# Comparison of Tacrolimus and Mizoribine in a Randomized, Double-blind Controlled Study in Patients with Rheumatoid Arthritis

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***ABSTRACT***. ***Objective.*** To compare the efficacy and safety of tacrolimus and mizoribine in patients with rheumatoid arthritis (RA).

***Methods.*** Adult patients with RA with an insufficient response to at least one disease modifying antirheumatic drug (DMARD) were randomized to receive 28 weeks of double-blind treatment with tacrolimus 3 mg once daily or mizoribine 50 mg three times daily. The primary efficacy endpoint was the American College of Rheumatology 20% (ACR20) response. Safety was evaluated by adverse events.

***Results.*** A total of 204 patients were enrolled for study (103 in the tacrolimus group, 101 in the mizoribine group). Significantly more patients receiving tacrolimus achieved an ACR20 response compared with mizoribine (48.5 vs 10.0%, respectively; $p = 0.001$). Tacrolimus was also superior to mizoribine in ACR50 and ACR70 response rate, tender and painful joint counts, swollen joint counts and patient and physician assessments of pain, disease activity, and patient's physical function assessment based on the Modified Health Assessment Questionnaire ($p < 0.001$). Adverse events were more frequent in the tacrolimus group than the mizoribine group (65.0 vs 59.4%); however, there were no statistically significant differences between treatment groups.

***Conclusion.*** Tacrolimus improves RA symptoms to a significantly greater extent than mizoribine in patients with RA inadequately controlled with at least one prior DMARD. Tacrolimus has the potential to be a useful and highly effective treatment for RA. (First Release Sept 1 2006; J Rheumatol 2006;33:2153–61)

*Key Indexing Terms:*
TACROLIMUS
MIZORIBINE
RHEUMATOID ARTHRITIS
RANDOMIZED CONTROLLED TRIAL

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*Supported by Astellas Pharma Inc., Tokyo, Japan (formerly Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). Drs. Kawai, Hashimoto, Kondo, Murayama, Kiuchi, and Abe have served as consultants to and/or received honoraria from Astellas Pharma Inc., the manufacturer of tacrolimus.*

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*Accepted for publication May 10, 2006.*

Rheumatoid arthritis (RA) is a chronic progressive inflammatory disease characterized by swelling and pain in multiple joints. The primary lesion of RA is considered to be in synovial membranes. Synovial cell proliferation gradually affects surrounding cartilage and bone, frequently leading to the disruption or deformation of joints. Physical symptoms other than those of joints include subcutaneous nodules or vascular inflammation, skin ulcers, and pulmonary fibrosis. RA is therefore considered not a joint disease but a systemic disease. Immune abnormalities underlie the pathology of RA, and the correction of these abnormalities is currently considered optimal therapy for RA.

Tacrolimus is a compound produced by *Streptomyces tsukubaensis*. The efficacy of this immunosuppressant has been consistently demonstrated in the field of transplantation involving the liver, kidney, heart, lung, and pancreas[1–6]. The clinical usefulness of tacrolimus has also been confirmed in atopic dermatitis (an allergic disease)[7,8] and intractable generalized myasthenia gravis (an autoimmune disease)[9,10].

Tacrolimus exerts its immunosuppressive effects primarily by interfering with the activation of T cells. Tacrolimus inhibits intranuclear translocation of nuclear factors in the cytoplasm of activated T cells by binding to tacrolimus-bind-

ing proteins in T lymphocytes and inhibiting calcineurin. Tacrolimus also suppresses the production of cytokines such as interleukin 2 (IL-2), IL-3, IL-4, interferon-γ, and tumor necrosis factor-α, resulting in immunosuppressive effects[11,12].

The usefulness of tacrolimus for RA has been demonstrated in double-blind, placebo-controlled, parallel-group studies[13–15], but has not yet been reported in controlled studies compared with active drug.

Mizoribine, an imidazole nucleoside, was first isolated from a soil fungus in 1974, and has proved to have beneficial effects in both clinical and experimental transplantation. Mizoribine blocks the proliferation of T cells to a variety of stimuli[16]. Mizoribine has been shown to have beneficial effects in the treatment of RA in several open trials and a placebo-controlled clinical trial[17]. Mizoribine has been available for 8 years and is indicated for the treatment of RA in patients with an insufficient response to at least one disease modifying antirheumatic drug (DMARD).

We carried out a double-blind, parallel-group, controlled study that compared tacrolimus with mizoribine in Japanese patients with RA with an insufficient response to at least one DMARD.

## MATERIALS AND METHODS

*Patients*. Patients were enrolled in the study if they met all the following eligibility criteria: (1) RA of at least 6 months' duration and diagnosed according to the American College of Rheumatology (ACR) 1987 criteria[18]; (2) age ≥ 20 and < 65 years; (3) insufficient response to at least 6 months' treatment with auranofin (3–6 mg/day) and sodium aurothiomalate (10–25 mg/day for 2-4 weeks initially, then 25–50 mg/day) or at least 3 months with one or more DMARD approved in Japan other than the aforementioned agents, tacrolimus, or mizoribine; and (4) currently active RA.

