Mizoribine Synchronized Methotrexate Therapy should be Considered when Treating Rheumatoid Arthritis Patients with an Inadequate Response to Various Combination Therapies

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Abstract

Objective The objective of this study was to confirm the efficacy of low-dose mizoribine (MZR), an inhibitor of inosine monophosphate dehydrogenase, as part of synchronized methotrexate (MTX) therapy for rheumatoid arthritis (RA) patients with an inadequate response to various combination therapies of MTX, other synthetic disease-modifying anti-rheumatic drugs (DMARDs) and biological DMARDs.

Methods Low-dose MZR was administered to 56 uncontrolled RA patients being treated with MTX and various biological DMARDs. The observation period was 12 months, and the disease activity was evaluated based on the Disease Activity Score in 28 joints (DAS28)-ESR, Simplified Disease Activity Index (SDAI) and serum MMP-3 level.

Results All of the disease activity indices were significantly improved within three months, and the serum MMP-3 levels were also significantly decreased around four months after starting low-dose MZR therapy. No patients experienced any adverse effects.

Conclusion The present preliminary findings suggest that low-dose MZR therapy with MTX should be considered for the treatment of RA patients with an inadequate response to various combination therapies including MTX, other synthetic DMARDs and biological DMARDs or in whom increasing the dose of MTX is difficult for reasons such as adverse effects and complications.

Key words: DMARDs, methotrexate, mizoribine, rheumatoid arthritis, remission

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Introduction

Mizoribine (MZR) is an immunosuppressive agent that is similar to mycophenolate mofetil (MMF) in its inhibitory effect on inosine monophosphate dehydrogenase, a rate-limiting enzyme in the de novo pathway of nucleic acid synthesis (1). The immunosuppressive effect has been suggested to be due to the inhibition of T and B cell proliferation (2). MZR was first isolated from the culture media of Eupenicillium brefeldianum M-2166 in 1974 in Japan (3).

Since MZR was first approved for use in renal transplantation patients (4), it has been thought to be safe and well-tolerated compared with other immunosuppressants, and recent studies have demonstrated its usefulness in the treatment of rheumatoid arthritis (RA) (5, 6), systemic lupus erythematosus (SLE) (7, 8), nephrotic syndrome (9) and immunoglobulin A (IgA) nephropathy (10). A previous report

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Data collection and analyses

The patient medical records were reviewed to identify the main clinical features in terms of the efficacy and safety of MZR therapy. The collected data were the age, sex and treatment history. For the safety evaluation, information about adverse events and the duration of therapy were also recorded. The disease activity was assessed by the swollen and tender joint counts, the erythrocyte sedimentation rate, the C-reactive protein level, the global assessment of the disease activity by the patient, the global assessment of the disease activity by the investigator, the Disease Activity Score in 28 joints (DAS28) following the ACR guidelines and the Simplified Disease Activity Index (SDAI)/the Clinical Disease Activity Index (CDAI) proposed by the ACR-EULAR (16), at the beginning of the study and at monthly intervals thereafter until the end of the study (12 months). In addition, we also assessed remission using the ACR/EULAR criteria, comparing our findings with the DAS28 remission results (17). Missing data were compiled using the last observation carried forward (LOCF) method.

Statistical analyses

The data are presented as the counts or means with the standard error (SE). In the statistical analyses, the paired t-test was used for comparisons between two groups, and the Wilcoxon signed-rank or two-way factorial analysis of variance (ANOVA) test was used for comparisons of the changes in the patients’ clinical course over time. The statistical analyses were performed by Kureha Special Laboratory Co. (Tokyo, Japan) using the SAS 9.4 software program (SAS Institute Inc., Cary, NC, USA). All p values were two-sided, and a p value of less than 0.05 was considered to indicate statistical significance.

Results

The patient characteristics at the start of the trial are summarized in Table 1. The mean age of patients was 61.3 (range: 48 to 74) years, and the mean disease duration was 7.5 (range: 0.6 to 14.9) years. Of the 56 patients evaluated by Steinbrocker’s radiological stage, 21 were stage I, 16 II, 3 III and 16 stage IV (18). The mean dosage of steroids was 4.2 mg/day (range: 3.0-5.5 mg/day). The mean dose of MTX was 7.5 mg/week (range: 4.0-10.5 mg/week). Other synthetic DMARDs were used in 32% (18/56) of patients, and the numbers of patients who used DMARDs were as follows: SASP was used by 9 patients, bucillamine (BUC) by 1 patient and tacrolimus (TAC) was used by 8 patients. In addition, biological agents were also used by 32% (18/56) of the patients, and the breakdown showed that 10 patients were treated with infliximab (IFX), 4 patients with etanercept (ETN), 1 patient with tocilizumab (TCZ) and 3 patients with abatacept (ABT). There was no dosage increase of MTX, other synthetic DMARDs or biologics within three months before starting this study. There were
Table 1. Baseline Patient Characteristics.

