Pediatrics International (2004) **46**, 444–449

Original Article

Efficacy of mizoribine in the treatment of systemic lupus erythematosus in children

KOUICHI YOSHIDOME, SYUJI TAKEI, HIROYUKI IMANAKA, NOBUAKI MAENO, TOSHIYA OHKAWA AND YOSHIFUMI KAWANO

Department of Pediatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

Abstract

Background: Mizoribine (MZR) is a novel immunosuppressant developed in Japan. As MZR is reported to be less toxic than other cytotoxic drugs, it is frequently used in Japan in the treatment of adult patients with rheumatoid arthritis or lupus nephritis. The objective of this study was to evaluate the efficacy of MZR in children with SLE. Nine female children with lupus nephritis who had undergone renal biopsy before starting MZR, were involved in this study. Their mean disease duration was 4.8 years at the time MZR treatment was initiated. Patients who had received intensive medications, such as methyl-prednisolone pulse therapy, intravenous cyclophosphamide pulse therapy, and/or other immunosuppressants, within the 4 months prior to the start of the study, were excluded.

Methods: Patients treated with 3 mg/kg per day of MZR were monitored every month for up to 1 year. The efficacy of MZR was evaluated by the changes from baseline values of serum C3, serum C4, anti-dsDNA antibody titer, erythrocyte sedimentation rate (ESR), urinary protein, dosage of prednisolone (PSL), and the sum of the scores defined by these parameters.

Results: Favorable changes were observed in C3 and ESR after 2 months and 3 months of MZR therapy, respectively. At 3 months of MZR therapy, the sum of scores defined by the parameters for disease activity indicated that MZR was more effective in non-class IV nephritis patients ($n = 5$) than in class IV nephritis patients ($n = 4$) ($P = 0.0197$). All nine children involved in the study tolerated the MZR therapy well during the study.

Conclusion: MZR was safe in lupus children, but its efficacy was limited in patients with non-class IV nephritis. Further study is necessary, in which higher dosages and/or earlier use of MZR is provided to a larger number of children.

Key words childhood, immunosuppressant, lupus nephritis, mizoribine, systemic lupus erythematosus, treatment.

Systemic lupus erythematosus (SLE) is known as a chronic inflammatory disease. Child-onset SLE is reported to be more acute and severe than adult SLE.[1] Consequently, children with SLE are generally given higher dosages of steroids and receive steroids for longer periods than adult patients do. To improve the quality of life for children with lupus, it is essential to reduce the side-effects of steroids.

Since immunosuppressant therapy was introduced into the treatment of children with SLE, the prognosis of childhood lupus has improved significantly. A pooled analysis of adults and children with lupus nephritis showed that patients receiving immunosuppressants had less renal deterioration, were less likely to have end-stage renal disease, and were less likely to die from kidney disease than patients receiving steroids alone.[2] However, various kinds of side-effects of the toxic agents in children and adolescents have been a major issue.

Mizoribine (MZR) is a novel immunosuppressant isolated from the culture medium of the mold *Eupenicillium brefeldianum* M-2166.[3] MZR has been found to suppress both humoral and cellular immunity. The immunosuppressive effect of MZR is attributed mainly to its inhibition of T-cell function.[4,5] Therefore, in Japan MZR has been used to treat patients with rheumatoid arthritis, lupus nephritis, or nephrotic syndrome, and patients who have undergone renal transplantation. Based on this experience, it has been

Correspondence: Kouichi Yoshidome, 8-35-1 Sakuragaoka, Kagoshima City 890-8520, Japan.
Email: tokino@m2.kufm.kagoshima-u.ac.jp

Received 3 November 2003; revised 1 December 2003; accepted 8 December 2003.

Table 1 Patients and clinical features

Patient no.	Sex	Age at onset (years)	Period from onset (years)	WHO classification	PSL (mg/kg per day)	MZR (mg/kg per day)	Period of MZR treatment (months)
1	F	13.7	8.5	IIa	0.3	3.1	12
2	F	8.1	2.0	IIIa	0.2	3.6	10
3	F	12.5	1.0	Ib	0.6	3.4	8
4	F	10.7	3.4	Va	0.3	2.7	12
5	F	13.1	2.8	Ib	0.2	2.4	12
6	F	11.2	8.0	IVd	0.3	3.5	12
7	F	9.1	7.7	IVc	0.4	2.4	12
8	F	9.8	2.0	IVb	0.4	2.8	5
9	F	12.5	8.1	IVc	0.1	1.9	12
Mean ± SD		11.2 ± 1.9	4.8 ± 3.1		0.3 ± 0.1	2.9 ± 0.6	10.6 ± 2.5

reported that MZR is less toxic than other immunosuppressants, especially with regard to both bone marrow and liver function.[6] In the present study, we examined the efficacy of MZR in the treatment of children with SLE.

