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ORIGINAL ARTICLE

Efficacy and safety of additional use of tacrolimus in patients with early rheumatoid arthritis with inadequate response to DMARDs—a multicenter, double-blind, parallel-group trial

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Abstract In this trial, we investigated the safety and efficacy of tacrolimus used in addition to standard antirheumatic drugs in patients with rheumatoid arthritis. Tacrolimus 3 mg or placebo was orally administered once daily for 52 weeks in a double-blind manner to patients with early active rheumatoid arthritis receiving other disease-modifying antirheumatic drugs (DMARDs). A total of 123 patients were randomized to the tacrolimus group (61 patients) and to the placebo group (62 patients). In the tacrolimus group, 70.5% achieved a clinical response according to American College of Rheumatology (ACR) 20 criteria, whereas 45.2% in the placebo group did so ($P = 0.005$). The tacrolimus group also showed significant improvement in terms of the European League Against Rheumatism (EULAR) response criteria of "good or moderate" versus the placebo group (86.9 vs. 56.5%, respectively). Likewise, significantly more patients in the tacrolimus group versus the placebo group achieved remission of the Disease Activity Score in 28 joints (DAS28) (45 vs. 21%). The mean changes in the Total Sharp Score and erosion score were lower in the tacrolimus group, but the differences between the two groups were not significant. There was no significant difference between the two groups in the incidence of adverse events. Based on these results, we can conclude that the additional use of tacrolimus in patients with early rheumatoid arthritis with inadequate response to other DMARD treatments is useful, and this could become one of the treatment options for these rheumatoid arthritis patients.

Keywords DMARD · Randomized controlled trial · Rheumatoid arthritis · Tacrolimus

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Introduction

Tacrolimus is an immunosuppressive agent with a macrolide structure; it is produced by the streptomycete *Streptomyces tsukubaensis* and it specifically suppresses T-cell activation by inhibiting calcineurin. In Japan, this agent is approved for various indications in transplantations and autoimmune diseases as an injectable drug, as encapsulated formulations, and as a granulated powder. It is also approved as an ointment for the indication of atopic dermatitis, and as an ophthalmic solution for spring catarrh.

Rheumatoid arthritis is a disease characterized by destructive synovial joint inflammation, which causes not only pain but also interference with the activities of daily living and decreased quality of life because of functional impairment. The involvement of immunocompetent T cells

has been reported as the pathogenic mechanism of rheumatoid arthritis [1] and the activation of autoreactive T cells is one cause of inflammatory cytokine production.

Tacrolimus, by inhibiting T-cell activation, inhibits the production of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6, which are inflammatory cytokines that participate in the pathogenesis of rheumatoid arthritis [2–4]. The efficacy of tacrolimus against collagen arthritis and adjuvant arthritis, which are animal models of rheumatoid arthritis, has also been ascertained [5, 6]. Furthermore, this efficacy has been confirmed in various clinical trials in patients with rheumatoid arthritis; all the clinical trials in Japanese patients with rheumatoid arthritis were implemented as monotherapy trials [7, 8]. In North America, trial results have also suggested that this drug used additionally with methotrexate (MTX) is effective [9]. When a single disease-modifying antirheumatic drug (DMARD) has been found to be insufficient in rheumatoid arthritis patients, combined therapy with other antirheumatic drugs has been recommended [10]. Furthermore, because joint destruction due to rheumatoid arthritis has been reported to occur during the early stages of the disease [11], treatments are required to start at an earlier stage than has previously been reported.

We thus conducted a double-blind, placebo-controlled trial to investigate the efficacy and safety of tacrolimus used in addition to MTX, salazosulfapyridine, or bucillamine, which are the standard antirheumatic drugs used in Japan. We also investigated the efficacy of tacrolimus against the progression of joint destruction during a double-blind trial in patients with early rheumatoid arthritis with significant progression of bone destruction.

Patients and methods

Patients

Our inclusion criteria included patients aged between 20 and 65 years diagnosed with rheumatoid arthritis based on the definition (1987) [12] of the American College of Rheumatology (ACR), with a disease duration of at least 6 months but no more than 3 years, with at least 6 tender joints and at least 3 swollen joints, a C-reactive protein (CRP) level higher than 1.0 mg/dL, and an erythrocyte sedimentation rate higher than 30 mm/h, despite continuous administration of either MTX (6–8 mg/week), salazosulfapyridine (1 g/day), or bucillamine (100–300 mg/day). Our criteria also included patients with erosions that had been observed in more than 1 joint by X-ray films of hands and feet.

Our main exclusion criteria included patients who had previously received tacrolimus; patients corresponding to Steinbrocker's functional classification class 4; patients who had received biological products with an inhibitory effect on the progression of joint destruction (such as infliximab or etanercept) or leflunomide within 12 weeks before administration of the study drug; patients whose daily oral glucocorticoid dose exceeded 7.5 mg (prednisolone equivalent) within 4 weeks before administration of the study drug; patients who used at least two nonsteroidal anti-inflammatory drugs (NSAIDs) (oral or suppository) concomitantly within 4 weeks before administration of the study drug; and patients with the complications of renal dysfunction, pancreatitis/glucose intolerance, hyperkalemia, advanced liver dysfunction, cardiac disorders (such as ischemic cardiac disease, arrhythmia requiring treatment or cardiac failure), severe respiratory disorders, severe infectious disease, severe drug hypersensitivity disorders, or a malignant tumor.

Study protocol

This trial was conducted in 32 facilities from April 2006 to October 2008. All participating institutions received the approval of their governing institutional board or equivalent, and the trial was implemented in accordance with the ethical principles of the Declaration of Helsinki and the good clinical practice (GCP) guidelines, as well as relevant laws or regulations promulgated by the Institutional Review Boards for clinical trials. This trial is registered at ClinicalTrials.gov (NCT00319917).

After obtaining written consent, we allocated rheumatoid arthritis patients who met the inclusion criteria and did not fall under the exclusion criteria in a double-blind manner, and administered tacrolimus 3 mg or placebo orally once daily after dinner. The treatment period was 52 weeks.