| Characteristic                  | Value                        |
|---------------------------------|------------------------------|
| Number of females/males         | 42/14                        |
| Age, years (range)              | 61.3 (48 to 74)              |
| Steinbrocker stage              | I: 21, II: 16, III: 3, IV: 16|
| Steinbrocker class              | I: 27, 2: 28                 |
| Disease duration, years (range) | 7.5 (0.6 to 14.9)            |
| PSL dose, mg/day (range)        | 4.2 (3.0 to 5.5)             |
| Number of patients treated with PSL | 37                               |
| MTX dose, mg/week (range)       | 7.5 (4.0 to 10.5)            |
| Number of patients treated with MTX | 56                               |
| Number of patients treated with SASP | 9                                |
| Number of patients treated with BUC  | 1                                |
| Number of patients treated with TAC  | 8                                |
| Number of patients treated with IFX | 10                               |
| Number of patients treated with ETN | 4                                |
| Number of patients treated with TCZ | 1                                |
| Number of patients treated with ABT | 3                                |

MTX: methotrexate, PSL: prednisolone, SASP: salazosulfapyridine, BUC: bucillamine, TAC: tacrolimus, IFX: infliximab, ETN: etanercept, TCZ: tocilizumab, ABT: abatacept

![Figure 1](image1.png)

**Figure 1.** The efficacy as determined by the 28-joint count Disease Activity Score (DAS28) and the Simplified Disease Activity Index (SDAI). (A) The DAS28-ESR and (B) the SDAI. The values are the means ± standard error (SE). Low-dose mizoribine (MZR) pulse therapy led to significant improvement in all of the disease activity indices: each month vs. 0 months (baseline); * p <0.05, ** p <0.01; on both the Wilcoxon signed-rank test and paired t-test for the DAS28-ESR and the Wilcoxon signed-rank test for the SDAI.

![Figure 2](image2.png)

**Figure 2.** The effects of MZR pulse therapy on patients treated with only synthetic DMARDs or synthetic and biologic DMARDs. A: A comparison of DAS28-ESR between low-dose (50-100 mg/week, n=6), moderate-dose (101-200 mg/week, n=32) and high-dose (201-400 mg/week, n=15) MZR pulse therapy; the solid, large broken and small broken lines represent low-, moderate- and high-dose MZR therapy, respectively. B: The effects of MZR pulse therapy on patients treated with only synthetic DMARDs or synthetic and biologic DMARDs; the broken and solid lines indicate therapy with and without biologic DMARDs, respectively. A stratified analysis of MZR therapy showed no significant differences between each dosage group; two-way factorial ANOVA tests. MZR pulse therapy showed no significant differences in the efficacy between patients using synthetic DMARDs (n=35) and synthetic/biologic DMARDs (n=18); Wilcoxon signed-rank test.

three incomplete patients due to a worsening of the disease activity within 12 months. None of the patients showed any adverse effects due to the medication throughout this prospective study.

The DAS28-ESR value decreased significantly after 3 months compared with baseline (p<0.01), and the effect continued for at least 12 months (p<0.01) (Fig. 1A). The mean DAS28-ESR was improved from 3.50 at baseline to 2.41 at 12 months. The SDAI value also decreased significantly after 3 months compared with baseline (p<0.01), and the effect continued for 12 months (p<0.01). In particular, the mean SDAI was improved from 10.51 at baseline to 3.13 at 12 months (Fig. 1B).
Table 2. EULAR Response in the Trial.

| DAS28-ESR Baseline | 6 Months | 12 Months |
|---------------------|----------|-----------|
|                     | good     | moderate  | no   | good     | moderate | no   |
| ≤3.2                | 11       | 8         | 2    | 11       | 10       | 0    |
| >3.2 and ≤5.1       | 0        | 29        | 0    | 0        | 28       | 0    |
| >5.1                | 0        | 5         | 0    | 0        | 4        | 0    |
| Total               | (20.0%)  | (76.4%)   | (3.6%) | (20.7%)  | (80.8%)  | (0.0%) |

Table 3. DAS28-ESR Remission Rate in the Trial.

| Remission (DAS28-ESR<2.6) | 6 Months | 12 Months |
|---------------------------|----------|-----------|
| Yes                       | 29 (52.7%) | 36 (67.9%) |
| No                        | 26 (47.3%) | 17 (32.7%) |

Table 4. The Stratified Analysis of the Trial Based on DAS28-ESR.

| DAS28-ESR | 0 | 2M | 4M | 6M | 8M | 10M | 12M |
|-----------|---|----|----|----|----|-----|-----|
| Remission(≤2.6) | 55.4% | 40.0% | 33.3% | 52.7% | 51.6% | 48.4% | 67.9% |
| Low (>2.6 but ≤3.2) | 8.9% | 16.7% | 26.7% | 29.1% | 25.8% | 9.7% | 10.1% |
| Moderate (>3.2 but ≤5.1) | 28.6% | 40.0% | 36.7% | 18.2% | 22.6% | 38.7% | 22.0% |
| High (>5.1) | 7.1% | 3.3% | 3.3% | 0.0% | 0.0% | 3.2% | 0.0% |
| Chi-squared test | p=0.042 | p=0.007 | p=0.001 | p=0.523 | p=0.016 | p=0.001 |

