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A multicenter trial of mizoribine compared with placebo in children with frequently relapsing nephrotic syndrome

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Background. The use of corticosteroids or cytotoxic/immunosuppressive agents such as cyclophosphamide, chlorambucil, and cyclosporine for the treatment of frequently relapsing nephrotic syndrome (FRNS) is limited because of their adverse effects. This study was conducted to evaluate the efficacy and safety of mizoribine, a relatively new immunosuppressive drug developed in Japan, in children with FRNS.

Methods. A double-blind, placebo-controlled, multicenter trial was carried out in children, from 2 to 19 years old, with FRNS. At relapse, patients were treated with prednisolone. According to a dynamic allocation, mizoribine or a placebo was concurrently administered to each patient. Prednisolone was gradually tapered and discontinued within 12 weeks. The test drug was maintained for 48 weeks. The primary end point was the relapse rate (the total number of relapses/the total treatment days for all patients). Analyses were performed according to the intention-to-treat principle.

Results. The primary analysis was conducted on 99 mizoribine- and 98 placebo-treated patients. The relapse rate was lower in the mizoribine group than in the placebo group (0.0055 vs. 0.0067; ratio 0.81, 95% CI, 0.61 to 1.05, $P = 0.12$). The hazard ratio of the cumulative remission rate between the two groups was 0.79 (95% CI, 0.57 to 1.08). In the subgroups consisting of patients 10 years old or younger, the relapse rate ratio between the mizoribine subgroup (54 patients) and the placebo subgroup (57 patients) was 0.66 (95% CI, 0.44 to 0.94, $P = 0.017$). The hazard ratio of the cumulative remission rate between the two subgroups was 0.56 (95% CI, 0.37 to 0.85, $P = 0.007$). Hyperuricemia was the most common adverse event with mizoribine (16%), but was transient.

Conclusions. Compared with the placebo, mizoribine significantly decreased the relapse rate and prolonged the remission period in the subgroup consisting of patients 10 years old or younger. This drug may be useful in young children with FRNS who generally relapse more frequently than older children.

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The introduction of corticosteroids markedly improved the prognosis of primary nephrotic syndrome in children. Once remission is achieved by steroid therapy, a significant proportion of patients exhibits subsequent relapses [1, 2]. Continuous or repeated use of steroids inevitably induces growth failure, cataracts, hypertension, and osteoporotic bone disease. Cytotoxic or immunosuppressive agents such as cyclophosphamide [3–5], chlorambucil [6], cyclosporine [7, 8], and levamisole [9] are used to induce lasting remission, thereby sparing the patient further exposure to corticosteroids. These drugs have acute and chronic side effects, including leukopenia, infection, hemorrhagic cystitis, alopecia, infertility, and late occurrence of malignancy for cyclophosphamide, central nervous system disorders, gonadal toxicity, and risk of hematological malignancy for chlorambucil, nephrotoxicity for cyclosporine [1, 2, 10–12], and neutropenia or rarely agranulocytosis in levamisole [9]. Effective and safe drugs are needed for the treatment of frequently relapsing nephrotic syndrome (FRNS).

Mizoribine (bredinin; 4-carbamoyl-1-β-D-ribofuranosylimidazolium) is an imidazole nucleotide originally isolated from a soil fungus, *Eupenicillium brefeldianum*, in Japan [13] and is an effective immunosuppressant [14, 15]. Mizoribine blocks the purine biosynthesis pathway [16, 17] and inhibits mitogen-stimulated T- and B-cell proliferation [16, 18–21]. Experimental studies showed that mizoribine favorably influenced the course of lupus nephritis in NZB/W F1 mice [21], antiglomerular basement membrane antibody-mediated nephritis in rabbits [22], and IgA nephropathy in ddy mice [23]. The clinical efficacy of mizoribine was first approved in renal transplant recipients [24]. Controlled trials conducted in Japan demonstrated that this drug prolonged graft survival without myelosuppression or hepatotoxicity [25]. Controlled studies further revealed the efficacy of mizoribine in the treatment of patients with lupus nephritis [26], rheumatoid arthritis [27], and steroid-resistant nephrotic syndrome [28].

Previously, we conducted an open-labeled trial in 44 children with FRNS (unpublished observations). Patients at the relapse were treated with prednisolone plus mizoribine. The steroid therapy was tapered and discontinued as described for the preceding relapse. Mizoribine was given at a daily dose of 2 to 5 mg/kg for 24 weeks, and the patients were followed up for an additional 24 weeks. The relapse rate (the number of relapses during the study period), compared between 48 weeks before and after the mizoribine therapy, was significantly decreased after the therapy (unpublished observations). No case showed any serious side effects. Therefore, we performed a multicenter, prospective, double-blind study to compare mizoribine with a placebo in the management of children with FRNS.