Active RA was defined as (1) erythrocyte sedimentation rate (ESR) ≥ 30 mm/h or C-reactive protein (CRP) concentration ≥ 1.0 mg/dl; (2) at least 6 of 48 joints assessed as tender or painful with pressure; and (3) at least 3 of 46 joints assessed as swollen. The "other DMARD" used in this study included methotrexate (MTX; n = 102, < 8 mg/wk), salazosulfapyridine (n = 64, < 1 g/day), bucillamine (n = 31, < 300 mg/day), actarit (n = 21, < 300 mg/day), D-penicillamine (n = 11, 600 mg/day), and lobenzarit disodium (n = 3, 240 mg/day). All drugs were used in the approved dose in Japan. Patients were excluded from the study if they met any of the following criteria: (1) previous treatment with tacrolimus or mizoribine; (2) surgery within 1 year prior to the initiation of the study and residual effects from surgery; (3) oral steroid use > 7.5 mg/day (prednisolone equivalent) within 4 weeks prior to initiation of the study; (4) use of ≥ 2 nonsteroidal antiinflammatories within 4 weeks prior to initiation of the study; (5) severely reduced bone marrow function (white blood cell count ≤ 3000/mm$^3$) or impaired renal function; (6) history of pancreatitis, impaired glucose tolerance, heart disease, serious hepatic disorders (alanine aminotransferase/aspartate aminotransferase levels ≥ 2.5 times the upper limit of normal), hyperkalemia, malignant tumors, severe infectious disease, marked drug hypersensitivity; and (7) women who were pregnant, lactating, or not using adequate birth control.

The institutional review board at each study site approved the protocol, and all patients gave written informed consent prior to enrollment. Patient eligibility was confirmed during a 1 to 4 week screening period during which patient characteristics (age, medical history, complications, etc.), concomitant therapy, disease activity, vital signs (blood pressure, body weight), hematology, blood chemistry, urinalysis, immunology, and electrocardiography were assessed. The randomization was stratified by either insufficient response to MTX or DMARD other than MTX. Eligible patients were randomized to receive either tacrolimus or mizoribine within each stratum. All patients and investigators were blinded to study medication until completion of the study. Patients in the tacrolimus group received three 1 mg capsules once daily after dinner and a mizoribine placebo tablet 3 times daily after each meal, and patients in the mizoribine group received a 50 mg tablet 3 times daily after each meal and 3 tacrolimus placebo capsules once daily after dinner for 28 weeks.

*Criteria for evaluation*. The primary efficacy endpoint was the ACR20 response. Secondary efficacy endpoints included ACR20 success (ACR20 responders who completed 28 weeks of treatment), ACR50 and ACR70 response, and change from baseline in individual ACR component scores [tender joint counts, swollen joint counts, CRP levels, ESR, patient's assessment of pain on a 100 mm visual analog scale (VAS), patient and physician global assessment of disease activity (100 mm VAS), and patient's physical function assessment based on the Modified Health Assessment Questionnaire (MHAQ)[19]] at the end of treatment. ACR20, ACR50, and ACR70 response are defined as a patient who achieves a 20%, 50%, or 70% improvement from baseline in tender and swollen joint counts and in at least 3 of the 5 other ACR components[20]. Clinical improvement was also assessed using the Disease Activity Score in 28 joints (DAS28), a validated composite index with measures of joint tenderness and swelling, global disease activity, and serum levels of acute-phase reactants[21-23]. Safety was evaluated in terms of adverse events, including concomitant symptoms, abnormal changes in laboratory values, and infection.

Discontinuation criteria were as follows: patient request; CRP < 1.0 mg/dl and ESR 30 mm/h at baseline; concurrent administration of prohibited drugs; experience of adverse reaction; constant elevated serum creatine (≥ 0.3 mg/dl from baseline), fasting blood sugar ≥ 125 mg/dl or blood sugar ≥ 200 mg/dl; $HbA_{1c}$ ≥ 6.5%; marked worsening of RA; or no response.

*Statistical methods*. All statistical tests were 2-sided with a type I error rate of 0.05. For efficacy analyses, intention to treat with last observation carried forward was used. Cochran-Mantel-Haenszel test stratified by DMARD failure status was used to compare the end of treatment difference between the groups for ACR20 response rate, ACR20 success rate, ACR50 and ACR70 response rate, and the change from baseline in individual ACR component scores. Confidence intervals (95%) of the treatment difference (tacrolimus-mizoribine) were also calculated for those variables. Fisher's exact test was used to compare the incidence of adverse events between the treatment groups.

## RESULTS

*Patient demographics and disposition*. A total of 204 patients were randomized to double-blind treatment with tacrolimus or mizoribine. This consisted of 102 patients with insufficient response to MTX (54 in the tacrolimus group, 48 in the mizoribine group) and 102 patients with insufficient response to DMARD other than MTX (49 in the tacrolimus group, 53 in the mizoribine group). Thirty-nine tacrolimus patients and 68 mizoribine patients discontinued the study; the reasons for discontinuation were "adverse events" in 22 (12 in the tacrolimus group, 10 in the mizoribine group), "no response/worsening" in 71 (19 in the tacrolimus group, 52 in the mizoribine group), "adverse events" and "no response/worsening" in one patient (mizoribine group), and "other" in 13 patients (8 in the tacrolimus group, 5 in the mizoribine group; Figure 1).