Patients and methods

The SLE patients enrolled in this study were all under 16 years of age at onset of SLE. They received MZR while visiting our outpatient clinic between 1984 and 2002. All of the patients were initially diagnosed with SLE according to the 1982 criteria for the classification of SLE patients by the American College of Rheumatology (ACR),[7] although all patients later met the ACR criteria that were updated in 1997.[8] Excluded from the study were patients who had received intensive treatment, such as methylprednisolone pulse therapy (iv mPSL), cyclophosphamide pulse therapy (iv CY), or another immunosuppressant within 4 months prior to starting MZR.

Nine female patients with childhood-onset SLE were enrolled in this study (Table 1). The mean age at onset was 11.2 years (8.1–13.7 years). At the initiation of MZR therapy, the disease duration was 4.8 years on average (1.0–8.5 years) and the mean dose of PSL was 0.3 mg/kg per day (0.1–0.6 mg/kg per day). At the initiation of MZR treatment, all patients were experiencing the adverse effects of steroid therapy. Remarkable obesity was observed in cases 1, 2, and 9, while growth impairment was found in cases 2, 3, 4, 5, and 8. Cases 1, 6, and 9 were also receiving topical remedy for steroid-induced cataract. In addition, cases 7 and 8 had persistent proteinuria of more than 1 g/day.

All patients had undergone renal biopsy within 6 months prior to starting MZR. Their histopathological findings, categorized by World Health Organization (WHO) classification criteria were: class I in two patients, class II in one, class III in one, class IV in four, and class V in one.

The average MZR dose was 2.9 mg/kg per day (from 1.9 to 3.6 mg/kg per day). Of the nine patients, six were monitored for the efficacy of MZR for 12 months, and one patient (case 2) was monitored for 10 months. The remaining two patients (cases 8 and 3) each experienced a recurrence of SLE and had to stop the MZR treatment at 5 and 8 months, respectively. Prior to the stoppage, these two patients were treated with iv CY or iv mPSL therapy, respectively.

The patients were retrospectively assessed for changes in the levels of serum C3, serum C4, anti-dsDNA antibody, erythrocyte sedimentation rate (ESR), urinary protein, categorized urinary protein, and dosage of PSL, throughout the MZR therapy. The urinary protein levels were categorized as negative (<50 mg/dL), 1 + (50– <100 mg/dL), 2 + (100–<200 mg/dL), 3 + (200– <300 mg/dL), and 4 + (>300 mg/dL).

To evaluate the efficacy of MZR, the changes in these parameters from baseline were categorized and scored as –1, 0, or + 1, as shown in Table 2.

Statistical analysis

As the data showed a non-parametric distribution, our statistical analysis included the Wilcoxon rank sum test for the paired data and the Mann–Whitney *U*-test for the unpaired data. A *P*-value of <0.05 was considered significant. The data are expressed in mean values ± standard deviation (M ± SD) throughout the paper.

Informed consent was obtained from all patients and/or their parents before MZR was initiated.

Results

Changes in each parameter

Figure 1 shows the changes in each parameter at 0, 1, 2, 3, 6, and 12 months of MZR treatment. Each figure indicates the data from each patient.

The mean serum C3 levels increased in all patients, from 75.9 mg/dL at baseline to 84.9 mg/dL at 12 months of MZR

Table 2 Definition of scores for each parameter

Score	Deteriorated – 1	No change 0	Improved + 1
C3	Decreased > 10 mg/dL	± 10 mg/dL	Increased > 10 mg/dL
dsDNA	Increased > 10 IU/mL	± 10 IU/mL	Decreased > 10 IU/mL
ESR	Increased > 10 mm/h	± 10 mm/h	Decreased > 10 mm/h
Categorized levels of urinary protein	Increased ≥ 1 level	No change	Decreased ≥ 1 level or maintained negative
Daily PSL dose	Increased > 2.5 mg	± 2.5 mg	Decreased > 2.5 mg

Changes from baseline in each parameter were categorized and scored from –1 to +1.