METHODS

Patients

A total of 210 patients from 57 centers throughout Japan were considered for enrollment in our study. Patients were eligible if they met all of the inclusion criteria as follows: primary nephrotic syndrome with three or more relapses in the past 12 months or two times or more in the past six months, and age over 2 and less than 18. Exclusion criteria included the following: previous treatment with cytotoxic drugs, other immunosuppressive agents, levamisole, or antihuman lymphocyte globulin in the recent six months; renal dysfunction (creatinine clearance of $\leq$50 mL/min/1.73 m^2 or serum creatinine of $\geq$1.5 mg/dL); secondary nephrotic syndrome; and pregnancy.

The definition and criteria of nephrotic syndrome used here are those defined by the International Study of Kidney Diseases in Children [29]. Remission was defined as a reduction in proteinuria to less than 10 mg/dL or qualitative resolution of proteinuria (negative by dipstick method) for three consecutive days with normal levels of serum albumin and cholesterol. Relapse was defined as the condition in which urinary protein in early morning urine exceeded 100 mg/dL or 2+ or above by the dipstick method for at least three consecutive days. Steroid-dependence was defined as the condition in which relapse occurred at the tapering of the steroid dosage or within 14 days after the termination of steroids.

Study design

We used centralized, computer-generated dynamic allocation to balance the following baseline characteristics between mizoribine group and placebo group: (*1*) the center, (*2*) age (10 years or younger, 11 years or older), (*3*) relapse frequency in the past, (*4*) history of immunosuppressive therapy including cyclosporine, and (*5*) steroid dependency (Table 1). The allocation code was not broken until all patients had completed the follow-up period. All eligible patients or their parents gave written informed consent to take part in the study. Appropriate ethics committee approval was obtained for each center.

Administration of the test drug was started when relapse occurred. After a relapse had been confirmed, patients were first treated with prednisolone at a dose of 1.0 to 2.0 mg/kg for consecutive 28 days. The dose was gradually reduced every week and discontinued within 12 weeks. Mizoribine or a matching placebo was started within two weeks after the initiation of the steroid therapy for relapse. According to the patient's height, the body weight was calculated based on the standard height-weight table in Japan 1988. Mizoribine or placebo at a daily dose of 4 mg/kg was orally given divided into two doses and was maintained for 48 weeks.

Patients were re-examined once a month after the

Table 1. Baseline characteristics of patients

	Mizoribine (N = 99)	Placebo (N = 98)	P value
Sex			
Male	72	70	0.84[a]
Female	27	28	
Age[e] *years*			
0–5	18	22	
6–10	36	35	0.46[b]
11–15	35	33	
16+	10	8	
Duration of illness *years*			
<1	19	21	
<3	24	29	0.46[b]
<6	28	22	
≥6	28	26	
Disease type[e]			
Steroid-dependent	90	90	0.82[a]
Nonsteroid-dependent	9	8	
Renal biopsy			
Not performed	57	55	
Performed[e]			0.76[a]
Minimal change	39	38	
Others[c]	3	5	
Number of previous relapses[e]			
2 times/24 weeks	26	31	
3 times/48 weeks	39	35	0.70[a]
≥4 times/48 weeks	34	32	
History of other immunosuppressants at least 6 months before the study[d] (some overlapped)			
Immunosuppressants[e]			
yes	39	42	0.62[a]
no	60	56	
Cyclosporine[e]			
yes	14	14	0.98[a]
no	85	84	
Cyclophosphamide			
yes	34	38	0.52[a]
no	65	60	

[a] χ^2 test
[b] Wilcoxon test
[c] Focal segmental glomerulosclerosis in 2 (1: mizoribine; 1: placebo) and mesangial proliferative glomerulonephritis in 6 (2: mizoribine; 4: placebo)
[d] Patients treated with immunosuppressants in the recent 6 months were excluded from the study
[e] Used as balancing factors for allocation

start of treatment. Urinalysis and blood pressure were checked at every visit. We assessed standard hematological and biochemical tests, including serum creatinine, blood urea nitrogen, and uric acid, every three months. The creatinine clearance and serum IgG level were also assessed every three months. Height and body weight were measured at the beginning and end of the study. The subjects were asked about adverse events and compliance at each visit. Withdrawal from treatment could result because of lack of efficacy, serious adverse events, or the patient's request. Whenever possible, those who had withdrawn were encouraged to attend the final study visit. Relapse during the study period was treated with prednisolone, similar to the protocol applied to the relapse at start of the trial. Patients were followed up for an additional six months after the discontinuation of treatment with the test drugs.

An interim analysis was performed at the point when half of the targeted number of patients was observed for 24 weeks. Continuation of the trial was determined under the recommendation of Independent Data Monitoring Committee (IDMC).

Statistical analysis

Based on the results of the phase II study, the accumulated remission rate after the 48-week treatment duration of test drugs was assumed to be 25% in the mizoribine group and 12% in the placebo group. The estimated sample size was 100 patients in each group at a 5% two-sided significance level with a power of 80%.

