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ORIGINAL ARTICLE

Study of the efficacy of mizoribine in lupus nephritis in Chinese patients

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Abstract We conducted a clinical study in China on the efficacy and safety of mizoribine (MZR) in lupus nephritis. Eleven subjects with proteinuria (≥2 g/day) who had undergone renal biopsy confirming a diagnosis of lupus nephritis (class III: 1 subject; class IV: 6 subjects; class V: 4 subjects) were enrolled. Nine of the subjects were treatment-naive patients who received remission induction therapy, and the other two were switched from cyclophosphamide (CTX) or mycophenolate mofetil due to lack of efficacy. MZR 150 mg was administered once a day. After 6 months, the remission rate was 72.7 % (2 subjects achieved complete remission, and 9 partial remission). After 3 and 6 months, significant reductions ($p < 0.01$) were obtained in 24-h proteinuria (g/day). In the subjects switched to MZR due to lack of efficacy with CTX, the dose was increased from MZR 150–200 mg due to inadequate improvement in proteinuria, and this dose escalation resulted in complete remission after 6 months. It is believed that this kind of dose escalation is one possible treatment option for lupus nephritis. In this study, no adverse events occurred in any of the subjects. We therefore concluded that this first use in China as remission induction therapy in lupus nephritis patients of MZR, which is recognized as an effective maintenance therapy in Japan, was effective. The results also suggest that MZR could be effective in patients for whom other drugs have been insufficiently effective.

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Introduction

Mizoribine (MZR) is an immunosuppressant that was developed in Japan and inhibits DNA synthesis in the S phase of cellular division by selectively inhibiting inosine monophosphate dehydrogenase (IMPDH) in the de novo pathway [1–3]. In Japan, MZR has been used for a long time, since its approval, in renal transplantation, lupus nephritis, rheumatoid arthritis, and nephritis syndrome and is known to be highly safe and affords a defined level of efficacy [4–6]. However, in Japan, steroids are the first-line treatment in patients with lupus nephritis, and MZR, azathioprine (AZP), or other antimetabolic agents are used only when steroids have afforded insufficient efficacy or when maintenance efficacy has not been obtained or when adverse drug reactions have occurred, or the objective is to reduce the steroid dose. Therefore, there is almost no evidence demonstrating the efficacy of MZR in remission induction therapy.

In China, as in Europe and the USA, immunosuppressants such as cyclophosphamide (CTX) and mycophenolate mofetil (MMF) have been being used concomitantly with steroids as remission induction therapy [7, 8]. However, these immunosuppressants are known to cause serious adverse drug reactions, including infections, or to afford insufficient efficacy [7, 8]. MZR is known to possess antiviral activity versus cytomegalovirus (CMV) and hepatitis C virus (HCV) [9–12] and to cause few significant adverse drug reactions, such as infections [6].

The objective of this study was to confirm the efficacy and safety of MZR in lupus nephritis and to clarify for the first time the efficacy and safety of MZR in remission

induction therapy and to determine whether MZR could become a treatment option for lupus nephritis in China.

Materials and methods

Subjects

The subjects were Chinese who received diagnoses of lupus nephritis between April 2009 and August 2010 at Nanjing Drum Tower Hospital (an affiliated hospital of Nanjing University Medical School) and Jiangsu Province Hospital (First Affiliated Hospital, Nanjing Medical University).

The inclusion criteria were (1) age ≥16 years, (2) a diagnosis of systemic lupus erythematosus (SLE) according to the SLE classification criteria (American College of Rheumatology 1997, revision) (3) proteinuria ≥2.0 g/day, and (4) an SLE disease activity index (SLEDAI) ≥10. The exclusion criteria were (1) serum creatinine >3.0 mg/dl or estimated Ccr <30 ml/min per 1.73 m^2; (2) leukocytes ≤3,000/mm^3; (3) pregnant or nursing; (4) CNS LN (cerebral lupus); (5) treated for hepatitis B, pulmonary tuberculosis, or fungal infection in the 3 months prior to the start of the study; and (6) serious cardiac disease or liver disease. Consent to study participation was obtained in advance from eligible patients.