The efficacy analysis was based on the full analysis set, which included all randomized patients who received at least one dose of study medication and had ACR20 evaluation (total 203 patients). The safety analysis set was defined as all

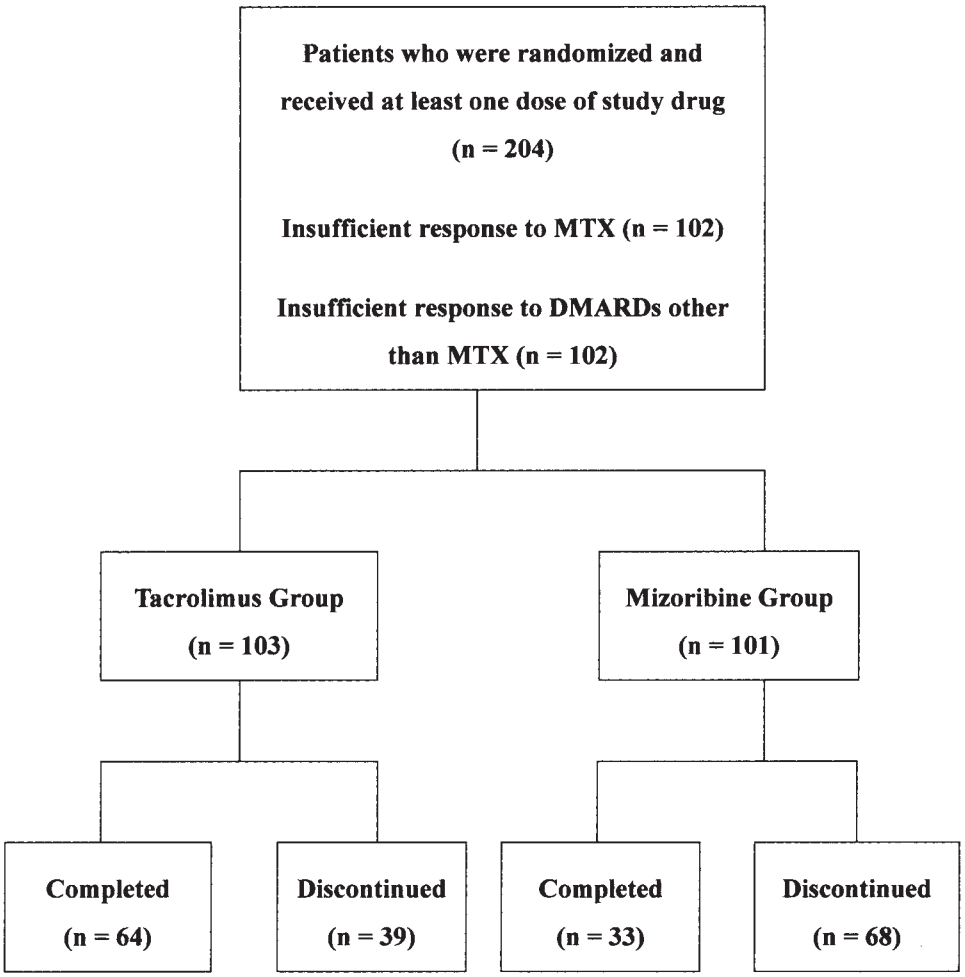


*Figure 1*. Disposition of patients in the trial. Completed: patients completing 28 weeks of treatment.

randomized patients who received at least one dose of study medication. Table 1 presents patient demographics, baseline disease characteristics, and prior therapy in the full analysis set.

*Efficacy*. The ACR20 response rate in the full analysis set was significantly higher in the tacrolimus group (48.5%; 50/103 patients) compared with the mizoribine group (10.0%; 10/100 patients; $p < 0.001$). The 95% CI for the difference between the groups was 28.1%–50.2%. In the subgroup analysis based on response to DMARD, the ACR20 response rate was 40.7% (22/54) in the tacrolimus group and 4.2% (2/48) in the mizoribine group in patients with an insufficient response to MTX, and 57.1% (28/49) in the tacrolimus group and 15.4% (8/52) in the mizoribine group in patients with an insufficient response to DMARD other than MTX (Figure 2A).

The ACR20 success rate (i.e., the proportion of patients achieving ACR20 and completing 28 weeks of therapy) in the full analysis set was significantly higher in the tacrolimus group (43.7%; 45/103 patients) than in the mizoribine group (8.0%; 8/100 patients; $p < 0.001$; Figure 2B). The 95% CI for the difference between the groups was 25.3%–47.0%. In the subgroup analysis based on response to DMARD, the ACR20 success rate was 37.0% (20/54) in the tacrolimus group and 4.2% (2/48) in the mizoribine group in patients with an insufficient response to MTX, and 51.0% (25/49) in the tacrolimus group and 11.5% (6/52) in the mizoribine group in patients with an insufficient response to DMARD other than MTX.

The ACR50 response rate in the full analysis set was also significantly higher in the tacrolimus group (27.2%; 28/103 patients) compared with the mizoribine group (2.0%; 2/100 patients; $p < 0.001$; Figure 2C). The 95% CI for the difference between the groups was 16.4%–34.4%. In the subgroup analysis based on response to DMARD, the ACR50 response rate was 22.2% (12/54) in the tacrolimus group and 2.1% (1/48) in the mizoribine group in patients with an insufficient response to MTX, and 32.7% (16/49) in the tacrolimus group and 1.9% (1/52) in the mizoribine group in patients with an insufficient response to DMARD other than MTX.