Analyses were performed according to the intention-to-treat principle. The primary end point of this study was the relapse rate, defined as the total number of relapses divided by the duration of observation for all patients in each treatment group. The ratio of the relapse rate of two groups was statistically tested by the permutation method. Four patients (two in each group) were excluded from this analysis because they did not achieve remission after allocation and continuous use of steroids with test drugs. The cumulative remission rate was calculated using the period until the first relapse with the Kaplan–Meier method and was compared with the log-rank test. In this analysis, the four patients who did not achieve remission after allocation were included and treated as they relapsed at the start of the observation period. The permutation test and log-rank test were adjusted for the number of previous relapses. The baseline characteristics of the patients between the two groups were compared by a chi-square test or Wilcoxon test. Since 0.2% of type I error was spent at the interim analysis, the two-sided significance level of the primary end point was decided to be 4.8% at the final analysis. All of the analyses were done with SAS software (version 6.10).

Relapse risk index

We searched for risk factors associated with predisposition to future relapse using logistic regression techniques. We included in the regression models those variables found in the baseline characteristics as follows: gender, age at time of treatment, duration of illness before treatment, steroid dependency, renal biopsy findings, the number of relapses in the past, dose of steroids taken by the patient at the latest relapse, and the history of other immunosuppressive therapy. As risk factors, three variables, the number of relapses in the past, duration of illness, and dose of steroids taken by a patient at the latest relapse were selected by the stepwise method. The relapse risk index was defined as the value of linear predictor calculated by the logistic regression method with these three variables for each patient. A larger value of the index indicated a higher risk of relapse.

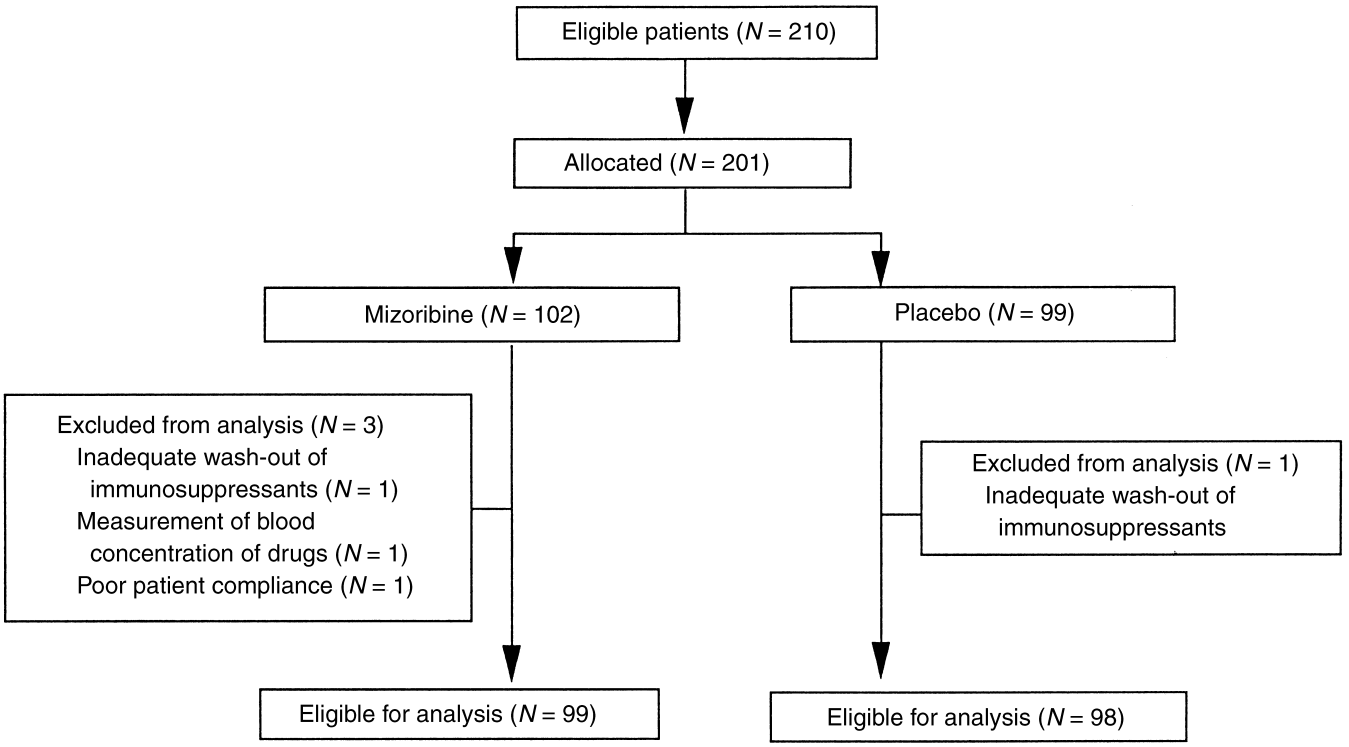

Fig. 1. Trial profile. Two patients in each group were excluded from the analysis of the relapse rates because they did not achieve remission after allocation.