Treatment protocol

Study subjects received oral prednisone 0.8–1.0 mg/kg (up to 60 mg/day) every day for 6–8 weeks. The daily dose was then reduced by 5 mg every 2 weeks, and once the dose reached 20 mg/day, the daily dose was reduced by 2.5 mg every 2 weeks to 10 mg/day, and then maintained at 10 mg/day. Subjects with serious lupus nephritis (WHO Class IV) received 1.0 mg/kg as the initial dose and were treated for 8 weeks. Subjects received MZR (Bredinin, Asahi Kasei Pharma, Tokyo) 150 mg once a day, starting either at the same time as the steroid treatment or 2 weeks after steroid treatment initiation. Blood biochemistry tests, urinalysis, immunological tests, and SLEDAI assessments were performed at 1, 2, 3, and 6 months. Treatment continuation beyond 6 months was permitted depending on the wishes of the patient.

Criteria of treatment efficacy

Complete remission was defined as proteinuria <0.4 g/24 h, serum albumin ≥3.5 g/dL, and normal serum creatinine. Partial remission was defined as a decrease in proteinuria of 50 % of more and proteinuria <3 g/24 h, serum albumin ≥3.0 g/L, and serum creatinine either normal or no worsening greater than 20 %.

Statistical analysis

The mean (SD) values were used for the data analysis tabulations. Statistical Package for Social Sciences (SPSS) Version 20.0 was used to analyze changes in the clinical efficacy assessment values. The Bonferroni multiple comparison test was used for comparisons of each assessment time point versus MZR treatment initiation, with $p < 0.05$ indicating a significant difference. The McNemar test was used to analyze the anti-dsDNA results, with $p < 0.05$ indicating a significant difference.

Results

Table 1 shows the baseline characteristics of subjects and MZR treatments of the 11 lupus nephritis patients. The patients were 18–60 years of age. All of the patients were female. The duration of SLE was 0.6–13.4 months, and the duration of lupus nephritis was 0.4–7.3 months. Nine of the eleven patients concomitantly received MZR during the remission induction phase. Two of the patients had received inadequate efficacy from the concomitant use of a steroid and other drugs (CTX and MMF). Renal biopsies (WHO criteria) revealed that 1 of the patients was class III, 6 were class IV, and 4 were class V. Class IV patients therefore accounted for a majority of the subjects. The initial daily dose of MZR was 150 mg, administered as a single dose. Four of the patients continued treatment for 9 months, at their request.

Table 2 shows the changes in daily proteinuria, serum creatinine, eGFR, serum albumin, and steroid dose. Daily proteinuria ranged from 2.0 to 4.2 g/24 g (mean: 3.65 g/24 h), but improved significantly ($p = 0.000$) after 3 and 6 months. Neither serum creatinine nor eGFR levels changed at 3 or 6 months, and renal function was maintained. Serum albumin ranged from 23 to 30 g/L (mean: 28.5 g/L), but improved significantly ($p = 0.000$) after 3 and 6 months. The steroid dose (mean: 41.8 mg/day) also improved significantly ($p = 0.000$) after 3 and 6 months and could be reduced to a mean level of 11.7 mg/day.

Table 3 shows the SLEDAI, anti-dsDNA, and clinical outcome results. SLEDAI ranged from 13 to 26 (mean: 20.6), but improved significantly ($p = 0.000$) at month 3 (mean: 6.4) and at month 6 (mean: 3.8). Although all subjects were positive for anti-dsDNA at initiation, after 6 months, 8 of the subjects (72.7 %) had turned negative, a significant improvement ($p = 0.016$). Improvement of 50 % or greater in proteinuria (at least partial remission) was obtained in 9 of the subjects, but since the percent change in the serum creatinine level of subject no. 9 exceeded 20 % (it was 29 %), this subject was assessed as treatment failure (TF) (Table 2). The remission rate after