In addition, the ACR70 response rate in the full analysis set was 11.7% (12/103 patients) in the tacrolimus group and 0%

*Table 1*. Demographics and baseline characteristics of patients in the full analysis set (FAS).

| | Tacrolimus Group, n = 103 | Mizoribine Group, n = 100 |
|---|---|---|
| Sex. no. female (%) | 91 (88.3) | 89 (89.0) |
| Age, yrs, mean ± SD | 51.1 ± 8.8 | 52.5 ± 8.4 |
| Weight, kg, mean ± SD | 52.8 ± 7.8 | 53.2 ± 8.2 |
| Steinbrocker stage (progression of RA) | | |
| I | 8 (7.8) | 6 (6.0) |
| II | 29 (28.2) | 21 (21.0) |
| III | 32 (31.1) | 25 (25.0) |
| IV | 34 (33.0) | 48 (48.0) |
| Steinbrocker class (functional status in RA) | | |
| 1 | 14 (13.6) | 15 (15.0) |
| 2 | 73 (70.9) | 63 (63.0) |
| 3 | 15 (14.6) | 22 (22.0) |
| 4 | 1 (1.0) | 0 (0.0) |
| Duration of RA, mo, mean ± SD | 120.7 ± 98.7 | 126.8 ± 104.9 |
| Anamnesis, no. (%) | 54 (52.4) | 63 (63.0) |
| Complications, no. (%) | 59 (57.3) | 65 (65.0) |
| Tender or painful joint count, mean ± SD | 13.6 ± 7.2 | 12.4 ± 6.4 |
| Swollen joint count, mean ± SD | 10.2 ± 6.2 | 9.6 ± 5.3 |
| ESR, mm/h, mean ± SD | 62.8 ± 28.1 | 60.4 ± 25.8 |
| CRP, mg/dl, mean ± SD | 3.37 ± 3.05 | 3.54 ± 2.52 |
| Treatment of RA | 103 (100.0) | 100 (100.0) |
| Immunosuppressants, no. (%) | 96 (93.2) | 90 (90.0) |
| Corticosteroids, no. (%) | 84 (81.6) | 78 (78.0) |
| NSAID, no. (%) | 93 (90.3) | 99 (99.0) |
| DMARD, no. (%) | 3 (2.9) | 2 (2.0) |
| Physiotherapy, no. (%) | 3 (2.9) | 3 (3.0) |

CRP: C-reactive protein level; ESR: erythrocyte sedimentation rate; NSAID: nonsteroidal antiinflammatory drug; DMARD: disease modifying antirheumatic drug.

(0/100 patients) in the mizoribine group. The 95% CI for the difference between the groups was 5.6%–18.1%. In the subgroup analysis based on response to DMARD, the ACR70 response rate was 7.4% (4/54) in the tacrolimus group in patients with an insufficient response to MTX, and 16.3% (8/49) in the tacrolimus group in patients with an insufficient response to DMARD other than MTX.

The greater efficacy of the tacrolimus group over the mizoribine group was highly significant for all individual ACR component scores ($p < 0.001$; Table 2).

The DAS28 response rate in the full analysis set was significantly higher in the tacrolimus group (60.8%; 62/102 patients) compared with the mizoribine group (25.5%; 25/98 patients; $p < 0.0001$). In the subgroup analysis based on response to DMARD, the DAS28 response rate was 50.9% (27/53) in the tacrolimus group and 14.9% (7/47) in the mizoribine group in patients with an insufficient response to MTX, and 71.4% (35/49) in the tacrolimus group and 35.3% (18/51) in the mizoribine group in patients with an insufficient response to DMARD other than MTX (Figure 2D).

*Safety*. The overall incidence of adverse events was numerically higher in the tacrolimus group (65.0%; 67/103 patients) than in the mizoribine group (59.4%; 60/101 patients); however, the difference was not statistically significant ($p = 0.471$). The discontinuation rate due to adverse events was similar between the groups, 11.7% (12/103) in the tacrolimus group and 9.9% (10/101) in the mizoribine group.

*Symptomatic adverse events*. There were 88 symptomatic adverse events in 49 patients (49/103; 47.6%) in the tacrolimus group, and 48 adverse events in 35 patients (35/101; 34.7%) in the mizoribine group. In the tacrolimus group, gastrointestinal system disorders were the most frequent, with 21 events reported, followed by 20 events classified as skin and limb disorders, and 10 events classified as body as a whole, general disorders. In the mizoribine group, the most frequent adverse events were skin and limb disorders, with 18 events reported, followed by 11 cases of gastrointestinal system disorders and 5 events classified as whole-body/general disorders (Table 3). The majority of adverse events in both groups were transient.