RESULTS

Study population

Of 210 patients screened for eligibility, 9 patients did not meet the inclusion criteria (Fig. 1). Allocation was performed in the 201 patients. At the end of the trial, four patients were excluded because of a lack of eligibility evaluated before key opening. The remaining 197 patients (99 patients in the mizoribine group, 98 patients in the placebo group) were analyzed on an intention-to-treat policy. The administration of test drugs was discontinued early in five patients in the mizoribine group and nine patients in the placebo group and was maintained for more than 12 weeks in the remaining 183 patients (92.9%).

The groups were well matched in terms of baseline characteristics (Table 1). As shown, 180 of the 197 patients (91.4%) were steroid dependent. Renal biopsy revealed minimal changes in 77 of 85 patients (90.6%). Immunosuppressive or cytotoxic drugs were previously administered in 81 of the 197 patients (41.1%).

Clinical efficacy

The relapse rates were calculated for a total of 193 patients (97 patients in the mizoribine group and 96 patients in the placebo group), excluding 4 patients (2 in each group) who did not achieve remission after the initiation of the test drug (**Methods** section). The relapse rate was 0.0055 in the mizoribine group and 0.0067 in the placebo group. The ratio of the relapse rates was 0.81 (95% CI, 0.61 to 1.05, $P = 0.12$). The cumulative remission rate was calculated on a total of 197 patients (99 patients in the mizoribine group and 98 patients in the placebo group). In this analysis, the four patients who did not achieve remission after allocation were included and treated as they relapsed at the start of the observation period. The hazard ratio of cumulative remission rate between the two groups was 0.79 (95% CI, 0.57 to 1.08, $P = 0.13$; Fig. 2).

Subgroup analysis

To identify the subgroups in which the test drug was effective, the relapse rate ratio, hazard ratio of the cumulative remission rate, and 95% confidence intervals were calculated depending on each baseline characteristic (Table 2). In the subgroup of patients aged 10 years or younger, the relapse rate was significantly lower in the mizoribine group (54 patients) than the placebo group (57 patients, ratio 0.66, 95% CI 0.44 to 0.94, $P = 0.017$). The hazard ratio of the cumulative remission rate between the mizoribine and placebo subgroups consisting of children aged 10 years or younger was 0.56 (95% CI, 0.37 to 0.85, $P = 0.007$; Fig. 3). In this subgroup, the daily dosage of steroids in the study period was significantly lower in the mizoribine group than in the placebo

A Relapse rate

	N	Relapse rate	Relapse rate ratio	*P* value
Mizoribine	97	0.0055	0.81	0.12
Placebo	96	0.0067		

B Kaplan-Meier plot

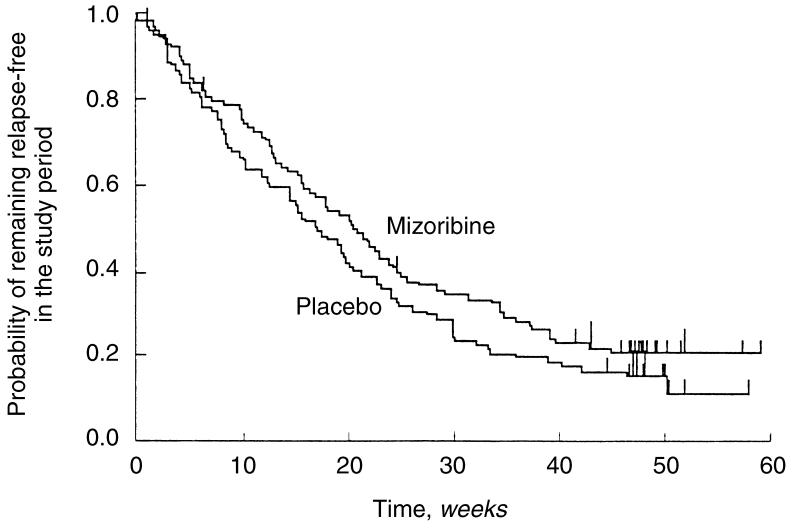

Fig. 2. Relapse rate (*A*) and cumulative remission rate (*B*) in mizoribine and placebo groups. Hazards ratio = 0.79; $P = 0.13$.