As a general rule, we did not make any changes to the allocated dosage of MTX, salazosulfapyridine, or bucillamine, or to the dosage of oral glucocorticoid and/or NSAID (oral or suppository) during the treatment period. However, oral glucocorticoid doses were allowed to be changed if they were equal to or less than 7.5 mg prednisolone equivalent daily. The average prednisolone equivalent doses during the study period fluctuated between 4.3 and 4.5 mg daily in the tacrolimus group and between 4.9 and 5.7 mg daily in the placebo group. The differences in the glucocorticoid doses between these 2 groups were not statistically significant. The new administration of antirheumatic drugs or oral glucocorticoid was not allowed during the treatment period.

Clinical response was evaluated based on the ACR criteria (ACR20, ACR50, or ACR70) [13], Disease Activity Score in 28 joints (DAS28), and the European League Against Rheumatism (EULAR) response criteria [14, 15].

Remission was defined as DAS28 <2.6, in accordance with the EULAR definition [16]. Furthermore, the Modified Health Assessment Questionnaire (MHAQ) Score [17] was determined, from answers to the questionnaire given by the patients.

Joint destruction was assessed based on the change from baseline in the Total Sharp Score (0–448), erosion score, and joint-space narrowing score, using the modified Sharp van der Heijde scoring system [18, 19]. We obtained X-ray films of the hands and feet before the first administration of the study drug, then at week 28, and at week 52 (or at the time of discontinuation). Interpretation of the radiographic images was conducted by two people, who evaluated each radiographic image independently, without knowing the time the radiograph was taken or information relating to the disease activity or the treatment group. These scores were calculated based on the average of both readers' results.

Safety was evaluated by determining the incidence of adverse events (AEs) and laboratory abnormalities. We also measured the concentration of tacrolimus in whole blood, using the microparticle enzyme immunoassay technique in a blind manner, within a period (mean ± SD) of 12 ± 4 h after every administration of the study drug.

Statistical analysis

Efficacy analyses were based on the full analysis set (FAS), which consisted of all randomized subjects with rheumatoid arthritis who received at least one dose of the randomized study drug and who had at least one set of post-randomization data. Safety analyses were performed for all patients who received at least one dose of the randomized study drug.

All reported *P* values are two-sided; those less than 0.05 were considered to indicate statistical significance. ACR20, ACR50, and ACR70 responses, EULAR response criteria (good or moderate response), and DAS28 remission (cut-off point of DAS28 <2.6) were compared between the treatment groups using logistic regression analyses, adjusted for additional MTX use. For the radiographic end points, changes from baseline in the modified Sharp Scores (Total Sharp Score, erosion score, joint-space narrowing score) over 52 weeks were compared. The radiographic progression was extrapolated to impute 52-week values if patients had discontinued treatment after 28 weeks of study drug administration. Analyses of covariance were performed with the additional use of MTX as the covariate. For the other efficacy endpoints, means and proportions were compared using analyses of covariance and logistic regression analyses, respectively. Frequencies of adverse events (AEs) were compared with the use of Fisher's exact test. The coding dictionary for this study was the medical dictionary for regulatory activities (MedDRA), version 11.1. It was used to summarize AEs by system organ class and preferred term.

Results

Patient characteristics

Figure 1 shows the patient disposition in this trial. Of the 157 patients who gave their consent, 123 patients (61 in the tacrolimus group, 62 in the placebo group) were randomized. All of them received the study drug to be analyzed for safety and efficacy. Ninety-five patients (56 in the tacrolimus group, 39 in the placebo group) completed the treatment.

Twenty-eight patients discontinued the study drug (5 in the tacrolimus group, 23 in the placebo group). Reasons for

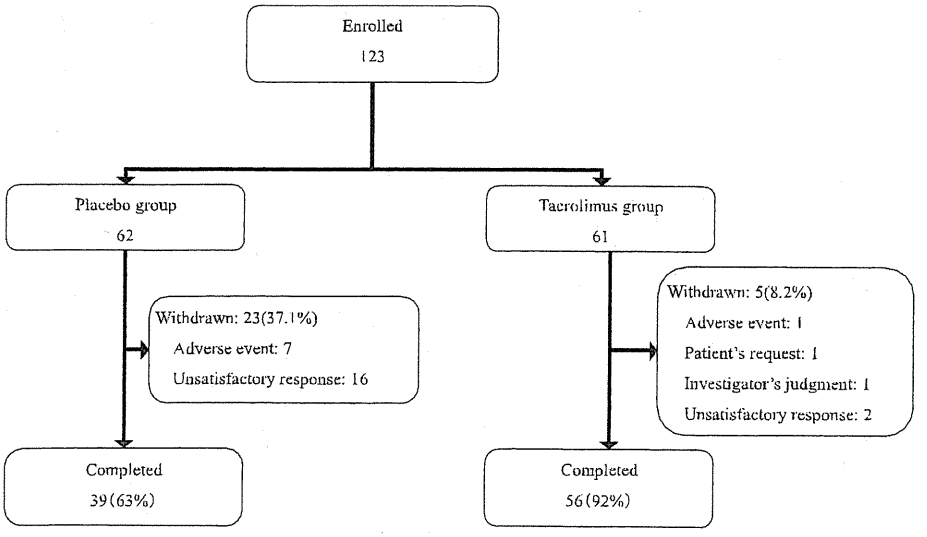

Fig. 1 Randomization, reasons for withdrawal, and numbers of patients who completed the trial