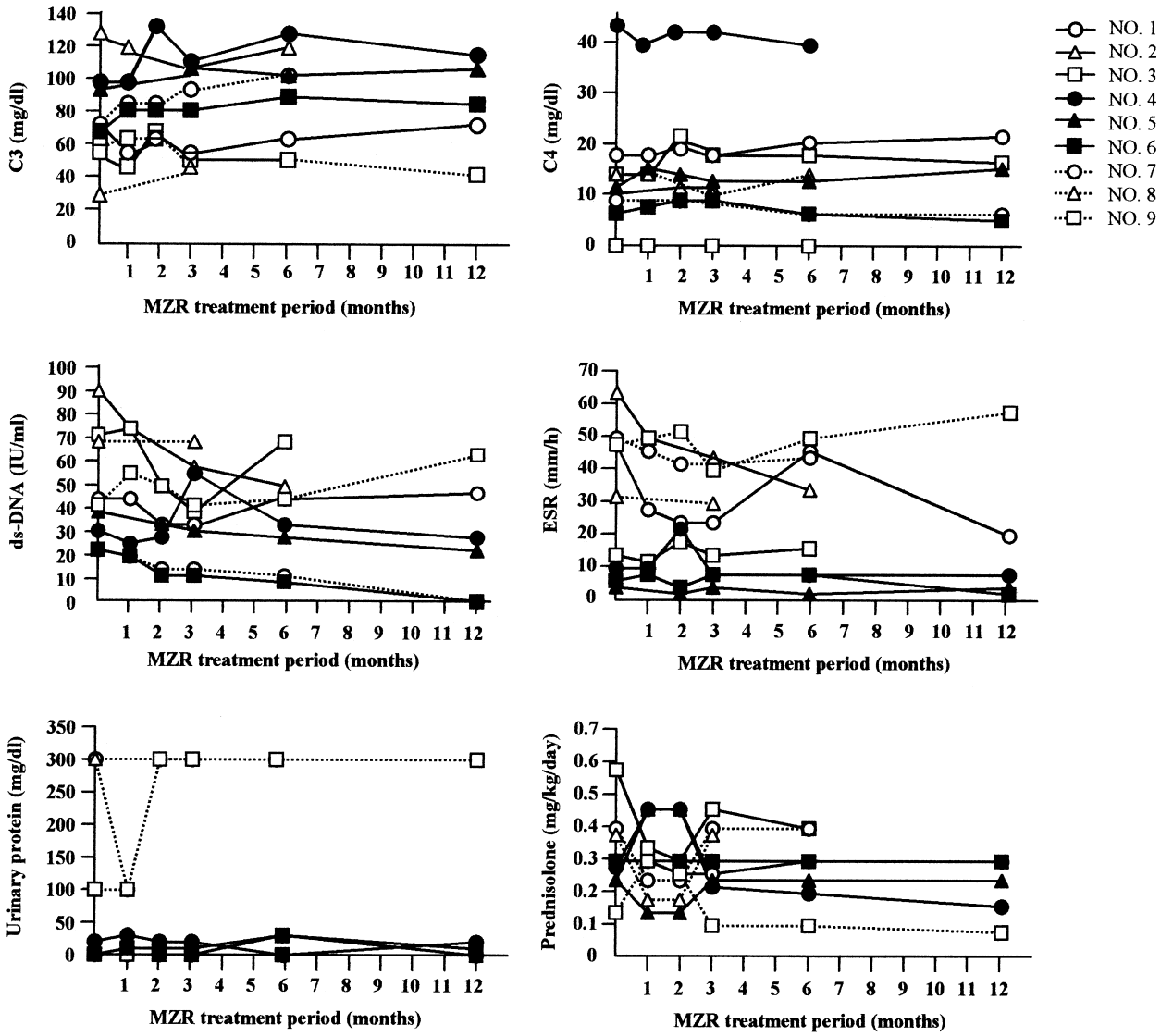

Fig. 1 Changes in each parameter during mizoribine therapy. Clinical parameters such as serum levels of C3 and C4, antidsDNA antibody, erythrocyte sedimentation rate, semiquantitatively assessed urinary protein, and prednisolone dosage were monitored at 0, 1, 2, 3, 6, and 12 months of mizoribine treatment in nine children with systemic lupus erythematosus. The figure shows the changes in each parameter in each patient. Cases 1, 2, 3, 4, and 5 were patients with non-class IV nephritis. Cases 6, 7, 8, and 9 were patients with class IV nephritis.