Table 2. Relapse rate ratio and 95% confidence intervals (CI) in the patients grouped according to the baseline characteristics

Baseline characteristic of patients	Relapse rate ratio (mizoribine/placebo)	95% CI
Total patients	0.81	0.61–1.05
Age		
≤10 years	0.66	0.44–0.94
≥11 years	1.12	0.73–1.72
Duration of illness		
<1 year	0.97	0.59–1.58
≥1 years	0.80	0.57–1.08
Number of previous relapse (times/weeks)		
2/24	0.72	0.42–1.11
3/48	0.96	0.60–1.55
≥4/48	0.78	0.46–1.22
History of immunosuppressants		
yes	0.74	0.45–1.16
no	0.87	0.61–1.21
Prednisolone dose at the last relapse *mg/kg/day*		
<0.4	0.80	0.54–1.14
≥0.4	0.85	0.55–1.27

group [0.65 ± 0.33 (SD) vs. 0.83 ± 0.44 (SD), $P = 0.017$]. There was no imbalance in baseline characteristics, including gender, duration of illness, steroid dependency, renal biopsy findings, number of previous relapses, or history of other immunosuppressive drugs, between the mizoribine and placebo subgroups consisting of children aged 10 years or younger. There was no significant difference in the relapse rate or cumulative remission period between the mizoribine group and the placebo group in the subgroups of patients aged 11 years or older.

Relapse risk index

To determine the reason that mizoribine showed a different efficacy according to the patients' age, the prevalence of relapses (relapse risk index) was investigated. The relapse risk index, calculated in each patient by multiple-regression models (**Methods** section), was similarly distributed in the mizoribine and placebo groups. Irrespective of which drug was given, mizoribine or placebo, the relapse risk was greater in the patients 10 years old or younger than in patients 11 years old or older (Fig. 4). There was no direct relationship between the patient's age and other factors such as the number of relapses in the past, the duration of illness, and steroid dosage at the latest relapse. It seems that the age collectively represents relapse risk generalized by these factors.

Safety

Adverse events were observed in 33 of 99 patients (33%) in the mizoribine group and 21 of 98 patients (21%) in the placebo group (Table 3). No significant differences were noted in the prevalence of adverse events except hyperuricemia, which occurred more fre-

A Relapse rate

	N	Relapse rate	Relapse rate ratio	*P* value
Mizoribine	54	0.0052	0.66	0.017
Placebo	57	0.0078		

B Kaplan-Meier plot

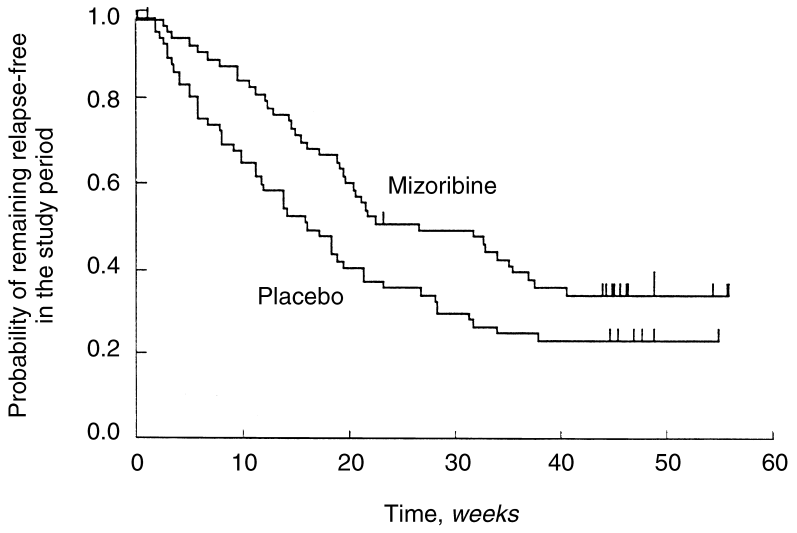

Fig. 3. Relapse rate (*A*) and cumulative remission rate (*B*) in the subgroup of patients aged 10 years or younger at the start of the trial. Hazards ratio = 0.56; $P = 0.007$.

quently in the mizoribine group (16 patients) than in the placebo group (4 patients). In the mizoribine group, hyperuricemia mostly occurred within eight weeks after the start of the trial. Mizoribine was discontinued in one patient because of the elevation of serum uric acid level to 21.2 mg/dL after the trial from 10.7 mg/dL before. The administration of the drug was temporarily suspended in two patients, and the dosage was reduced in one patient, while the initial dosage was maintained in the remaining 12 patients. The serum uric acid level gradually returned to the normal level in the 15 patients, and then the trial safely continued. Renal calculi were noticed in one patient of the mizoribine group who had renal stones in the past history but not on allocation. No serious adverse events were noted in either group.

DISCUSSION

This trial was planned to confirm and extend the promising results of the previous pilot (phase II) study. To obtain a large sample size estimated from the pilot study, the patients ranging from 2 years to 19 years were included in this trial. The primary analysis, conducted on 97 mizoribine- and 96 placebo-treated patients, showed that the relapse rate was lower in the mizoribine group than in the placebo group, but was not significantly different.