Table 1 Baseline characteristics of subjects and MZR treatment received

Subject No.	Age	Sex	SLE duration (months)	LN duration (months)	Renal histology (WHO)	Previous immunosuppressive drugs	Initial dose of MZR (mg/day) (administered as a single dose)	Duration of MZR therapy (months)
1	60	F	11.3	7.3	IV	CTX	150	9
2	40	F	2.4	0.4	III		150	9
3	30	F	1.2	1.2	IV		150	9
4	41	F	13.4	5.3	IV		150	9
5	37	F	3.1	0.3	IV		150	6
6	48	F	1.4	0.4	V		150	6
7	42	F	0.6	0.6	IV		150	6
8	41	F	2.5	0.5	V		150	6
9	38	F	2.4	0.5	V		150	6
10	18	F	3.1	1.1	IV		150	6
11	54	F	4.8	4.2	V	MMF	150	6
Mean	40.8		4.2	2	III; 1, IV; 6,			
SD	11.3		4.2	2.4	V; 4			

MZR mizoribine, *SLE* systemic lupus erythematosus, *LN* lupus nephritis, *CTX* cyclophosphamide, *MMF* mycophenolate mofetil, *SD* standard deviation

Table 2 Changes in proteinuria, serum creatinine, eGFR, serum albumin, and steroid dose in subjects with lupus nephritis receiving MZR

Subject No.	Proteinuria (g/day)			Serum creatinine (mg/dL)			eGFR (mL/min/1.73 m^2)			Serum albumin (g/L)			Steroid dose (mg/day)		
	Baseline (B)	3 (m)	6 (m)	Baseline (B)	3 (m)	6 (m)	Baseline (B)	3 (m)	6 (m)	Baseline (B)	3 (m)	6 (m)	Baseline (B)	3 (m)	6 (m)
1	3.7	0.8	0.3	1.01	1.14	1.12	65.8	56.3	57.7	23	36	36	45	30	5
2	3.6	1.5	1	0.95	1.04	1	76.1	68	71.8	30	35	35	45	17.5	15
3	4.2	2.5	1.5	0.98	1.04	0.98	76.8	71.7	76.8	30	34	35	45	15	10
4	3.7	2.7	1.9	1.14	1.17	1.1	60.2	58.8	63.3	29	32	33	45	12.5	12.5
5	3.6	2.1	1.5	1.15	0.98	0.96	60.8	74	76.2	29	32	35	45	30	20
6	3.7	2.8	2.2	1.01	1.04	1.02	68.6	65.9	67.7	29	31	33	45	20	15
7	3.7	2.1	1.5	1.1	1	0.97	63.1	71.2	73.2	29	34	35	45	17.5	15
8	4.1	2.3	1.5	1.15	1.07	1.03	59.6	65.5	68.6	30	35	37	45	17.5	15
9	4.2	2.5	2	0.78	1.1	1.01	97.9	64.3	71.5	29	35	35	40	15	7.5
10	3.6	2.2	1.1	1.15	1.01	1.02	69.2	81.8	80.7	30	35	36	40	10	10
11	2	0.2	0.2	0.53	0.63	0.59	147.4	120.1	130.1	26	43	46	10	8	4
Mean	3.65	1.97**	1.34**	1	1.02	0.98	76.9	72.5	76.2	28.5	34.7**	36.0**	41.8	17.5**	11.7**
SD	0.57	0.79	0.62	0.18	0.14	0.13	24.7	16.5	18.1	2.12	3.09	3.34	7.16	6.76	4.68

eGFR estimated glomerular filtration rate

Comparison versus baseline value * $p < 0.05$; ** $p < 0.01$

6 months was therefore 72.9 % [2 subjects (18.2 %) with complete remission and 6 subjects (54.5 %)] with partial remission). By tissue classification, the remission rate for class IV was 83.3 % (5 of 6 subjects; 1 subject achieved complete remission, and 4 subjects achieved partial remission), and the remission rate for class V was 50 % (2 of 4 subjects; 1 patient achieved complete remission, and 1 patient achieved partial remission). The improvement rate was therefore lower for class V than for class IV (Table 4).