Table 1 Baseline characteristics of the patients

	Placebo ($n = 62$)	Tacrolimus ($n = 61$)	P value
Gender [number, female (%)]	50 (80.6)	55 (90.2)	0.202[a]
Age (years)	50.0 ± 11.6	47.1 ± 9.9	0.144[b]
Weight (kg)	56.4 ± 12.7	53.4 ± 8.1	0.130[b]
Disease duration (years)	1.7 ± 0.7	1.6 ± 0.7	0.276[b]
Steinbrocker stage [number (%)]			
I	1 (1.6)	0 (0.0)	0.792[c]
II	43 (69.4)	42 (68.9)	
III	16 (25.8)	19 (31.1)	
IV	2 (3.2)	0 (0.0)	
Steinbrocker class [number (%)]			
1	14 (22.6)	12 (19.7)	0.970[c]
2	45 (72.6)	48 (78.7)	
3	3 (4.8)	1 (1.6)	
4	0 (0.0)	0 (0.0)	
Concomitant therapy at baseline			
Glucocorticoids [number (%)]	31 (50.0)	32 (52.5)	0.857[a]
Prednisolone equivalent dose (mg/day)	4.9 ± 1.6	4.4 ± 1.9	0.328[b]
MTX [number (%)]	42 (67.7)	42 (68.9)	1.000[a]
Dose (mg/week)	7.3 ± 1.0	7.2 ± 1.0	
SASP [number (%)]	12 (19.4)	14 (23.0)	0.664[a]
Dose (mg/day)	1.0 ± 0.0	1.0 ± 0.0	
BUC [number (%)]	8 (12.9)	5 (8.2)	0.559[a]
Dose (mg/day)	150 ± 54	190 ± 55	
Tender joint count	11.7 ± 4.9	13.3 ± 7.5	0.152[b]
Swollen joint count	9.7 ± 5.1	10.9 ± 5.7	0.225[b]
CRP (mg/dL)	2.2 ± 2.1	1.8 ± 1.6	0.186[b]
ESR (mm/h)	46.3 ± 25.1	44.9 ± 21.7	0.738[b]
MHAQ	0.5 ± 0.4	0.5 ± 0.5	0.473[b]
Total Sharp Score	17.2 ± 20.6	17.7 ± 18.7	0.889[b]
Erosion score	8.3 ± 10.1	10.1 ± 10.9	0.354[b]
Joint-space narrowing score	8.8 ± 12.9	7.6 ± 9.5	0.537[b]
Yearly progression	10.8 ± 12.2	12.7 ± 15.2	0.433[b]
Rheumatoid factor (IU/mL)	128.6 ± 140.2	105.3 ± 137.4	0.353[b]

Plus-minus values are means ± SD

MTX methotrexate, *SASP* salazosulfapyridine, *BUC* bucillamine, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *MHAQ* modified health assessment questionnaire

[a] Fisher's exact test

[b] *t*-test

[c] Wilcoxon rank sum test

discontinuation in the tacrolimus group were "AE", "patient's request", and "investigator's judgment" in one patient each, and "unsatisfactory response" in two patients. In the placebo group, seven patients discontinued the trial due to "AEs" and 16 patients discontinued due to "unsatisfactory response".

Table 1 shows the patient characteristics. The age range (mean ± SD) was 47.1 ± 9.9 years in the tacrolimus group and 50.0 ± 11.6 years in the placebo group; disease duration (mean ± SD) was 1.6 ± 0.7 years in the tacrolimus group, and 1.7 ± 0.7 years in the placebo group. The Total Sharp Score according to the modified Sharp van der Heijde scoring system was 17.7 ± 18.7 in the tacrolimus group, and 17.2 ± 20.6 in the placebo group; yearly progression was 12.7 ± 15.2 in the tacrolimus group, and 10.8 ± 12.2 in the placebo group. There were no major differences between the two groups.

Of the antirheumatic drugs used, MTX was the most commonly used: in 42 patients (68.9%) in the tacrolimus group, and 42 patients (67.7%) in the placebo group; salazosulfapyridine was used by 14 patients (23.0%) in the tacrolimus group, and 12 patients (19.4%) in the placebo group; bucillamine was used by five patients (8.2%) in the tacrolimus group and eight patients (12.9%) in the placebo group. Oral glucocorticoids (prednisolone, <7.5 mg per day) were used in 32 patients (52.5%) patients in the tacrolimus group and 31 patients (50.0%) in the placebo group.

Efficacy

Clinical responses

In the tacrolimus group, 70.5% (43/61 patients) achieved an ACR20 response at the end of treatment, compared with 45.2% (28/62 patients) in the placebo group; with a significantly higher response rate in the tacrolimus group ($P = 0.005$). Although the ACR50 and ACR70 response rates were higher in the tacrolimus group, the difference between the two groups was not statistically significant ($P = 0.085$, $P = 0.166$, Table 2).

According to the EULAR criteria, the incidence of a "good or moderate" response at the end of treatment was 86.9% (53/61 patients) in the tacrolimus group, and 56.5% (35/62 patients) in the placebo group, with the incidence being significantly higher ($P < 0.001$) in the tacrolimus group. Likewise, for a "good" response according to the EULAR criteria, the rate was 55.7% (34/61 patients) in the tacrolimus group, compared with 29.0% (18/62 patients) in the placebo group. Furthermore, the incidence of a "good" and that of a "good or moderate" response according to the EULAR criteria was significantly higher in the tacrolimus group ($P < 0.001$ to $P = 0.009$, Fig. 2) at any time after 8 weeks during the assessment period.

The percentage of patients achieving DAS28 remission (DAS28 < 2.6) after study drug administration was 45.0% (27/60 patients) in the tacrolimus group and 21.0% (13/62 patients) in the placebo group; there was a significantly greater response in the tacrolimus group ($P = 0.005$, Table 3).

Regarding MHAQ Scores, improvement was observed after the start of study drug administration in the tacrolimus group. The improvement effect at the end of treatment was significantly higher in the tacrolimus group than in the placebo group ($P < 0.05$) (Fig. 3).

The efficacy parameters, including Total Sharp Score, were compared in the patients with and without glucocorticoid use in the tacrolimus group. There were no significant differences between these 2 groups in any of the parameters (data not shown). The efficacy parameters were also compared in the patients in the tacrolimus group receiving MTX, salazosulfapyridine, or bucillamine therapies. We could not find any significant differences among these 3 groups either (data not shown).