We analyzed the subgroups in which the test drug was effective. Dividing the group by patient age, one of the balancing factors for allocation, we found that the relapse rate and cumulative remission rate significantly improved after mizoribine administration in the subgroup consisting of patients aged 10 years or younger. There was no difference in the effect between mizoribine and placebo in the older subgroup, probably because the relapse risk of children with nephrotic syndrome decreases with age as a natural course. Our analysis using the relapse risk index showed that younger children had a higher risk of relapse. The age was not confounded with other factors. Previous studies pointed out that in children with steroid-sensitive nephrotic syndrome, a younger age is a predisposing factor to a lengthy relapsing course [30, 31]. Barratt et al analyzed baseline characteristics that might influence the relapse rate in patients with FRNS treated with cyclophosphamide [5]. They found that age at the time of treatment affected the time to relapse (longer remission in older children). Studies by Tarshish et al showed that patients with minimal-change nephrotic syndrome who were initially frequent relapsers tended to achieve an infrequent or nonrelapsing course with age [32]. Based on these observations, it is likely that the older subgroup of our study contained patients with a tendency toward long-term remission without immunosuppressive drugs.

For the treatment of frequent relapsing and steroid-

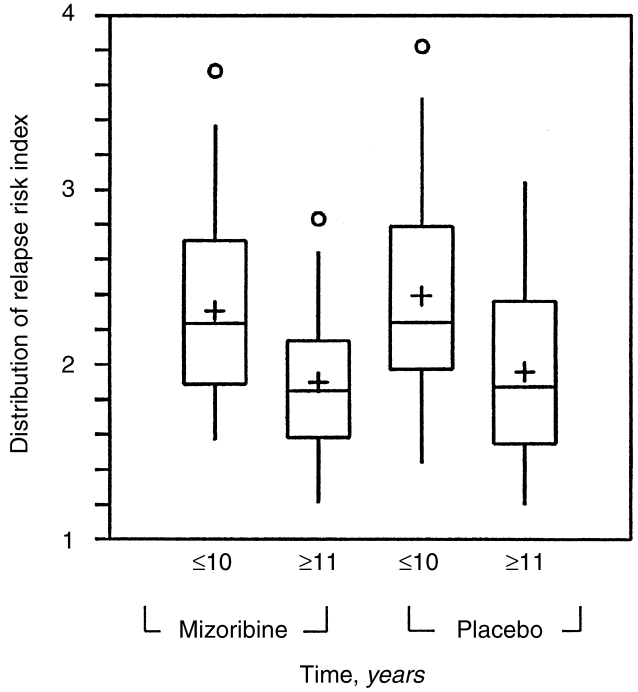

Fig. 4. Distribution of the relapse risk index in the subgroup of patients by age at the start of the trial.

Table 3. Adverse events

	Mizoribine (N = 99)	Placebo (N = 98)
Hyperuricemia	16	4
Hepatic dysfunction	9	9
Alopecia	3	2
Leukocytopenia	2	1
Leukocytosis	1	2
Anemia	0	1
Hyperlipidemia	0	2
Headache	2	0
Hypertension	1	0
Aphthous stomatitis	1	1
Nausea	1	0
Chest discomfort	1	0
Finger tremor	1	0
BUN increased	1	0
Serum creatinine increased	1	0
Hypocomplemetemia	1	0
Edema	0	1
Urticaria	0	1
Tonsillitis	1	0
Varicella	0	1
Urinary tract infection	1	0
Ureteral calculus	1	0

The occurrence of adverse events was 33% (33/99) in the mizoribine group and 21% (21/98) in the placebo group.

dependent nephrotic syndrome, cytotoxic or immunosuppressive drugs such as cyclophosphamide, chlorambucil, cyclosporine, and levamisole have been used to induce a lasting remission and prevent further exposure to steroids. There are few placebo-controlled studies to elucidate the relapse reducing effect of an immunosuppressive/cytotoxic drug in children with FRNS [9, 33]. A multicenter, double-blind study in Japan [33], including 108 children (under 15 years old) with FRNS, reported that during 24-week administration of the test drugs, 50% patients in the cyclosporine group (N = 49) and 70% patients in the placebo group (N = 59) developed relapse. The relapse rate during the study period was 0.0054 and 0.0099 in the cyclosporine group and the placebo group, respectively, thus reaching a relapse rate ratio of 0.55 (95% CI, 0.37 to 0.82, P = 0.003) in this placebo-controlled cyclosporine study. The data in our subgroup consisting of children aged 10 years or younger (relapse rate 0.0052 in mizoribine vs. 0.0078 in placebo, ratio 0.66, 95% CI, 0.44 to 0.94, P = 0.017) seemed to be close but not superior to the cyclosporine data. In the previous study comparing levamisole with placebo in children (N = 61) with steroid-dependent nephrotic syndrome, 55 and 87% patients had relapsed by the end of the 16-week test period [9]. Compared with the result in our subgroup (children ≤10 years old), the percentage in remission by the time to relapse in the levamisole study was low in the test drug group as well as the placebo group. Mizoribine may be compared with other immunosuppressive drugs in its ability to decrease the relapse rate. However, the baseline characteristics of patients, duration of the study period, and numbers of patients differed from study to study. A comparative study between immunosuppressive/cytotoxic agents would be desirable to clarify the superiority among these drugs.