*Laboratory data*. Abnormal changes in laboratory values occurred in 24.3% of patients (25/103) in the tacrolimus group and 21.8% of patients (22/101) in the mizoribine group. There was no significant difference between the groups ($p = 0.740$). Overall, the incidence of abnormal renal function tests was higher in the tacrolimus group compared with the mizoribine group. Abnormal tests included increased blood urea nitrogen (8.7% in the tacrolimus group vs 2.0% in the mizoribine

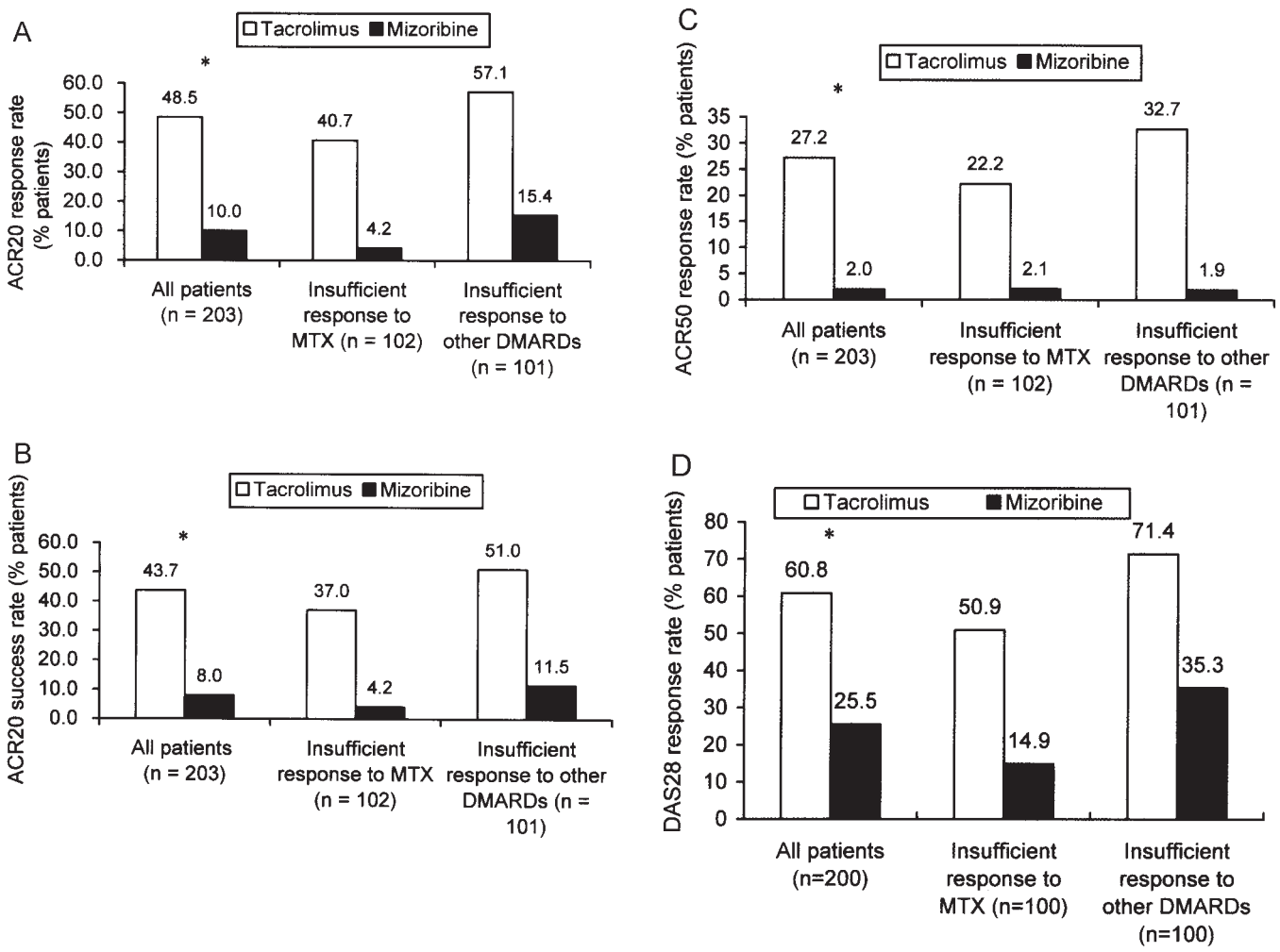


*Figure 2*. Percentage of patients in the full analysis set (n = 200) achieving ACR 20% and 50% improvement in prespecified criteria[17] following 28 weeks of treatment with tacrolimus or mizoribine. A. ACR20 response rate. B. ACR20 success rate. C. ACR50 response rate. D. DAS28 response rate. *p < 0.001, Cochran-Mantel-Haenszel test stratified by DMARD failure status.

group), increased creatinine (1.9% vs 0%), increased uric acid (1.9% vs 0.0%), and increased ß$_2$-microglobulin (2.9% vs 3.0%). Two patients in the tacrolimus group experienced increases in creatinine level; in a female patient, serum creatinine level increased from 0.8 mg/dl at baseline to 1.2 after 24 weeks. In a male patient serum creatinine level increased from 0.8 mg/dl at baseline to 1.3 after 12 weeks. Increased $HbA_{1c}$ was 2.0% in the tacrolimus group and increased blood glucose was 1.0% in the mizoribine group. Discontinuations due to abnormal changes in laboratory values were similar between the groups: 1.0% (1/103 patients) in the tacrolimus group and 1.0% (1/101 patients) in the mizoribine group (Table 3). Except for the following patients, the abnormal changes in laboratory values in the tacrolimus group resolved within the period of observation after discontinuation of tacrolimus. One patient with hepatic function abnormalities in the tacrolimus group did not recover; AST and ALT were 60 and 71 at baseline, 75 and 94 after 15 days, 94 and 100 after 22 days, and 73 and 100 after 64 days (47 days from discontinuation), respectively. Similarly, a patient with renal function abnormalities in the mizoribine group did not recover: ß$_2$-microglobulin was 2.4 at baseline, 4.1 after 15 days, 4.0 after 85 days, and 2.8 after 183 days (98 days from discontinuation).