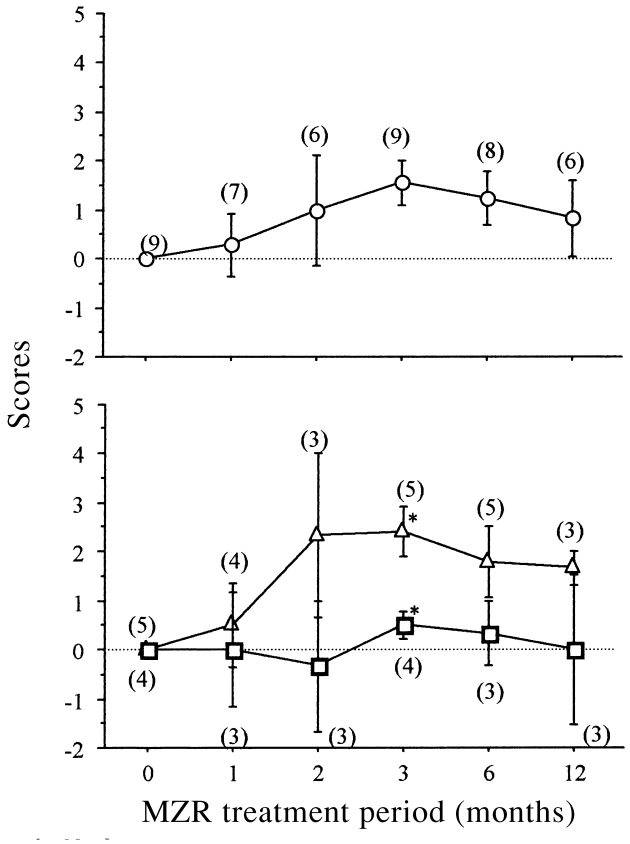

Fig. 2 Changes in scores of disease activity during mizoribine therapy. The changes in each parameter were categorized and scored. The scores of patients with non-class IV nephritis increased after initiation of mizoribine treatment and peaked at 3 months of mizoribine therapy. In contrast, the scores of patients with class IV nephritis did not change during the course of mizoribine therapy. *A significant difference was observed in the scores between the two groups at 3 months of mizoribine treatment ($P = 0.0197$).

treatment; a statistical difference was observed only at 2 months ($P = 0.0464$).

In patients with non-class IV nephritis, the mean serum C4 levels tended to increase, from 19.0 mg/dL at baseline to 22.0 mg/dL at 6 months of MZR therapy. However, no statistical difference from baseline was obtained at any point during the MZR treatment.

In patients with non-class IV nephritis, the mean anti-dsDNA antibody level decreased from 44.6 U/mL at baseline to 25.0 U/mL at 12 months of MZR therapy. However, for this parameter, no statistical difference from baseline was obtained at any point during the MZR treatment.

ESR improved with the duration of MZR treatment, especially in patients with non-class IV nephritis. Statistical differences from baseline were observed at 3 months of MZR treatment in all nine patients ($P = 0.0357$).

No significant changes in urinary protein levels were observed during the MZR treatment. Of six patients with excessive levels of urinary protein at the start of the MZR treatment, four maintained the same levels of urinary protein excretion, and the other two had increased levels of urinary protein excretion at the last evaluation. The three remaining patients, who were negative for proteinuria at the start of MZR, remained negative throughout the MZR treatment.

The mean dose of PSL was 0.3 mg/kg per day at baseline and 0.2 mg/kg per day at 12 months of MZR treatment. At the last evaluation, however, the daily dose of PSL was decreased in three of the nine patients.