Figure 1 shows the clinical course of treatment for subject no. 1. This subject was a female, 60 years of age, with diffuse glomerulonephritis (class IV). This subject received steroid (45 mg/day every day) and CTX 0.8 g/body once a month, but virtually no improvement in the subject's proteinuria had been obtained after 2 months, CTX treatment was discontinued, and treatment was initiated with MZR 150 mg once a day. After 1 month of treatment with MZR, the subject's proteinuria had

Table 3 Changes in SLEDAI, anti-dsDNA, and clinical outcome in subjects with lupus nephritis receiving MZR

Subject No.	SLEDAI			Anti-dsDNA		Clinical outcome
	Baseline (B)	3 (m)	6 (m)	Baseline (B)	6 (m)	6 (m)
1	16	4	0	Positive	Negative	CR
2	24	8	8	Positive	Positive	PR
3	17	6	3	Positive	Negative	PR
4	16	6	6	Positive	Negative	TF
5	24	10	4	Positive	Negative	PR
6	23	7	4	Positive	Negative	TF
7	24	10	6	Positive	Negative	PR
8	19	6	3	Positive	Negative	PR
9	25	8	6	Positive	Positive	TF[a]
10	26	5	2	Positive	Positive	PR
11	13	0	0	Positive	Negative	CR
Mean	20.6	6.4**	3.8**	Positive; 11 (100)	Positive; 3 (18.2)	Remission rate (CR + PR)
SD	4.3	2.7	2.4	Negative; 0 (0)	Negative; 8 (72.7)*	72.9 % (CR; 2, PR; 7)

SLEDAI systemic lupus erythematosus disease activity index, *Anti-dsDNA* anti-double stranded DNA antibodies, *CR* complete remission, *PR* partial remission, *TF* treatment failure

[a] Assessed as TF because the percent change in serum creatinine exceeded 20 %

Comparison versus baseline value * $p < 0.05$; ** $p < 0.01$

Table 4 Relationship of renal histology and clinical outcome after 6 months of MZR therapy

Before therapy		After therapy		
Renal histology	Number of subjects	Clinical outcome	Number of subjects	Remission rate (CR + PR) (%)
III	1	PR	1	100.0
IV	6	CR	1	83.3
		PR	4	
		TF	1	
V	4	CR	1	50.0
		PR	1	
		TF	2	

CR complete remission, *PR* partial remission, *TF* treatment failure

improved to approximately 2 g/24 h, but since no further improvement was obtained after 2 months, the MZR dose was increased to 200 mg/day. This resulted in an improvement in the subject's proteinuria to 0.3 g/24 h after 6 months. Treatment was continued for 9 months per the subject's wishes, and the proteinuria fell to 0.1 g/24 h. Following MZR treatment initiation, it was possible to reduce the dose of the concomitant steroid to 5 mg/day after 6 months. Steroid treatment was continued at this dose level through the end of the study (9 months), and the subject did not experience recurrence. Serum albumin also improved, and normalized, following MZR dose escalation. The subject's SLEDAI score also improved following dose escalation and was 0 after 6 months, indicating the disappearance of disease activity. The subject became anti-dsDNA negative after 2 months and remained so thereafter. Renal function (eGFR) remained virtually unchanged. Therefore, MZR dose escalation resulted in improved efficacy in a patient for whom CTX had been insufficiently effective.

Not a single subject experienced any adverse drug reactions in this study.

Discussion

In China, lupus nephritis treatment consists of 4–8 weeks of steroid therapy at a dose level of 0.8–1.0 mg/kg, after which the dose is reduced. In the case of diffuse lupus nephritis, steroid pulse therapy is also an option. In this study, none of the subjects received steroid pulse therapy. The class IV subjects generally received 1.0 mg/kg for 2 weeks longer than the other subjects (class III or class V subjects). In addition to the steroid therapy, subjects received CTX concomitantly in the remission induction period. The MZR that was used in this study has been used for a long time in Japan in the maintenance phase following remission induction for the purpose of reducing the steroid dose, preventing recurrence, aiding patients for whom other drugs have been insufficiently effective, and preventing adverse drug reactions [14–16]. Therefore, virtually no data are available on the use of MZR in the induction period. However, it is believed that the results of