*Infections*. The incidence of infection was 17.5% (18/103 patients) in the tacrolimus group and 24.8% (25/101 patients) in the mizoribine group, with no significant differences between the groups (p = 0.232). These infections generally resolved in both treatment groups upon treatment discontinuation. Two cases with severe infection (urinary infection, cellulitis) in the tacrolimus group and one case with severe infection (pericarditis) in the mizoribine group were hospitalized and treated with intravenous antibiotics such as meropenem, ceftriaxone, and clarithromycin, respectively.

## DISCUSSION

This 28-week randomized, double-blind study compared the

*Table 2.* Change from baseline to end of treatment for individual ACR component scores of the full analysis set of patients.

| ACR Component | Drug | Baseline | Change from Baseline | p* |
|---|---|---|---|---|
| Tender/painful joint count | Tacrolimus | 13.6 ± 7.2 | –5.5 ± 6.7 | < 0.001 |
| | Mizoribine | 12.4 ± 6.4 | –1.0 ± 7.8 | |
| Swollen joint count | Tacrolimus | 10.2 ± 6.2 | –4.0 ± 5.5 | < 0.001 |
| | Mizoribine | 9.6 ± 5.3 | –1.0 ± 5.6 | |
| CRP, mg/dl | Tacrolimus | 3.37 ± 3.05 | –1.13 ± 2.89 | < 0.001 |
| | Mizoribine | 3.54 ± 2.52 | +1.29 ± 3.09 | |
| ESR, mm/h | Tacrolimus | 62.8 ± 28.1 | –12.6 ± 24.2 | < 0.001 |
| | Mizoribine | 60.4 ± 25.8 | +11.7 ± 23.5 | |
| Patient assessment of pain, mm | Tacrolimus | 58.2 ± 23.4 | –21.0 ± 30.5 | < 0.001 |
| | Mizoribine | 61.2 ± 22.9 | +6.4 ± 23.9 | |
| Patient global assessment of disease activity, mm | Tacrolimus | 59.7 ± 23.5 | –20.9 ± 30.6 | < 0.001 |
| | Mizoribine | 66.3 ± 21.5 | +1.8 ± 23.7 | |
| Physician global assessment of disease activity, mm | Tacrolimus | 59.8 ± 18.6 | –22.0 ± 26.1 | < 0.001 |
| | Mizoribine | 61.5 ± 16.9 | –0.3 ± 21.3 | |
| Modified HAQ | Tacrolimus | 0.85 ± 0.52 | –0.21 ± 0.42 | < 0.001 |
| | Mizoribine | 0.85 ± 0.62 | +0.17 ± 0.46 | |

* Cochran-Mantel-Haenszel test stratified by DMARD failure status. ACR: American College of Rheumatology; CRP: C-reactive protein level; ESR: erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire.

efficacy and safety of tacrolimus 3 mg/day and mizoribine 150 mg/day in adult patients with RA with an insufficient response to at least one DMARD. The ACR20 response rate was significantly higher in the tacrolimus group compared with the mizoribine group ($p < 0.001$). The ACR20 response rate was higher in both the tacrolimus group and the mizoribine group for the subgroup of patients with an insufficient response to DMARD other than MTX.

In this study, 33% to 48% of enrolled patients had Steinbrocker stage IV RA (Steinbrocker stage I being mild and IV severe). One reason that a relatively higher percentage of patients with advanced stages of RA took part in this study might be the enrollment of patients with an insufficient response to DMARD including MTX. However, the baseline characteristics of the 2 groups of patients were similar, so the reason for this result remains to be elucidated.

The ACR20 success rate and ACR50 and ACR70 response rate were also significantly higher in the tacrolimus group than in the mizoribine group ($p < 0.001$ for both). The combination of these results indicates the superior efficacy of tacrolimus compared with mizoribine.

The standard dosage regimen of mizoribine approved by the Ministry of Health, Labour and Welfare in Japan is 150 mg, i.e., oral administration of 50 mg mizoribine 3 times daily, for adult patients with RA. Although up to 300 mg daily mizoribine was allowed, efficacy rates of 150 mg and 300 mg mizoribine were 21.1% and 25.5%, respectively (from the mizoribine package insert). Thus, we decided to use the standard dose of mizoribine in our study.

The study included 54 patients in the tacrolimus group and 48 patients in the mizoribine group who had experienced an insufficient response to MTX prior to enrollment. The highest approved dose of MTX in Japan is 8 mg/week, whereas it is 15 mg/week or more in the US[24]. It may be possible that some patients with insufficient response to MTX might have responded with a higher dose of MTX and then would not be considered DMARD failures. Since the clinical utilization of DMARD such as MTX is considerably different between the US and Japan, data in our study may not be immediately adaptable to countries outside of Japan.

Progression of RA may lead to destruction of joints, resulting in markedly decreased quality of life as a result of impaired joint function. Greater emphasis is being placed on quality of life and patients' physical function assessment (by MHAQ) in the treatment of RA[25]. Our study showed significantly greater improvements in MHAQ scores in the tacrolimus group compared with the mizoribine group and baseline scores. Tacrolimus improved all indicators according to the ACR criteria; in particular, a vast improvement was obtained in physical function assessed by patients via the MHAQ. This is considered meaningful as many patients in the study had insufficient response to MTX, which is regarded as the first-line drug for treatment of RA[26].