Table 2 ACR20, ACR50, and ACR70 response rates at week 28, week 52, and end of the treatment

	Placebo $n = 62$ n (%)	Tacrolimus $n = 61$ n (%)
ACR20		
Week 28	22 (44.0)	39 (67.2)*
Week 52	23 (59.0)	42 (75.0)
End of treatment	28 (45.2)	43 (70.5)**
ACR50		
Week 28	15 (30.0)	19 (32.8)
Week 52	16 (41.0)	29 (51.8)
End of treatment	20 (32.3)	29 (47.5)
ACR70		
Week 28	7 (14.0)	10 (17.2)
Week 52	9 (23.1)	16 (28.6)
End of treatment	10 (16.1)	16 (26.2)

ACR American College of Rheumatology

* $P < 0.05$, ** $P < 0.01$ compared with placebo (logistic regression analysis)

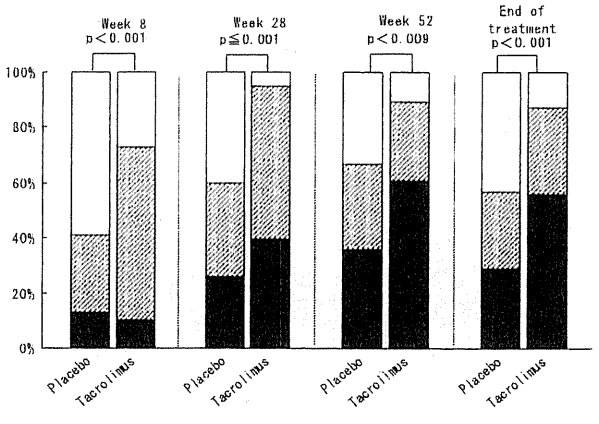

Fig. 2 European League Against Rheumatism (EULAR) response criteria. *Open bars* indicate "no response", *shaded bars* indicate "moderate response", and *solid bars* indicate "good response". EULAR response criteria (good or moderate response) were compared between treatment groups, using logistic regression analyses

Joint destruction

The change from baseline in the Total Sharp Score (mean value ± SD) at 52 weeks was 6.16 ± 10.84 in the tacrolimus group and 7.73 ± 12.23 in the placebo group. Although the score was lower in the tacrolimus group, this −1.44 difference between the two groups was not statistically significant ($P = 0.485$). The change from baseline in

Table 3 The proportions of patients in DAS28 remission

	Placebo	Tacrolimus	*P* value
Week 28	7 (14.0)	16 (28.1)	0.084
Week 52	11 (28.2)	27 (49.1)	0.041
End of treatment	13 (21.0)	27 (45.0)	0.005

No. of patients (%)

Disease Activity Score in 28 Joints (DAS28) remission, defined as DAS28 <2.6, was compared between treatment groups using logistic regression analyses

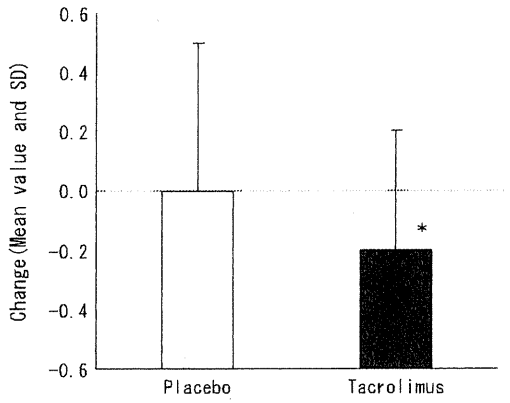

Fig. 3 Change in the Modified Health Assessment Questionnaire (MHAQ) Score from baseline to end of treatment. $^*P < 0.05$, analysis of covariance (ANCOVA)

the erosion score (mean value ± SD) at 52 weeks was 2.50 ± 4.56 in the tacrolimus group and 4.27 ± 7.53 in the placebo group; although the score was lower in the tacrolimus group, this −1.91 difference between the groups was not significant ($P = 0.090$). The joint-space narrowing scores were 3.67 ± 7.03 in the tacrolimus group and 3.46 ± 5.33 in the placebo group; there was no significant difference between these groups. ($P = 0.665$, Table 4).

Figure 4 shows the cumulative probability plots for the modified Sharp Scores (Total Sharp Score, erosion score, joint-space narrowing score). The proportion of patients with change from baseline of the Total Sharp score of ≤0 over 52 weeks was 14/58 patients (24.1%) in the tacrolimus group, higher than the 7/50 patients (14.0%) in the placebo group. Similarly, the proportion of patients with change from baseline of the erosion score of ≤0 over 52 weeks was 21/58 patients (36.2%) in the tacrolimus group, and 11/50 patients (22.0%) in the placebo group, also being higher in the tacrolimus group.

Safety

The incidence of AEs was 86.9% (53/61 patients) in the tacrolimus group, and 79.0% (49/62 patients) in the placebo group; the incidence of discontinuation of the study drug due to AEs was 3.3% (2/61 patients) in the tacrolimus group and 11.3% (7/62 patients) in the placebo group. There was no significant difference between the two groups ($P = 0.338$ and $P = 0.163$, respectively). Furthermore, the incidence of serious AEs (SAEs) was significantly higher in the placebo group ($P = 0.017$, Table 5).

Table 4 Radiographic analysis

	Placebo ($n = 50$)	Tacrolimus ($n = 58$)	*P* value
Total Sharp Score			
Mean ± SD	7.73 ± 12.23	6.16 ± 10.84	0.485
Median (IQR)	3.50 (0.75–8.00)	3.25 (0.50–7.00)	
Erosion score			
Mean ± SD	4.27 ± 7.53	2.50 ± 4.56	0.090
Median (IQR)	2.00 (0.50–5.00)	1.00 (−0.50 to 4.50)	
Joint-space narrowing score			
Mean ± SD	3.46 ± 5.33	3.67 ± 7.03	0.665
Median (IQR)	1.75 (0.00–4.00)	0.50 (0.00–5.50)	

Changes from baseline to end of treatment in radiographic outcomes. *P* values for between-group differences in change were calculated by analysis of covariance (ANCOVA)

IQR interquartile range

One SAE was observed in the tacrolimus group (benign giant cell bone tumor), with nine SAEs (in nine patients) observed in the placebo group (peritonitis, deep vein thrombosis, thrombotic stroke, organizing pneumonia, pyoderma gangrenosum, subarachnoid hemorrhage, herpes zoster, retinal break, and colon cancer). All the events (with the exception of organizing pneumonia and colon cancer with unchanged outcome) that were observed in the placebo group were resolved by appropriate treatment. The benign giant cell bone tumor that occurred in a patient in the tacrolimus group was identified as multiple microcystic lesions in the left patella during an X-ray examination on day 85 after the first administration of the study drug. In this patient, administration of tacrolimus had been withdrawn since day 57 due to the occurrence of other AEs, and administration was discontinued after the tumor was identified. We then conducted nidus curettage and bone cement filling and the patient recovered on day 234. The investigator determined that the possibility of a causal relationship with the study drug could not be ruled out.