Change in score during mizoribine therapy

The sum of the scores had increased (improved) at 3 months of MZR treatment in all patients with non-class IV nephritis, and maintained improved levels for the rest of the treatment period (Fig. 2). In contrast, the scores of patients with class IV nephritis were unchanged throughout the therapy. At 3 months of MZR therapy, scores in patients with non-class IV nephritis were: 4 (case 1), 3 (case 2), 3 (case 3), 1 (case 4), and 2 (case 5), while scores in class IV nephritis patients were 0 (case 6), 1 (case 7), 0 (case 8), and 0 (case 9). As a result, a significant difference was observed in the scores between the two groups at 3 months of MZR treatment ($P = 0.0197$).

Side-effects

All patients tolerated the MZR therapy well; no side-effects were observed during the study period in any of the SLE children.

Discussion

In the present study, we have shown the efficacy of MZR in the treatment of SLE children with non-class IV nephritis. Several reports have examined the efficacy and safety of MZR in adult SLE patients.[9–14] However, only three published reports have examined the usefulness of MZR in the treatment of children with SLE. According to those reports, MZR was effective in three children with SLE[15] and in four children with lupus nephritis[16] in combination with oral steroid therapy. Miyamae *et al*. indicated that MZR was useful as a maintenance therapy after iv mPSL in seven patients with child-onset lupus nephritis.[17,18] However, these studies did not consider the influence of concomitant therapy with MZR or the influence of therapies administered prior to the MZR treatment. In the present study therefore, patients who had been treated with iv mPSL, iv CY, or other immunosuppressants within 4 months prior to the start of MZR

therapy, or at any time during it, were excluded to avoid distortions in the data resulting from the efficacy of these intensive treatments.

In order to evaluate the efficacy of MZR in this study, we monitored several parameters, including C3, C4, ESR, dsDNA, urinary protein, and PSL dosage. Of these parameters, it has been reported that changes in C3, C4, ESR, and dsDNA levels correlated well with disease activity,[19,20] and urinary protein levels and daily PSL dosage directly reflected the effectiveness of the treatment. Although our scoring system was locally developed and locally used, we believe it reasonably offers objective evidence in evaluating the efficacy of MZR in the treatment of children with SLE.

Except for urinary protein levels, all other parameters showed favorable changes after the MZR treatment was started. However, significant changes in these parameters were observed only in ESR and C3 levels at 2 or 3 months of MZR therapy, respectively. This may be explained by the small number of patients in the present study. To overcome this problem, we employed a scoring system to evaluate the comprehensive clinical efficacy of MZR. The resultant changes in the score indicated that MZR induced clinical improvement after 3 months of MZR therapy in non-class IV nephritis patients, while no clinical improvement was observed during MZR therapy in class IV nephritis patients. These results suggest that MZR is more effective for children with mild cases of SLE than for children with more severe cases. In addition, the efficacy of MZR appears to emerge a few months after the treatment is initiated.

The limited efficacy of MZR, especially in severe cases of SLE, might be attributable to the low dosages of MZR used in this study. Several reports have indicated that the efficacy of MZR was dose-dependent in adult lupus nephritis[9] and rheumatoid arthritis patients.[21] In the present study, patients were treated with 2.9 mg/kg per day MZR, on average. In our experience in juvenile rheumatoid arthritis children treated with 3 mg/kg per day MZR, about half of the patients did not reach a serum level of 1.0 μg/mL; the threshold for effective inhibition of T-cell function *in vitro* (data not shown). As MZR is reported to be a less toxic immunosuppressant,[3,22–24] increased dosages of MZR may be safe and effective in the treatment of children with class IV lupus nephritis.

In conclusion, MZR was effective in SLE children with non-class IV nephritis. Further study, using higher dosages and/or earlier initiation of MZR in a larger number of children with SLE, is necessary.

References

1 Tucker LB, Menos S, Schaller JG, Isenberg DA. Adult- and childhood-onset systemic lupus erythematosus: a comparison of onset, clinical features, serology, and outcome. *Br. J. Rheumatol.* 1995; **34**: 866–72.

2 Felson DT, Anderson J. Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis. Results of a pooled analysis. *N. Engl. J. Med.* 1984; **311**: 1528–33.

3 Mizuno K, Tsujino M, Takada M *et al.* Studies on bredinin. I. Isolation, characterization and biological properties. *J. Antibiot.* 1974; **27**: 775–82.

4 Yoshizawa M, Tsujino M, Mizuno K, Hayano K. Immunosuppressive effect of mizoribine. I. Effect on immune response cells. *Clin. Immunol.* 1982; **14**: 256–62 (in Japanese with English abstract).