The number of patients discontinuing treatment due to "no response/worsening" was greater in the mizoribine group (52 patients) than in the tacrolimus group (19 patients). Patients discontinuing treatment due to "no response/worsening" within 8 weeks in the mizoribine group totalled 19.2% (10/52) and this incidence was about 2-fold greater than in the tacrolimus group [10.8% (2/19)]. Although it is known that efficacy of mizoribine is usually observed at 2 to 4 months, patients discontinuing treatment due to "no response/worsening" within 16 weeks in the mizoribine group totalled 80.8% (42/52), while patients discontinuing treatment due to "no response/worsening" within 16 weeks in the tacrolimus group were 52.6% (10/19). CRP and ESR values, which are objec-

*Table 3.* Incidence of adverse events in the 2 treatment groups (safety analysis set). Values in parentheses are percentages, rounded and reported to the nearest whole number. More than 1 adverse event could be reported by a single patient.

| Adverse Events, No. of Patients (%) | Tacrolimus Group, n = 103 | Mizoribine Group, n = 101 | p* |
|---|---|---|---|
| Any system | 67 (65.0) | 60 (59.4) | 0.471 |
| Symptomatic events | 49 (47.6) | 35 (34.7) | 0.066 |
| Nervous system | 4 (3.9) | | 0.121 |
| Headache | 2 (1.9) | | 0.498 |
| Psychiatric | 1 (1.0) | | 1.0 |
| Visual | 4 (3.9) | 1 (1.0) | 0.369 |
| Dysacusis | 1 (1.0) | | 1.0 |
| Respiratory | 6 (5.8) | 3 (3.0) | 0.498 |
| Pharynx pain | 3 (2.9) | | 0.246 |
| Cardiovascular | 2 (1.9) | 2 (2.0) | 1.0 |
| Heart beat | 1 (1.0) | | 1.0 |
| Vascular (extracardiac) | | 1 (1.0) | 0.495 |
| Gastrointestinal | 21 (20.4) | 11 (10.9) | 0.083 |
| Stomach ache | 4 (3.9) | 1 (1.0) | 0.369 |
| Stomach dysphoria | 3 (2.9) | 1 (1.0) | 0.621 |
| Diarrhea | 3 (2.9) | 2 (2.0) | 1.000 |
| Stomatitis | 1 (1.0) | 3 (3.0) | 0.366 |
| Urinary system | 1 (1.0) | | 1.0 |
| Musculoskeletal | 2 (1.9) | 3 (3.0) | 0.681 |
| Skin and appendages | 20 (19.4) | 18 (17.8) | 0.858 |
| Eczema | 1 (1.0) | 6 (5.9) | 0.064 |
| Itch | 3 (2.9) | 1 (1.0) | 0.621 |
| Body as a whole | 10 (9.7) | 5 (5.0) | 0.284 |
| Fever | 3 (2.9) | 2 (2.0) | 1.000 |
| Lesions | 2 (1.9) | | 0.498 |
| Laboratory data | 25 (24.3) | 22 (21.8) | 0.740 |
| Blood urea nitrogen increased | 9 (8.7) | 2 (2.0) | 0.058 |
| Creatinine | 2 (1.9) | | 0.498 |
| Uric acid | 2 (1.9) | | 0.498 |
| ß$_2$-microglobulin increased | 3 (2.9) | 3 (3.0) | 1.0 |
| Urinary NAG increased | 3 (2.9) | 8 (7.9) | 0.132 |
| Hemoglobin decreased | 1 (1.0) | 5 (5.0) | 0.117 |
| Platelets increased | 1 (1.0) | 5 (5.0) | 0.117 |
| Triglycerides increased | 4 (3.9) | | 0.121 |
| Potassium increased | 3 (2.9) | 1 (1.0) | 0.621 |
| Magnesium decreased | 3 (2.9) | | 0.246 |
| Infections | 18 (17.5) | 25 (24.8) | 0.232 |
| Cold | 8 (7.8) | 15 (14.9) | 0.125 |
| Upper airway infection | 2 (1.9) | 3 (3.0) | 0.681 |

* Fisher exact test. NAG: N-acetyl-3b-d-glucosaminidase.

tive indicators for RA activity, showed a lower response profile in the mizoribine group compared with the tacrolimus group. These results confirmed the efficacy results, showing a difference in the beneficial effects of treatment in favor of tacrolimus over mizoribine.

The dose of tacrolimus used in this study was 3 mg/day. For this dose, our results are consistent with those from dose-finding studies in Japan[13] and the US[15]. In a randomized, double-blind, placebo-controlled Japanese study[13], 212 patients were randomized to receive tacrolimus 3 mg/day, tacrolimus 1.5 mg/day, or placebo for 16 weeks. The resulting ACR20 response rates were 48.3%, 24.6%, and 14.1%, respectively, with a significantly higher response rate in the 3 mg/day group compared to the placebo group. Further, no statistically significant differences were noted in the incidence of adverse events among the 3 groups, and the optimal dosage was thus concluded to be 3 mg/day[13]. Similarly, in a randomized, double-blind, placebo-controlled US study[15], 268 patients with RA who were resistant to or intolerant of MTX were randomized to receive tacrolimus 5 mg/day, 3 mg/day, 1 mg/day, or placebo for 24 weeks. ACR20 response rates were 50%, 34.4%, 29%, and 15.5%, respectively, with significantly higher rates in 5 mg and 3 mg tacrolimus groups compared with the placebo group. However, 12.5%–15.6% of patients in the 5 mg and 3 mg tacrolimus groups discontinued the study because of adverse events. Of these, 28.1% of patients receiv-

ing tacrolimus 5 mg/day, 18.8% receiving tacrolimus 3 mg/day, 8.7% receiving tacrolimus 1 mg/day, and 7% receiving placebo had an increase in creatinine levels of more than 40% compared with baseline. Based on this result, the optimal tacrolimus dosage was estimated to be 1 to 3 mg/day.