To confirm the MZR pulse dose-dependent efficacy, high-dose (201-400 mg/week), moderate-dose (101-200 mg/week) and low-dose (50-100 mg/week) MZR pulse regimens were statistically compared (Fig. 2A). The stratified analysis of MZR therapy showed no significant differences between each dosage group using a two-way factorial ANOVA test. The DAS28-ESR of patients with or without biologics was then statistically analyzed to confirm the effect of biologic DMARDs. MZR pulse therapy showed efficacy in patients using both only synthetic DMARDs and synthetic/biologic DMARDs without any significant differences as determined by a Wilcoxon signed-rank test (Fig. 2B). Both the EULAR response (Table 2) and DAS28 remission rate (Table 3) improved at 6 and 12 months compared with the baseline, respectively. A stratified analysis based on the DAS28-ESR showed that the proportion of patients with remission/low disease activity increased while the proportion of patients with moderate/high disease activity decreased (Table 4). In particular, the proportion of patients in remission was significantly higher at 12 months into the study than at baseline (0 months), as determined by the chi-squared test (p<0.001).

We next assessed the changes in the serum MMP-3 levels, and found that there was a statistically significant reduction in the serum MMP-3 levels after 4 months (p<0.05 to 0.01) (Fig. 3). The mean serum MMP-3 levels dropped from 233.9 ng/mL at baseline to 140.3 ng/mL at 12 months. The serum MMP-3 levels in female patients were higher than in males; however, the differences were not significant, and both groups showed significant reductions in the serum MMP-3 levels (data not shown).

We also showed that the efficacy of MZR was not dependent on prednisolone (PSL) compared with the dose between the just before starting this study and at the end points of the observation period. The mean dosage of PSL significantly decreased during the observation period, from 4.23 mg/day at baseline to 3.39 mg/day at 12 months (p<0.001) (Fig. 4). The dosages of MTX and biologics did not significantly change throughout the observation period (data not shown). Both SASP and BUC were discontinued in all patients, and NSAIDs were discontinued in seven patients (data not shown).

**Discussion**

There are some limitations associated with this study due to its non-controlled nature and the small number of patients enrolled. However, this prospective study demonstrated that low-dose intermittent MZR pulse therapy synchronized with MTX led to re-remission in patients with relapsing RA, which led to significant improvements in the DAS28-ESR and SDAI after three months (Fig. 1). The serum MMP-3 levels were also significantly reduced after four months (Fig. 3). Those data are similar to the results of several pre-
vicious reports (12, 13). The efficacy of low-dose intermittent MZR therapy was previously proven in patients with RA that showed an inadequate response to MTX monotherapy or to IFX, a chimeric antibody against tumor necrosis factor-alpha (13, 19). Our study indicates that MZR pulse therapy was effective against RA in patients with an inadequate response to combination therapy, such as MTX plus other DMARDs or biological agents such as SASP, BUC, TAC, IFX, ETN (a dimeric fusion protein against tumor necrosis factor-alpha), TCZ (a humanized monoclonal antibody against the interleukin-6 receptor [IL-6R]) and ABT (a cytotoxic T lymphocyte-associated antigen 4 immunoglobulin fusion protein). While biological agents are extremely effective in improving the disease activity of RA, they did not affect the efficacy of MZR pulse therapy (Fig. 2).

The doses of MTX and steroids used during the study period were not significantly increased throughout the course, and the observed improvement in the disease activity in this study was due solely to the addition of MZR pulse therapy (Fig. 4). These present and previous findings regarding concurrent medications suggest that MZR pulse therapy might be able to salvage RA patients with a wider insufficient response to MTX alone, MTX with other DMARDs and MTX with various biological agents. Additionally, there were no adverse effects throughout the duration of this study in patients of any age, and efficacy was also shown in patients treated with low-dose MTX therapy. We also demonstrated that MZR pulse therapy was effective against MTX-resistant RA, regardless of the MTX amount (4-10.5 mg/week). These results further indicated that MZR pulse therapy could be used safely even in aged patients and patients expected to have an adverse reaction if the MTX dose were increased.

Based on the efficacy, convenience, safety and cost of this treatment, the present preliminary results suggest that low-dose MZR therapy synchronized with MTX should be considered for the treatment of RA patients with an inadequate response to various combination therapies including MTX, other DMARDs and biological agents, or in whom increasing the dose of MTX is difficult due to adverse effects and complications.

The authors state that they have no Conflict of Interest (COI).

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![Figure 3. The effects of MZR pulse therapy on the serum MMP-3 levels. There were statistically significant reductions in the serum MMP-3 levels after four months. Each month vs. 0 months (baseline); * p <0.05, ** p <0.01; Wilcoxon signed-rank test.](image1)

![Figure 4. The alteration of steroid dosage throughout this prospective study. The dose of prednisolone (PSL) was significantly decreased during the observation period (p <0.001); Wilcoxon signed-rank test.](image2)
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