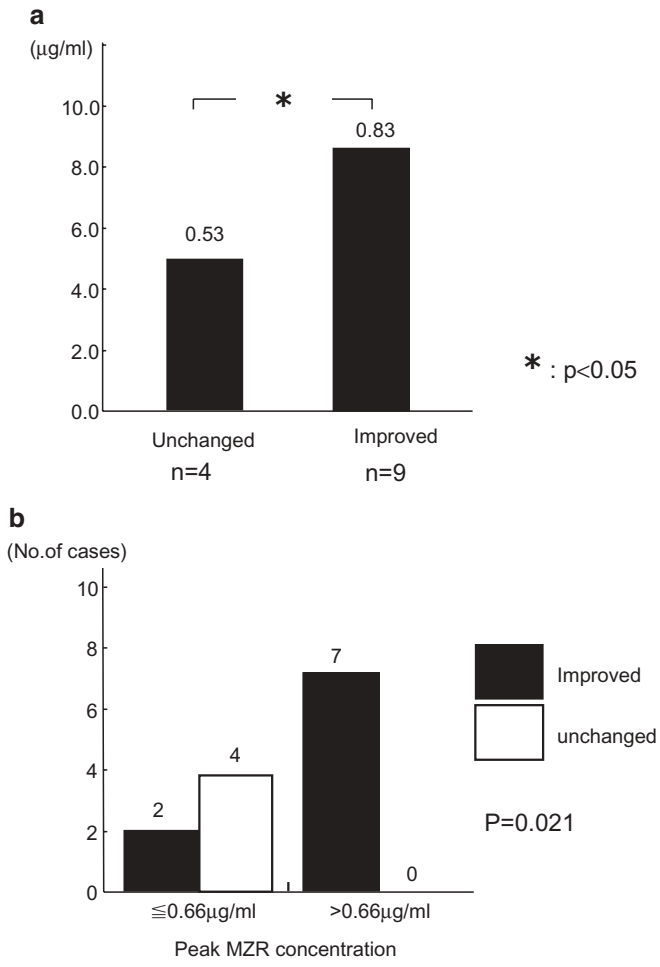

Fig. 4. **a** Peak mizoribine (MZR) concentration and clinical efficacy. **b** Peak MZR concentration and clinical efficacy

Table 3. Analysis of mizoribine concentration and clinical efficacy

Cut off (mg/ml)	PPV	NPV	Sensitivity	Specificity
≥1.55	100.0	33.3	11.1	100.0
≥1.08	100.0	36.4	22.2	100.0
≥0.95	100.0	40.0	33.3	100.0
≥0.79	75.0	33.3	33.3	75.0
≥0.73	80.0	37.5	44.4	75.0
≥0.72	83.3	42.9	55.6	75.0
≥0.67	8507	50.0	66.7	75.0
≥0.66	87.5	60.0	77.8	75.0
≥0.65	70.0	33.3	77.8	25.0
≥0.44	7207	50.0	88.9	25.0
≥0.39	75.0	100.0	100.0	25.0
≥0.33	69.2	30.8	100.0	0.0

PPV, positive predictive value; NPV, negative predictive value

Adverse effects of MZR

Only one patient (7.7%) had any indication of MZR toxicity. The white blood cell count was significantly reduced. The administration of the drug was temporarily suspended in that patient, and the white blood cell count rapidly recovered.

The patient could continue MZR treatment with the dosage of 150 mg daily. No other adverse reactions such as hair loss, bone marrow suppression, amenorrhea, hemorrhagic cystitis, or renal dysfunction were observed in any patients during the observation period.

Discussion

Mizoribine is the first imidazole nucleoside with demonstrated biological activity. It was shown that MZR had inhibitory effects on T- and B-lymphocyte activity in vivo, and the pharmacokinetics and immunosuppressive effects of MZR have also been measured in vivo.[2,3] In view of the inhibition of purine synthesis and lack of toxicity, MZR has been widely used as part of immunosuppressive drug regimens in Japan.

