Oral mizoribine pulse therapy for patients with steroid-resistant and frequently relapsing steroid-dependent nephrotic syndrome

Yukihiko Kawasaki, Mitsuaki Hosoya, Schogo Kobayashi, Shinichirou Ohara, Noriko Onishi, Ai Takahashi, Masato Isome and Hitoshi Suzuki

Department of Pediatrics, Fukushima Medical University School of Medicine, 1 Hikariga-oka, Fukushima City, Fukushima 960-1295, Japan

Abstract

Background. We investigated the efficacy of oral mizoribine pulse therapy (mizoribine-pulse) for cyclosporin (CyA)-dependent, steroid-resistant nephrotic syndrome (SRNS) and frequently relapsing, steroid-dependent nephrotic syndrome (FR-SDNS).

Methods. One child with CyA-dependent SRNS and eight children with CyA-dependent FR-SDNS were treated with mizoribine-pulse (daily dose: 10 mg/kg; maximum total dose 500 mg). We compared clinical manifestations before and after mizoribine-pulse, and studied the changes in serum mizoribine concentration in each patient on days when mizoribine was administered.

Results. Four patients had no subsequent relapses (responders). Two of the four responders discontinued prednisolone and CyA, the other two discontinued CyA. Although each of the five other patients (non-responders) experienced single subsequent relapses, the dosages of prednisolone and CyA after mizoribine-pulse were decreased significantly compared with before mizoribine-pulse. The peak blood concentration of mizoribine in the responders was higher than in the non-responders (3.6 ± 0.9 vs 1.8 ± 0.4 μg/ml).

Conclusions. Mizoribine-pulse may be effective for some patients with CyA-dependent SRNS and FR-SDNS.

Keywords: children; clinical; mizoribine oral pulse therapy; nephrotic syndrome; steroid-resistant nephrotic syndrome

Introduction

Patients with steroid-resistant nephrotic syndrome (SRNS) and frequently relapsing, steroid-dependent nephrotic syndrome (FR-SDNS) often experience significant morbidity due to the complications of their disease, such as acute renal failure, infection, thrombosis and progression to end-stage renal disease. There is also a significant risk of toxicity secondary to the regimens used to treat these patients [1–3].

There have already been several reports on the efficacy of cyclosporin (CyA) in the treatment of SRNS and FR-SDNS. Our hospital has used CyA to achieve remission in patients with SRNS, but it has been difficult to wean many of them off CyA without a relapse. Treatment options for patients whose FR-SDNS or CyA-dependent SRNS persists after receiving a 12 week course of cyclophosphamide include high-dose pulse steroids or initiation/continuation of CyA; however, both options have significant risks of side effects. Although CyA is effective in treating such difficult cases of nephrotic syndrome, its use can be complicated by long-term renal toxicity as well as persistence of relapses after withdrawal [2,3].

Mizoribine (MZB) is a new immunosuppressive agent that has been developed in Japan. Its actions have been reported to consist of selective inhibition of inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, which inhibits T-cell and B-cell proliferation [4]. In addition to being used after renal transplantation, it is used to treat childhood nephrotic syndrome, and its efficacy and safety have been demonstrated in recent studies [5]. However, MZB is used much less often clinically than cyclophosphamide or CyA.

According to preliminary reports, the peak blood concentration of MZB during regular oral use, i.e. 2.5–4.0 mg/kg daily divided into two or three doses, is <2.0 μg/ml [6]; however, it has been reported subsequently that peak blood concentrations ranging from...
3.0 to 6.0 μg/ml are required to effectively inhibit the human mixed lymphocyte reaction [7]. There have been studies on the efficacy of using MZB-pulse for systemic lupus erythematosus; however, there have been few reports on MZB-pulse used for SRNS and FR-SDNS [8]. In the present study, we evaluated the efficacy of MZB-pulse for CyA-dependent SRNS and CyA-dependent FR-SDNS.

**Subjects and methods**

The study was performed at Fukushima Medical University Hospital. All patients or their parents gave their informed consent.

**Patients**

One child (case 1) with CyA-dependent SRNS and eight children with CyA-dependent FR-SDNS were enrolled in the study. The mean period from the onset of nephrotic syndrome to CyA treatment start was 20.3 ± 19.8 months. All patients experienced frequent relapses associated with the tapering of CyA, so CyA could not be discontinued in them. Clinical features and laboratory data were investigated before and after MZB-pulse therapy, and the changes in each patient’s serum MZB concentration on the days when MZB was administered were monitored.