The acute side effects and long-term risks of treatment with steroids or immunosuppressive/cytotoxic agents are a matter of concern. Our study as well as others showed that mizoribine is a safe drug [26–28]. The most common side effect was an increase in serum uric acid levels, which was noted in previous studies [28], as well as our open trial (unpublished observations) and this study. Hyperuricemia tended to develop in the early phase of the treatment and soon disappeared in most cases without stopping the drug. The increase in uric acid in the plasma can be derived from the action of this drug to inhibit the synthesis of guanine nucleotide [16, 17]. There were no significant differences in the prevalence of other adverse events between mizoribine and placebo groups. Low frequency in serious side effects of mizoribine may be attributed to its pharmaceutical characteristics. Mizoribine is metabolized to its monophosphate form by adenosine kinase [34] and competitively inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthase, enzymes required for de novo synthesis of guanine nucleotides from inosine monophosphate [16, 17]. Purine synthesis in lymphocytes depends exclusively on the de novo pathway. Thus, mizoribine strongly inhibits the proliferation of lymphocytes, but rarely affects the proliferation of other cells, where a

salvage pathway is used for purine synthesis. Uptake of mizoribine into nucleic acids does not occur because phosphorylation ceases at the level of monophosphates [35, 36]. Therefore, it is less likely that mizoribine shows such carcinogenicity as reported for azathioprine and cyclophosphamide. In conclusion, this drug can be worth considering as an alternative to the treatment with alkylating or immunosuppressive agents.