Regarding AEs which led to discontinuation of the trial for other reasons, two events (vomiting and high blood pressure) in four patients in the tacrolimus group and four events (hypoglycemia, headache, glucose-positive urine, tendon rupture) in two patients in the placebo group were observed. All events observed in the tacrolimus group were resolved by discontinuation of the study drug.

Regarding AEs in the tacrolimus group, the highest incidence was observed in "infectious and parasitic diseases", followed by "laboratory data" and "gastrointestinal

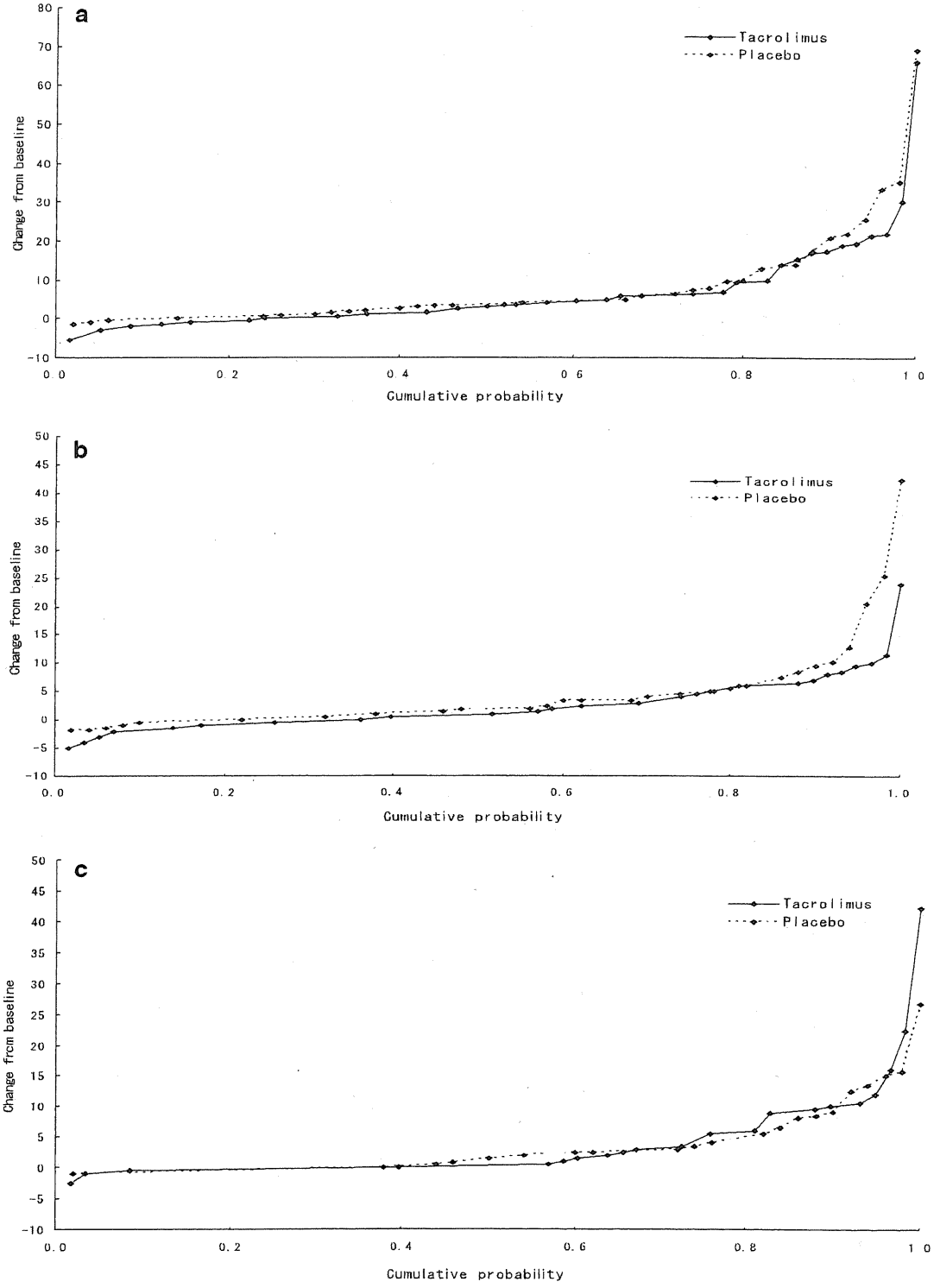

Fig. 4 Cumulative probability plots of radiographic changes from baseline to week 52 for patients treated with tacrolimus or with placebo. The space between the curves indicates the different treatment effects with a considerable difference in favor of the tacrolimus group. **a** Total Sharp Score, **b** Erosion score, **c** Joint-space narrowing score

Table 5 Treatment-related adverse events (AEs)