In our study, a tacrolimus concentration > 10 ng/ml of blood was found in 10 patients and, of these, 7 experienced adverse events [decreased magnesium, diarrhea, vomiting, stomach ache, weight loss, urinary tract infection, elevated $HbA_{1c}$, herpes, epigastrium, headache, dizziness, skin eruption, flu, LDH increase, elevation of urinary NAG (N-acetyl-3b-d-glucosamine), elevated blood urea nitrogen, and retrobulbar neuritis]. However, these adverse events were not serious. It has been reported that tacrolimus blood concentrations correlate closely with adverse events in renal transplant recipients. Higher incidences of adverse events were observed in patients with higher tacrolimus blood concentrations (≥ 10 ng/ml)[27]. In our study, mean blood concentrations of tacrolimus in patients with adverse events were not different from those in patients without adverse events, so we did not find a clear relationship between tacrolimus blood concentrations and adverse events. This lack of correlation may be due to the small sample size and/or lower tacrolimus blood concentrations, as in the previous study of tacrolimus in elderly patients with RA[28].

In our study, the incidence of adverse events was numerically higher in the tacrolimus group than in the mizoribine group, but the difference was not statistically significant, and the discontinuation rates due to adverse events were comparable between the groups (tacrolimus 12 patients vs mizoribine 10 patients). One patient in the tacrolimus group did not recover from abnormal hepatic function and one patient in the mizoribine group did not recover from abnormal renal function. The most common events were gastrointestinal system disorders in the tacrolimus group and skin and limb disorders in the mizoribine group. The incidence of abnormal renal function, an adverse event specific to tacrolimus, was relatively high in the tacrolimus group. Since most of the adverse events in the tacrolimus group resolved, we suggest that tacrolimus can be used safely in clinical settings, as long as patients are closely monitored for clinical and laboratory adverse events, and there is an appropriate action plan in place if an event does develop. On the other hand, the incidence of impaired glucose tolerance as a specific adverse event associated with tacrolimus was not different between the 2 groups.

These results indicate that tacrolimus may be useful and highly valuable in treating patients with RA with insufficient response to existing DMARD including MTX.

## ACKNOWLEDGMENT

The members of the study group were as follows: Michio Minami, MD (Hokkaido Orthopedics Memorial Hospital, Hokkaido); Koji Taneichi, MD (Kitami Red Cross Hospital, Hokkaido); Makoto Nishinarita, MD (Taga General Hospital, Ibaraki); Hitoaki Okazaki, MD (Jichi Medical School Hospital, Tochigi); Kimihiro Suzuki, MD (National Defense Medical College Hospital, Saitama); Tsutomu Takeuchi, MD (Saitama Medical School Hospital, Saitama); Hirobumi Kondo, MD (Kitasato University School of Medicine, Kanagawa); Takashi Murayama, MD (Kanazawa Rehabilitation Hospital, Ishikawa); Yuichi Nishioka, MD (Yamanashi Prefectural Central Hospital, Yamanashi); Eisuke Shono, MD (Fukuoka University Hospital, Orthopedics, Fukuoka); Masakazu Kondo, MD (Kondo Clinic of Rheumatology and Orthopaedy, Fukuoka); Hideyuki Ikematsu, MD (Haradoi Hospital, Fukuoka); Shigeto Touma, MD (National Sagamihara Hospital, Kanagawa); Tadao Mitsui, MD (Aichi Medical University Hospital, Aichi); Takashi Kanno, MD (Ohta Nishinouchi Hospital, Fukushima); Takayuki Sumita, MD (Tsukuba University Hospital, Ibaraki); Tsukasa Matsubara, MD, PhD, Keisuke Hashimoto, MD, PhD (Matsubara Mayflower Hospital, Hyogo); Osamu Saiki, MD (National Hospital Organization, Osaka Minami Medical Center, Osaka); Seigaku Hayashi, MD (Fukui General Hospital, Fukui); Hisaaki Miyahara, MD (National Hospital Organization Kyushu Medical Center, Fukuoka); Katsuji Shimizu, MD (Gifu University School of Medicine and Gifu University Hospital, Gifu); Shoji Kumaki, MD (Hokushin General Hospital, Nagano); Kenji Maeda, MD (Ureshino Medical Center, Saga); Michishi Tsukano, MD (Kumamoto Orthopedics Hospital, Kumamoto); Fumihiko Imai, MD (Imai Medical Clinic, Saitama); Terufumi Fukui, MD (Fukui Clinic, Tokyo); Shigeru Hosaka, MD (Hosaka Clinic, Tokyo); Naoya Takashina, MD (Takashina Clinic, Tokyo); and Ryuji Nagamine, MD (Kyushu Rosai Hospital, Fukuoka, Japan).

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