Several reports have shown that MZR is significantly effective in the treatment of patients with childhood-onset SLE and SLE patients with lupus nephritis.[11,12]

Mizoribine is considered to be a relatively mild immunosuppressant with low clinical toxicity. It has been reported that the inhibition of human mixed-lymphocyte reaction requires peak MZR blood concentrations ranging between 3.0 μg/ml and 6.0 μg/ml. Tanaka et al.[12] speculated that oral pulse therapy might produce high enough peak MZR blood concentrations to prevent immune cells from exiting G1 phase and entering S phase by blocking T-cell proliferation. They showed that MZR oral pulse therapy could be an alternative to increasing the dosage of prednisolone in a study of patients with remission of lupus nephritis.

Renal involvement is a major concern in SLE. From 1986 through 1988, Honma et al.[19] initiated a 24-week prospective, randomized, single-blinded trial to evaluate the efficacy and safety of MZR in patients with lupus nephritis that were refractory to treatment. Forty-nine patients who had been treated with corticosteroids were assigned randomly to receive either MZR 150 mg or placebo daily. The results of this study with 23 patients in each group were evaluated. The global improvement rate was significantly higher in the MZR group (26.1%) than in the placebo group (4.3%). The Cr and Ccr levels of the MZR group were significantly improved, and the urinary protein, blood urea nitrogen (BUN), and serum TP levels and other clinical symptoms such as eruption, tended to improve in the MZR group. Adverse effects were reported by four patients in the MZR group, each one experiencing of rash, leukopenia, infection, or gastrointestinal distress, but not serious enough to require special treatment. In our study, 13 patients were treated with MZR, and the levels of total protein, Ccr, C3, and anti-ds DNA antibodies were significantly improved. Additionally, the dosage of prednisolone could be significantly decreased in these patients.

On the other hand, in part because of its relatively low efficacy, the clinical use of MZR for the treatment of lupus nephritis is not as widespread as that of cyclosporine A. A recent study revealed that oral MZR pulse therapy, which is associated with increased serum levels of MZR, may be

associated with higher clinical efficacy without any significant increase in clinical toxicity when compared with conventional MZR therapy in patients with lupus nephritis, RA, and steroid-resistant nephritic syndrome.[12,20–22] In our study, we used approximately 25 mg/daily of prednisolone in combination with MZR. A combination of prednisolone with MZR might be the reason for the discrepancy between our in vivo data and our previous in vitro data. Our data also revealed that even though the prednisolone was tapered to approximately 15 mg/daily, MZR treatment was still effective. However, the general use of MZR and the correlation between the clinical effect and the blood concentration of MZR have not been fully investigated. In this study, we revealed that peak blood levels of MZR more than 0.66 μg/ml was important for the improvement of the clinical signs and symptoms of lupus nephritis. Although spontaneous remission or late response to prior prednisolone in combination with regular daily MZR therapy cannot be excluded, the patient's clinical course, as described, strongly suggested a correlation with the concentration of MZR in the blood. We consider that enough peak levels of MZR concentration were important for the treatment of lupus nephritis. The treatment of oral MZR pulse therapy was a method used to increase the peak blood level of MZR.[12,23] These earlier reports suggested that the peak MZR concentration of approximately 2.5–4.8 μg/ml was effective with lupus nephritis in childhood without severe adverse effects.[11,12] A similar peak concentration of MZR could be effective with adult lupus nephritis. As for the clinical efficacy of MZR, two dosages of MZR, between 150 mg/day and 300 mg/day, were compared. The patients in the treated group receiving 300 mg of MZR showed a significant improvement in the number of swollen joints, Lansbury activity index, and the final global assessment in patients with juvenile arthritis.[24] Unfortunately, peak MZR levels in the blood were not measured in this study. However, one could predict that the high concentration of MZR is rather better than the low concentration for clinical efficacy. We demonstrated that the peak MZR concentration was important in patients with adult lupus nephritis. In this study, we showed that there was a significant correlation between the peak MZR concentration of more than 0.66 μg/ml and clinical improvement; however, this was studied only for 13 cases. Further studies will reveal the effective peak concentration of MZR treatment. Additionally, further study will be necessary between trough MZR level and clinical efficacy because these correlations have not been fully investigated.

In conclusion, our results suggest that optimal MZR blood concentration was important for the treatment of lupus nephritis, and further evaluation is warranted for the use of MZR therapy in long-term follow-up studies.

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