	Placebo ($n = 62$)	Tacrolimus ($n = 61$)
All	49 (79.0)	53 (86.9)
Serious AEs	9 (14.5)*	1 (1.6)
Infections and infestations	22 (35.5)	26 (42.6)
Vascular disorders	3 (4.8)	3 (4.9)
Respiratory, thoracic, and mediastinal disorders	9 (14.5)	8 (13.1)
Gastrointestinal disorders	10 (16.1)	25 (41.0)**
Hepatobiliary disorders	1 (1.6)	0 (0.0)
Skin and subcutaneous tissue disorders	10 (16.1)	14 (23.0)
Musculoskeletal and connective tissue disorders	3 (4.8)	4 (6.6)
Renal and urinary disorders	5 (8.1)	2 (3.3)
Neoplasms: benign, malignant, and unspecified (including cysts and polyps)	1 (1.6)	1 (1.6)
Reproductive system and breast disorders	0 (0.0)	3 (4.9)
Congenital, familial, and genetic disorders	0 (0.0)	1 (1.6)
General disorders and administration-site conditions	3 (4.8)	4 (6.6)
Laboratory data	16 (25.8)	26 (42.6)
Injury, poisoning, and procedural complications	6 (9.7)	2 (3.3)
Blood and lymphatic system disorders	1 (1.6)	5 (8.2)
Metabolic and nutritional disorders	0 (0.0)	3 (4.9)
Psychiatric disorders	1 (1.6)	0 (0.0)
Nervous system disorders	4 (6.5)	7 (11.5)
Visual disorders	1 (1.6)	5 (8.2)

Data are numbers of patients (%). * $P < 0.05$ compared with tacrolimus, ** $P < 0.01$ compared with placebo (Fisher's exact test)

disorders". Aside from these events, only "gastrointestinal disorders" was observed at a significantly higher incidence than in the placebo group ($P = 0.003$). In addition, nasopharyngitis, which occurred in 24.6% (15/61 patients), was the AE with the highest incidence in the tacrolimus group. The incidence of nasopharyngitis in the placebo group was 29.0% (18/62 patients). Events observed in at least five patients were diarrhea (seven patients), upper respiratory tract inflammation (seven patients), oral inflammation (six patients), upper abdominal pain (five patients), alanine and aminotransferase elevations (five patients), and abnormality in liver function tests (five patients)in the tacrolimus group , and upper respiratory tract inflammation (six patients) in the placebo group.

Serum creatinine had increased by more than 40% after study drug administration compared with the pre-dose value in 12/61 patients (19.7%) in the tacrolimus group, and in 1/62 patients (1.6%) in the placebo group ($P = 0.001$). This number was higher in the tacrolimus group, but no patient discontinued the trial for this reason. Changes in blood pressure and laboratory data results did not show any notable differences between the two groups.

Blood concentration of tacrolimus

The mean and median tacrolimus blood concentration values in each evaluation period (weeks 2–52) were in the range of 4.9–6.1 ng/mL and 4.5–5.5 ng/mL, respectively. Though the blood concentration in one patient increased to 20 ng/mL or higher (day 253 after administration of the study drug, 23.8 ng/mL), it was only a transient increase and the value measured at any other time point was not higher than 10 ng/mL. Moreover, in the patients with AEs or side effects, there was no trend in which the blood concentrations prior to the onset of the AEs or side effects, or the mean blood concentration values during the treatment period were higher than those in the patients without AEs or side effects.

Discussion

The efficacy of tacrolimus monotherapy in Japanese patients with rheumatoid arthritis has been demonstrated previously [7, 8]. On the contrary, in this double-blind experiment, we showed for the first time sufficient efficacy and safety of tacrolimus administered in addition to the standard DMARDs used in Japan (MTX, salazosulfapyridine, or bucillamine) in patients with early rheumatoid arthritis with an inadequate response to previous treatment.

Regarding efficacy in terms of disease activity, 70.5% of the tacrolimus group in the present study achieved an ACR20 response, compared with 45.2% in the placebo

group; the incidence of a "good or moderate" response according to the EULAR criteria was 86.9% in the tacrolimus group, and 56.5% in the placebo group at the end of treatment; furthermore, 45.0% of the tacrolimus group and 21.0% of the placebo group achieved DAS28 remission (DAS28 < 2.6). The patients in the tacrolimus group exhibited significantly better results in comparison with the placebo group. The difference between the group with the additional use of tacrolimus and the placebo group (DMARD only) was significant, and sufficient efficacy of the additional use of tacrolimus is considered to be proved.

It has been shown previously that the use of biological products in addition to MTX is more effective than MTX monotherapy [20–22]. In the present study, the difference in the DAS28 remission rate between the tacrolimus group and the placebo group (the existing therapy group) was found to be comparable to that in studies using biological products. In recent years, combined treatment with antirheumatic drugs has been recommended for rheumatoid arthritis patients with a poor prognosis[10] and it has been noted that the ultimate goal of antirheumatic treatment is to approach clinical remission [23–25]. Considering these facts, the result of the present trial is clinically significant.

The inhibition of joint destruction has been reported mainly with biological products, including TNF inhibitors, and also with other products such as oral antirheumatic MTX, leflunomide, and salazosulfapyridine [20, 26–30]. During the present trial, there was no significant difference in Total Sharp Score, erosion score, or joint-space narrowing score for joint destruction between the tacrolimus group and the placebo group. Regarding the change in erosion score from before study drug administration, the erosion score showed a lower value at 52 weeks in the tacrolimus group than that in the placebo group ($P = 0.090$), and this suggested the possibility that the progress of erosion was delayed. Furthermore, 24.1% (14/58 patients) of patients in the tacrolimus group showed inhibition of joint destruction over 52 weeks (Total Sharp Score ≤ 0). This was higher than the 14.0% (7/50 patients) in the placebo group. In addition, 36.2% (21/58 patients) of patients in the tacrolimus group and 22.0% (11/50 patients) in the placebo group had a change in the erosion score of ≤ 0 from baseline ; this percentage was also higher in the tacrolimus group. The inhibitory effect of joint destruction with the additional use of tacrolimus was unclear partly because the number of the patients was small. However, it appears that the use of tacrolimus with other DMARDs can delay erosion, and further investigation is considered to be necessary.