5 Yoshizawa M, Tsujino M, Mizuno K, Hayano K. Immunosuppressive effect of mizoribine. II. Suppression of humoral and cellular immune response. *Clin. Immunol.* 1982; **14**: 561–70 (in Japanese with English abstract).

6 Kusaba R, Otubo O, Sugimoto H *et al.* Immunosuppresive effect of bredinin in the management of patients with renal transplantation. *Proc. Eur. Dial. Transplant. Assoc.* 1981; **18**: 420–5.

7 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1982; **25**: 1271–7.

8 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; **40**: 1725.

9 Shibazaki T, Murai S, Gomi H, Sakai N, Miyahara T. Clinical trial of mizoribine on refractory lupus nephritis. *Clin. Report* 1988; **22**: 5821–6 (in Japanese).

10 Kondo H, Okada J, Kashiwazaki S. Clinical trial of long-term administration of mizoribine in the treatment of lupus nephritis. *Clin. Immunol.* 1989; **21**: 643–50 (in Japanese with English abstract).

11 Ishikawa T, Iizuka T, Yamagata K *et al.* The effect of mizoribine (Bredinin) (2). Clinical evaluation in patients with systemic lupus erythematosus. *Kidney Dialysis* 1989; **27**: 137–41 (in Japanese).

12 Homma M, Akizuki M, Yokohari R *et al.* Clinical evaluation of mizoribine on lupus nephritis. Multicenter single-blind comparative study with inactive placebo. *J. Clin. Therapeutics Med.* 1989; **5**: 795–824 (in Japanese with English abstract).

13 Homma M, Kondo H, Akizuki M *et al.* Clinical evaluation of mizoribine on lupus nephritis (II). Long-term administration. *J. Clin. Therapeutics Med.* 1989; **5**: 825–35 (in Japanese with English abstract).

14 Ishikawa H, Yokohari R. Evaluation of mizoribine in the treatment of systemic lupus erythematosus. *Rheumatology* 1989; **1**: 250–6 (in Japanese with English abstract).

15 Aihara Y, Ibe M, Mituda T *et al.* Effects of combined administration of prednisolone and mizoribine in the course of remission for SLE children with nephritis. *Ryumachi* 1994; **34**: 64–70 (in Japanese with English abstract).

16 Tanaka H. Effects of mizoribine on various types of glomerulonephritis in children. *Jpn. J. Pediatrics* 1995; **48**: 2295–300 (in Japanese).

17 Miyamae T, Nakashima S, Imagawa T *et al.* Improvement of the maintenance therapy after methylprednisolone pulse therapy – Effect of prednisolone combined with immunosuppressants. *Ryumachi* 1999; **39**: 829–35 (in Japanese with English abstract).

18 Aihara Y, Miyamae T, Ito S *et al.* Mizoribine as an effective combined maintenance therapy with prednisolone in child-onset systemic lupus erythematosus. *Pediatr. Int.* 2002; **44**: 199–204.

19 Lehman TJ, Hanson V, Singsen BH, Kornreich HK, Bernstein B, King K. The role of antibodies directed against double-stranded DNA in the manifestation of systemic lupus erythematosus in childhood. *J. Pediatr.* 1980; **96**: 657–61.
20 Magil AB, Puterman ML, Ballon HS *et al.* Prognostic factors in diffuse proliferative lupus glomerulonephritis. *Kidney Int.* 1988; **34**: 511–17.
21 Kashiwazaki S, Kondo H, Koike T *et al.* Dose comparison of Bredinin on rheumatoid arthritis: a multicenter post-marketing surveillance study. *Jpn. J. Inf.* 1996; **16**: 269–89 (in Japanese with English abstract).
22 Kamata K. A study of immunosuppressive effect of Bredinin. *Ishoku* 1982; **17**: 46–55 (in Japanese).
23 Inou T. Immunosuppressive effects of Bredinin on renal transplantation. Summarized by Bredinin research committee. *Ishoku* 1982; **17**: 547–61 (in Japanese with English abstract).
24 Matsumoto K, Morino T, Yano J *et al.* Comparative toxicity studies of mizoribine (Bredinin) and azathioprine in the beagle dogs. *Ishoku* 1982; **17**: 603–14 (in Japanese with English abstract).