**Definitions**

The definitions of nephrotic syndrome, remission and relapse are those of the International Study of Kidney Disease in Children [6]. SRNS was defined as failure to respond, by either complete or partial remission, to an initial course of daily steroid therapy (prednisone 2 mg/kg per day) for 4–8 weeks. Steroid dependence was defined as recurrence of proteinuria following reduction of the dose of prednisolone, or within 2 weeks of discontinuation of therapy. FRNS was diagnosed when two or more relapses were documented within a 6 month period of the initial positive response, or when four or more relapses occurred within any 12 month period. CyA dependence was defined as recurrence of proteinuria when the dose of CyA was reduced, or within 2 weeks of discontinuation of CyA.

**Treatment**

**Steroid therapy.** Initial episodes and first relapses in our cohort were usually treated with prednisolone 60 mg/m² per day (maximum total dose: 60 mg/day) for 4 weeks, followed by 60 mg/m² every 2 days for 4 weeks; thereafter, prednisolone was tapered and discontinued over 4 weeks. At each subsequent relapse, patients were usually treated with up to 60 mg/m² of prednisolone a day for 4 weeks, followed by alternate-day tapering, according to their previous response to prednisolone, the dose of prednisolone at relapse and the clinical course before relapse.

**CyA treatment.** The dose of CyA (Sandimmune oral solution, CyA; Novartis Pharma K.K., Tokyo, Japan) at the start was 100–150 mg/m² daily (2.5–3 mg/kg body weight) in two divided doses, and was adjusted to a target blood trough level of 100 ng/ml, as measured by monoclonal antibody fluorescence polarization immunoassay.

**MZB-pulse.** MZB-pulse had been approved by our hospital ethics committee. MZB was given orally in a dose of 10 mg/kg body weight a day (maximum total dose: 500 mg) in three divided doses, on 2 days a week for 12–24 months.

**Tissue pathology**

The specimens of first kidney biopsy were examined by light and immunofluorescence microscopy. The specimen of the first kidney biopsy was fixed in 20% neutral formalin, and embedded in paraffin, sliced at 2–3 μm thickness, and stained with haematoxylin and eosin, or periodic acid–Schiff and periodic acid–methenamine-silver (PAM) reagent. The histological diagnosis of idiopathic nephrotic syndrome was based on the criteria of the International Study of Kidney Disease in Children [6]. CyA-induced tubulointerstitial lesions were defined as characteristic striped tubulointerstitial lesions (tubular atrophy associated with interstitial fibrosis).

**Laboratory examination**

Leukocyte counts (white blood cell counts), liver function tests, renal function tests and uric acid measurements were made each week after the start of MZB-pulse and then at monthly intervals. The mean doses of prednisolone and CyA were calculated as the mean daily dose of prednisolone and CyA during the 1 year before MZB-pulse and the 1 year after the start of MZB-pulse. The serum concentrations of MZB were measured by Asahi-Kasei Company.

**Statistics**

Data are expressed as mean values ± SD and were analysed with statistical analysis software (Stat View, Abacus Concepts, Berkeley, CA). Differences in laboratory findings between groups were assessed by means of Mann–Whitney rank sum test.

**Results**

**Comparison of the baseline characteristics of the nine children with CyA-dependent SRNS or CyA-dependent FR-SDNS (Table 1).**

Mean age at the onset of symptoms was 5.0 ± 2.4 years and the mean duration of illness before MZB-pulse was 68 ± 25 months. The number of relapses before MZB-pulse was 14.1 ± 4.6. All patients had previously been treated with prednisolone and CyA; five patients had been treated with cyclophosphamide, and one patient had been treated with MZB. Cyclophosphamide and MZB had not been effective in any of the patients.

Light microscopic examination of the initial biopsy specimens revealed minimal change in all patients, and examination of the second biopsy specimens, after CyA therapy, revealed chronic CyA-induced tubulo-interstitial lesions in all patients.
Comparison of the clinical features of the nine patients with SRNS or FR-SDNS and treatment before and after MZB-pulse (Table 1)

We determined that four patients (cases 1–4) had responded to MZB-pulse. CyA was discontinued 6 months after starting MZB-pulse in two of the four patients, and prednisolone and CyA were discontinued 3 and 5 months after the start of MZB-pulse in two of the four patients. All four patients who responded remained in remission. The other five patients (cases 5–9) did not remain in remission: each of them experienced one relapse during the observation period. In the non-responder group, the intervals until the relapses after MZB-pulse were 13 months in case 5, 15 months in case 6, 11 months in case 7, 12 months in case 8, and 11 months in case 9. In the responder and non-responder groups, the mean dosages of steroid and CyA after MZB-pulse were less than those before MZB-pulses.