In the present study, the incidence of AEs was 86.9% (53/61 patients) in the tacrolimus group and 79.0% (49/62 patients) in the placebo group; there was no significant difference between the two groups. When classifying the AEs by organ, "infectious and parasitic diseases" occurred with a high incidence in the tacrolimus group, followed by "laboratory data" and "gastrointestinal disorders". The incidence of "gastrointestinal disorders" was significantly higher in the tacrolimus group than that in the placebo group ($P = 0.003$), but there were no patients who discontinued the trial because of AEs or severe events. This result is not significantly different from those of tacrolimus monotherapy trials [7, 8, 31, 32] in patients with rheumatoid arthritis, and in the present study, good tolerability of tacrolimus was shown in patients with early rheumatoid arthritis who also received other DMARDs. Moreover, the main side effects, such as infections, gastroenterological disorders, abnormal variations in renal function test valuesand abnormal variations in glucose tolerance test valueshave been observed in previous studies [7, 8, 32]. However, in the present trial, no significant abnormal variations in renal function test values or abnormal variations in glucose tolerance test values were observed. The number of patients with an increased creatinine level after study drug administration was larger in the tacrolimus group, which is similar to results previously reported from trials conducted elsewhere [33–35]. However, there was no serious increase in serum creatinine in patients who discontinued tacrolimus administration in our study.

Tacrolimus is a drug metabolized in the liver, yet hepatic dysfunction has never been a major problem in trials of tacrolimus monotherapy . However, hepatic dysfunction is known as a side effect of MTX [36, 37]. Because MTX was used together with tacrolimus in about 70% of patients in the present trial, we looked closely at the occurrence of hepatic disorders due to the additional use of tacrolimus. Regarding abnormal liver function test values and variations in various liver function test values (alanine aminotransferase [ALT] increased, aspartate aminotransferase [AST] increased, γ-guanosine triphosphate [GTP] increased, alkaline phosphatase [ALP] increased), 15 events occurred in 12 patients in the tacrolimus group, and seven events occurred in 10 patients in the placebo group among patients who concomitantly received MTX. The tacrolimus group had a slightly higher incidence of events, but most of the events were "slight". These results suggest a low possibility of perturbation of liver function even with the additional use of tacrolimus, suggesting that, in this respect, combined tacrolimus and MTX therapy is not significantly different from tacrolimus monotherapy. Therefore, it is concluded that the combination therapy can be used without major problems.

The safety profile of the additional use of tacrolimus with DMARDs (MTX, salazosulfapyridine, or bucillamine) was almost the same as that of the tacrolimus monotherapy which was reported previously [7, 8, 32, 33]. Based on the results mentioned above, we can conclude that the

additional use of tacrolimus in patients with early rheumatoid arthritis with an inadequate response to other DMARD treatments is useful, and this could become one of the treatment options for these rheumatoid arthritis patients.

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Incidence and Risk Factors for Serious Infection in Patients with Rheumatoid Arthritis Treated with Tumor Necrosis Factor Inhibitors: A Report from the Registry of Japanese Rheumatoid Arthritis Patients for Longterm Safety

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ABSTRACT. ***Objective.*** To compare tumor necrosis factor-α (TNF-α) inhibitors to nonbiological disease-modifying antirheumatic drugs (DMARD) for the risk of serious infection in Japanese patients with rheumatoid arthritis (RA).

Methods. Serious infections occurring within the first year of the observation period were examined using the records for patients recruited to the Registry of Japanese Rheumatoid Arthritis Patients for Longterm Safety (REAL), a hospital-based prospective cohort of patients with RA. The analysis included 1144 patients, 646 of whom were treated with either infliximab or etanercept [exposed group: 592.4 patient-years (PY)]. The remaining 498 patients received nonbiological DMARD with no biologics (unexposed group: 454.7 PY).

Results. In the unexposed group, the incidence rate for all serious adverse events (SAE) was 9.02/100 PY and for serious infections, 2.64/100 PY. In the exposed group, SAE occurred in 16.04/100 PY and serious infections in 6.42/100 PY. The crude incidence rate ratio comparing serious infections in the exposed group with the unexposed group was 2.43 (95% CI 1.27–4.65), a significant increase. A multivariate analysis revealed that the use of TNF inhibitors is a significant independent risk factor for serious infection (relative risk 2.37, 95% CI 1.11–5.05, p = 0.026).

Conclusion. Our study has provided the first epidemiological data on Japanese patients with RA for the safety of TNF inhibitors compared to nonbiological DMARD for up to 1 year of treatment. Anti-TNF therapy was associated with a significantly increased risk for serious infections, compared to treatment with nonbiological DMARD. (First Release April 15 2011; J Rheumatol 2011; 38:1258–64; doi:10.3899/jrheum.101009)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
TUMOR NECROSIS FACTOR-α
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The introduction of tumor necrosis factor-α (TNF) inhibitors for treatment of rheumatoid arthritis (RA) is a major therapeutic breakthrough[1]. Because biologics, including TNF inhibitors, have become important and widely used clinical tools for treatment of RA, assessment of their safety is important. There are significant concerns relating to the association between opportunistic infections and TNF inhibitors. One example of this association is the observed reactivation of latent tuberculosis[2]. Serious bacterial, granulomatous, and fungal infections have also been reported to be associated with TNF inhibitor use[3,4].

To develop the safety profiles of biologics, several groups from Europe and the United States have established registries for patients receiving these drugs. Some of these have reported elevated risk for infections in patients with RA treated with biologics, including TNF inhibitors, compared to treatment with nonbiological disease-modifying antirheumatic drugs (DMARD)[5,6,7,8,9,10,11]. To date, there has been no comparable report on the safety of biologics for Asian patients with RA. Because racial and geographic differences occur in morbidities of such infections as *Mycobacterium tuberculosis*, the *Coccidioides* species, and *Pneumocystis jirovecii*, the development of a defined safety profile for treatment with biologics in each geographic area is crucial for clinicians[12,13,14,15].

In Japan, postmarketing surveillance programs of all cases treated with infliximab and etanercept were implemented, revealing several important safety concerns for these TNF inhibitors during the first 6 months of the therapy. These studies identified infection as the most important serious adverse event (SAE) during treatment with the TNF inhibitors[16,17]. These studies, however, had serious deficiencies related to the absence of appropriate comparator groups and the short tracking period. We therefore established the Registry of Japanese Rheumatoid Arthritis Patients for Longterm Safety (REAL) database in 2005 to compare the safety of midterm to longterm treatment with biological DMARD to treatment with nonbiological DMARD.