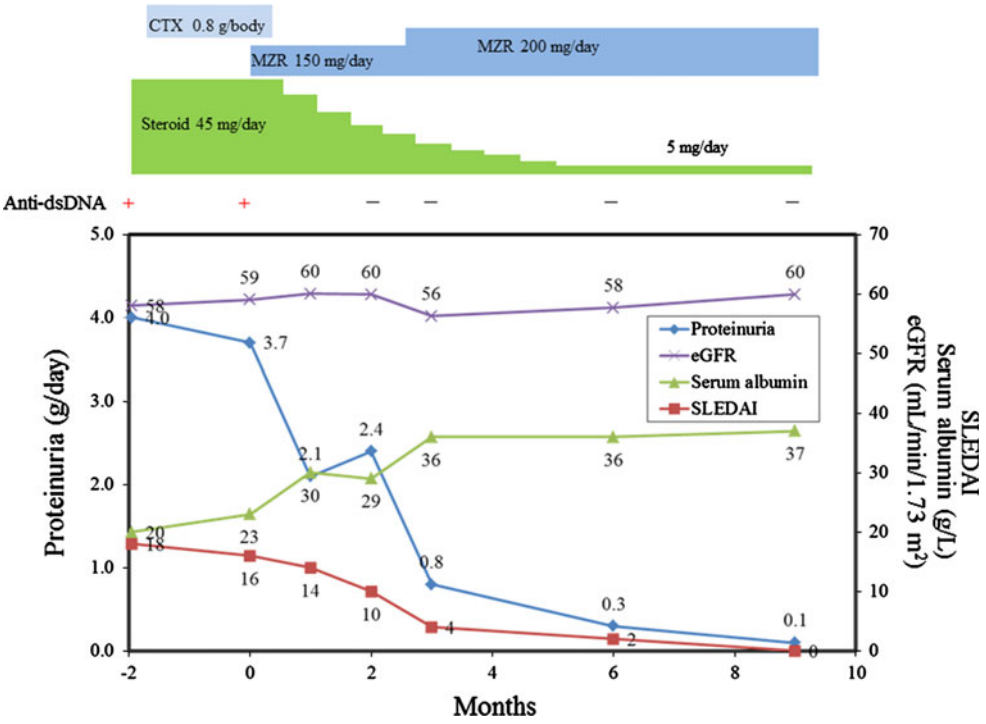

Fig. 1 Clinical course and treatment in Subject No. 1. The subject was female, 60 years of age, with diffuse glomerulonephritis (class IV)

this study, which show that MZR was effective in the induction period as well, provide important evidence supporting positioning MZR as a lupus nephritis treatment option. The remission rate in this study (complete + partial remission) was 72.9 %, which is similar to the remission rates of 53–72 % that have been reported for CTX in other studies, supporting the contention that MZR possesses efficacy comparable to that of CTX [7, 8, 13].

In addition to inhibiting DNA synthesis by selectively inhibiting IMPDH in the de novo pathway, MZR also acts on 14–3–3 proteins, and binds to glucocorticoid receptors (GR), potentiating GR transcriptional activity in a dose–dependent manner [17]. MZR is therefore expected to act to potentiate steroid efficacy. In this study as well, the steroid dose could be reduced almost exactly as specified in the protocol, and it was possible to reduce the dose to a mean dose level of 11.7 mg/day after 6 months. Furthermore, not a single subject experienced recurrence.

Mizoribine is held to have few serious adverse reactions and to be very safe. In this study as well, not a single adverse drug reaction was reported, supporting the contention that MZR is highly safe.

In subject no. 1, MZR dose escalation resulted in increased efficacy. A correlation between the MZR blood concentration and efficacy has already been demonstrated [18, 19]. It has also been reported that changing the dosing method from BID or TID dosing to once daily dosing improved efficacy [20]. Therefore, the increased efficacy obtained in this subject clearly appears to be due to the increase in the dose, and to the elevated blood levels. This is thought to be one method of using MZR in lupus nephritis in subjects experiencing insufficient efficacy. Furthermore, MZR is said to not cause clinically significant adverse drug reactions even when the Cmax (maximum blood concentration) is reached [5, 19, 20]. No adverse drug reactions due to dose escalation were reported in this subject.

Future topics of study for MZR are performing a comparative study versus CTX, for example, in the induction phase, and confirming efficacy in the maintenance phase in China as well. In this study, the sample size was small, the term of the study was short, and none of the subjects experienced adverse drug reactions. It is believed that these results need to be confirmed in a larger number of subjects.

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