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REFERENCES

1. Barratt T, Clark G: Minimal change nephrotic syndrome and focal segmental glomerulosclerosis, in *Pediatric Nephrology* (3rd ed), edited by Holliday M, Barratt T, Avner E, Baltimore, Williams & Wilkins, 1994, pp 767–787
2. Broyer M, Meyrier A, Niaudet P, Habib R: Minimal changes and focal segmental glomerular sclerosis, in *Oxford Textbook of Clinical Nephrology*, edited by Cameron S, Davison A, Grunfeld J-P, Ritz E, Oxford, Oxford University Press, 1992, pp 298–339
3. Anonymous: Effect of cytotoxic drugs in frequently relapsing nephrotic syndrome with and without steroid dependence. *N Engl J Med* 306:451–454, 1982
4. Anonymous: Prospective controlled trial of cyclophosphamide therapy in children with nephrotic syndrome: Report of the International Study of Kidney Disease in Children. *Lancet* 2:423–427, 1974
5. Barratt TM, Bercowsky A, Osofsky SG, Soothill JF: Cyclophosphamide treatment in steroid-sensitive nephrotic syndrome of childhood. *Lancet* 1:55–58, 1975
6. Williams SA, Makker SP, Ingelfinger JR, Grupe WE: Long-term evaluation of chlorambucil plus prednisone in the idiopathic nephrotic syndrome of childhood. *N Engl J Med* 302:929–933, 1980
7. Niaudet P, Broyer M, Habib R: Treatment of idiopathic nephrotic syndrome with cyclosporin A in children. *Clin Nephrol* 35(Suppl 1):S31–S36, 1991
8. Tejani A, Butt K, Trachtman HMS, Rosenthal CJ, Khawar MR: Cyclosporin-induced remission of relapsing nephrotic syndrome in children. *J Pediatr* 111:1056–1062, 1987
9. British Association of Paediatric Nephrology: Levamisole for corticosteroid-dependent nephrotic syndrome in childhood. *Lancet* 337:1555–1557, 1991
10. Cameron S: Chlorambucil and leukemia. (letter) *N Engl J Med* 296:1065, 1977
11. Myers BD: Cyclosporine nephrotoxicity. *Kidney Int* 30:964–974, 1986
12. Mihatsch MJ, Antonovych T, Bohman S-O, Habib R, Helmchen U, Noel LH, Olson S, Sibley RK, Kemeny E, Feutren G: Cyclosporin nephropathy: Standardization of the evaluation of kidney biopsies. *Clin Nephrol* 41:23–32, 1994
13. Mizuno K, Tsujino M, Takeda M, Hayashi M, Atsumi K, Asano K, Matsuda T: Studies on bredinin. I. Isolation, characterization and biochemical properties. *J Antibiot (Tokyo)* 27:775–782, 1974
14. Gerber DA, Bonham CA, Thomson AW: Immunosuppressive agents: Recent developments in molecular action and clinical application. *Transplant Proc* 30:1573–1579, 1998
15. Suthanthiran M, Morris RE, Strom TB: Immunosuppressants: Cellular and molecular mechanisms of action. *Am J Kidney Dis* 28:159–172, 1996
16. Mitchell BS, Dayton JS, Turka LA, Thompson CB: IMP dehydrogenase inhibitors as immunomodulators. *Ann NY Acad Sci* 685:217–224, 1993
17. Turka L, Dayton J, Sinclair G, Thompson C, Mitchell B: Guanine ribonuleotide depletion inhibits T-cell activation: Mechanism of action of the immunosuppressive drug mizoribine. *J Clin Invest* 87:940–948, 1991
18. Hirohata S, Yanagida T: Inhibition of expression of cyclin A in human B cells by an immunosuppressant mizoribine. *J Immunol* 155:5175–5183, 1995
19. Kamada H, Inoue N, Takaoka Y, Nakagami K, Mori H, Nagai H: Effect of mizoribine on effector T cell-mediated immune responses in mice. *Biol Pharm Bull* 19:1136–1140, 1996
20. Kamada H, Itoh H, Shibata H, Koshio T, Hayashi A, Nakagami K: Inhibitory mechanism of mizoribine on the antibody production of mouse B cells stimulated with lipopolysaccharide. *Jpn J Pharmacol* 74:323–330, 1997
21. Kamata K, Okubo M, Uchiyama T, Masaki Y, Kobayashi Y, Tanaka T: Effect of mizoribine on lupus nephropathy of New Zealand black/white F1 hybrid mice. *Clin Immunol Immunopathol* 33:31–38, 1984
22. Kobayashi Y, Shigematsu H, Masaki Y: Modification of crescentic Masugi nephritis in the rabbit by Bredinin, a new immunosuppressant. *Virchows Arch B Cell Pathol Incl Mol Pathol* 43:103–119, 1983
23. Shimizu M, Shou I, Tsuge T, Abe M, Tomino Y: Effect of mizoribine on glomerulonephritis of early-stage IgA nephropathy in ddY mice. *Nephron* 79:67–72, 1998
24. Inoue T, Kusaba R, Takahashi I, Sugimoto H, Kuzuhara K, Yamada Y, Yamauchi J, Otsubo O: Clinical trial of Bredinin in renal transplantation. *Transplant Proc* 13:315–318, 1981
25. Kokado Y, Ishibashi M, Jiang H: Low dose cyclosporin, mizoribine and prednisone in renal transplantation: A new triple therapy. *Clin Transplant* 4:191–197, 1990
26. Homma M, Akizuki R, Yokohari R, Hashimoto H, Kashiwazaki S, Kondo H, Irimajiri S: Clinical evaluation of mizoribine on lupus nephritis: Multicenter single-blind comparative study with inactive placebo (in Japanese). *Rinsyo-Iyaku* 5:795–824, 1989
27. Shiokawa Y, Homma M, Shichikawa K, Miyamopto T, Hirose S, Nobunaga T, Mizushima Y, Sugawara S, Warabi H, Kondo H, Ogawa N: Clinical effectiveness of mizoribine on rheumatoid arthritis: A double-blind comparative study using lobenzarit disodium as a standard drug (in Japanese). *Igakunoayumi* 156:811–831, 1991
28. Koshikawa S, Sato M, Narita M, Sakai O, Nakajima M: Clinical evaluation of an immunosuppressive drug, mizoribine (HE-69) on steroid-resistant nephrotic syndrome: A multicenter double-blind comparison study with placebo (in Japanese). *Jin Tohseki* 34:631–650, 1993
29. International Study of Kidney Disease in Children: Primary nephrotic syndrome in children: Clinical significance of histopathologic variants of minimal change and of diffuse mesangial hypercellularity. *Kidney Int* 20:765–771, 1981
30. Trompeter R, Lloyd B, Hicks J, White R: Long-term outcome for children with minimal-change nephrotic syndrome. *Lancet* 1:368–370, 1985
31. Kabuki N, Okugawa T, Hayakawa H, Tomizawa S, Kasahara T, Uchiyama M: Influence of age at onset of the outcome of steroid-sensitive nephrotic syndrome. *Pediatr Nephrol* 12:467–470, 1998
32. Tarshish P, Tobin J, Bernsstein J, Edelmann C Jr: Prognostic significance of the early course of minimal change nephrotic syndrome: Report of the International Study of Kidney Disease in Children. *J Am Soc Nephrol* 8:769–776, 1997
33. Tohjoh S, Narita M, Koyama T, Miyahara M, Nagasawa T, Kitagawa T, Maki S, Ohhashi Y: Clinical evaluation of cyclosporin in the treatment of nephrotic syndrome: Multi-center double blind study. *Jin Tohseki* 37:565–608, 1994
34. Horie T, Mizushina Y, Takemura M, Sugawara F, Matsukage A, Yoshida S, Sakaguchi K: A 5′-monophosphate form of bredinin selectively inhibits the activities of mammalian DNA polymerases in vitro. *Int J Mol Med* 1:83–90, 1998
35. Sakaguchi K, Tsujino M, Yoshizawa M, Mizuno K, Hayano K: Action of bredinin on mammalian cells. *Cancer Res* 35:1643–1648, 1975
36. Mizushina Y, Matsukage A, Sakaguchi K: The biochemical inhibition mode of bredinin-5′-monophosphate on DNA polymerase beta. *Biochim Biophys Acta* 1403:5–11, 1998