Changes in serum MZB concentrations in the nine patients on days when MZB was administered (Figure 1)

The peak serum concentration of MZB was >3.0 μg/ml in the responder group and 1.4–2.3 μg/ml in the non-responder group, and the morning trough serum MZB concentration was 0–0.3 μg/ml. The MZB concentrations were higher in the responder group than in the non-responder group (3.6±0.9 vs 1.8±0.4 μg/ml).

Side effects of MZB-pulse

One patient developed uricacidaemia, which was well controlled with allopurinol. No patients developed leukopenia, liver dysfunction or alopecia.

Discussion

MZB is the first imidazole nucleoside to have been demonstrated to have biological activity. In the early 1980s, Japanese studies began to demonstrate that MZB had an inhibitory effect on T- and B-lymphocyte activity in vitro and to examine the pharmacokinetics and immunosuppressive effects of MZB in vivo [4]. Because of its inhibition of purine synthesis and relative lack of toxicity, during the past decade MZB has been increasingly used instead of azathioprine as part of immunosuppressive drug regimens in Japan. MZB was first approved in 1984 by the Ministry of Health and Welfare of Japan as a drug indicated for the prevention of rejection after renal transplantation, and the indications were extended to ‘primary nephritic syndrome’ [5].

Yoshioka et al. showed that MZB significantly decreased the relapse rate and prolonged remission in a subgroup of nephrotic syndrome patients ≤10 years old, and that it can be useful in young children, who
generally experience relapses more frequently than older children [5].

MZB is considered to be a relatively mild immuno-suppressant, with low toxicity, and it has recently been reported that concentrations of MZB effective enough to inhibit the human mixed lymphocyte reaction require peak blood concentrations ranging between 3.0 and 6.0 μg/dl. Tanaka et al. showed that MZB-pulse could be of benefit to some patients with flare-ups of lupus nephritis, as a therapeutic alternative to increasing the dosage of corticosteroids [9].

In the present study, four of the nine patients with steroid- and CyA-dependent nephrotic syndrome who received MZB-pulse therapy remained in remission, and the other five patients did not. There has been no report on changes in serum MZB concentration over time in patients receiving an MZB-pulse. Since the peak serum MZB concentrations in our study were in the 2.5–4.8 μg/dl range in the responders, and in the 1.3–2.3 μg/dl range in the non-responders, we speculated that it is important to raise the serum concentration of MZB sufficiently twice a week to prevent relapses of nephrotic syndrome.

The five patients in the non-responder group did not remain in remission, but each patient had only one relapse; the mean interval between remission and the one relapse was longer than before MZB-pulse therapy, and the dosages of steroids or CyA were lower. We previously found that the peak serum MZB concentration was <0.8 μg/dl during regular use of MZB (unpublished); thus the higher serum MZB concentrations achieved by pulse therapy may have been of a relative efficacy in the non-responder group.

The precise mechanism of the effect of MZB-pulse on the lymphocyte cell cycle remains a matter of speculation. To reduce toxicity, MZB-pulse was scheduled to be given only twice a week, which resulted in a lower cumulative dose per week than regular administration, as previously described [7].

It has recently been reported that 14-3-3 proteins, which are MZB-binding proteins, interact with the glucocorticoid receptor, and that the interaction may enhance the transcriptional activity of the receptor, suggesting a steroid-sparing effect of MZB [10]. That effect of oral MZB pulse therapy may have made it possible to taper prednisolone and CyA and prevent relapses of steroid- and CyA-dependent minimal-change nephrotic syndrome in our patients.

In conclusion, the results of our study suggest that MZB-pulse may be effective for some patients with steroid- and CyA-dependent nephrotic syndrome.

In the present study, the efficacy of MZB-pulse therapy was limited due to the small number of patients and the short observation period. Further study is necessary to confirm the efficacy of MZB-pulse therapy in long-term follow-up studies.

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Conflict of interest statement. None declared.

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