The primary purpose of our study was to use the REAL database to compare the incidence of serious infections between TNF inhibitor-treated and nonbiological DMARD-treated patients with RA. A second objective was to identify independent risk factors for serious infections in this population.

MATERIALS AND METHODS

Data source. The REAL database is a hospital-based prospective cohort of patients with RA administered by the Department of Pharmacovigilance of the Tokyo Medical and Dental University. The ethics committee of the Tokyo Medical and Dental University Hospital and those of the participating institutions approved our study. Twenty-three institutions participate in REAL, including 15 university hospitals and 8 referring hospitals. Enrollment to the REAL database began in June 2005.

The criteria for admission to the REAL database include those patients (1) meeting the 1987 American College of Rheumatology criteria for RA; (2) ≥ 20 years old and able and willing to provide written informed consent and comply with the requirements of the protocol, or, for those patients < 20 years, having parents or legal guardians willing and able to provide written informed consent and to comply with the requirements of the protocol; and (3) starting treatment with biologics (the exposed group) or nonbiological DMARD (the nonexposed group) at the time of study entry. In addition, patients receiving treatment with nonbiological DMARD at the time of study entry are also enrolled as the nonexposed group. Exclusion criteria include (1) patient participation in a clinical trial for approval of drugs at the time of enrollment or during the followup in the study, and (2) patients withdrawing consent to join the study. We identified all patients who were registered from the participating hospitals of our study to the postmarketing surveillance programs for each biological DMARD that were implemented by the corresponding pharmaceutical companies. Participating physicians at each hospital enrolled all of these patients to the REAL database. In addition, patients who fulfilled the inclusion criteria were consecutively recruited for both groups by participating physicians at each hospital.

Exposed group. Because infliximab was introduced in Japan in 2003, etanercept in 2005, and adalimumab and tocilizumab in 2008, few data for patients receiving adalimumab or tocilizumab were available in the REAL database at the time we conducted our study. We therefore included only

those patients with RA who had started infliximab or etanercept at enrollment in the REAL database. Nonbiological DMARD were used for these patients at the attending physicians' discretion. Six hundred forty-six patients were enrolled in the exposed group. Patients who switched from infliximab to etanercept or etanercept to infliximab were included in the analysis using the combined time of the treatment. For those patients no longer receiving either infliximab or etanercept, only the time of actual use of these TNF inhibitors was analyzed.

Unexposed group. Four hundred ninety-eight patients were enrolled in the unexposed group. At the time of enrollment in our study, 57.6% of the patients in the unexposed group were being treated with methotrexate (MTX), 20.3% with salazosulfapyridine, 18.7% with tacrolimus, and 13.9% with bucillamine. Nonbiological DMARD used in fewer than 10 patients were leflunomide, actarit, gold salt, auranofin, mizoribine, D-penicillamine, and cyclosporine. Sixty-four patients (12.9%) of the unexposed group were given combination therapy with > 1 nonbiological DMARD agent during the observation period. Some patients who were initially enrolled in the unexposed group received biologics when clinically indicated; the time period following this change was excluded from the analysis.

Data collection. Each patient's recorded baseline data included demography, disease activity, comorbidities, treatments, and laboratory data at the start of the observation period. The same followup forms were used for both groups and included queries about RA disease activity, treatments, laboratory data, and occurrence and details of SAE. The followup forms were submitted every 6 months by the participating physicians to the REAL Data Center at the Department of Pharmacovigilance of Tokyo Medical and Dental University. The participating physicians in each hospital confirmed their submitted data to the REAL Data Center. Data were retrieved from the REAL database on November 30, 2008, for our study.

Baseline characteristics of patients. The observation period for 646 patients in the exposed group was 592.4 patient-years (PY). For 498 patients in the unexposed group, the observation period was 454.7 PY. In the exposed group, 300 patients (272.1 PY) received infliximab but not etanercept and 343 patients (320.3 PY) received etanercept but not infliximab. Three patients were switched from infliximab to etanercept during the observation period. The median length of the observation period was 1 year in both groups, and the percentage of patients followed up for a year was 83.1% in the exposed and 82.1% in the unexposed group. Minimal duration of followup was 2 months in the unexposed group and 3 months in the exposed group. The primary reason for not having at least a full year of followup in about 18% of the patients was that they were enrolled in the REAL database for < 1 year before November 30, 2008, when the data were retrieved from the database. Baseline data at the start of the observation period for the patients are shown in Table 1. Compared to the unexposed group, the exposed group was younger ($p < 0.001$), had more severe disease activity ($p < 0.001$), was treated with higher dosages of MTX ($p < 0.001$) and corticosteroids ($p = 0.001$), and had failed a larger number of DMARD ($p < 0.001$). Percentages of the patients on their first DMARD at baseline were 30.1% for the unexposed group and 24.0% for the exposed group ($p < 0.012$). Significantly more patients having comorbidities, including chronic pulmonary diseases ($p = 0.046$) and diabetes ($p = 0.024$), were seen in the exposed group compared to the unexposed group.

Definition of SAE. Our definition of an SAE was based on events described in the report by the International Conference on Harmonization[18]. In addition, bacterial infections that required intravenous administration of antibiotics, as well as opportunistic infections, including tuberculosis, *P. jirovecii* pneumonia (PCP), systemic fungal infection, cytomegalovirus infection, and herpes zoster were also regarded as SAE. The diagnosis of infections was based on a physician's clinical diagnosis, a comprehensive evaluation based on physical findings, laboratory data, and radiological examinations. The detection of infectious pathogens was not mandatory for making a diagnosis of infection. SAE were classified using the System Organ Class (SOC) of the Medical Dictionary for Regulatory Activities (MedDRA; version 11.1).

Table 1. Comparison of patients with rheumatoid arthritis (RA) treated with (exposed) and without (unexposed) the tumor necrosis factor-α (TNF) inhibitors infliximab or etanercept at the start of the observation period. Values are mean ± SD unless otherwise stated.

Characteristics	Exposed Group, n = 646	Unexposed Group, n = 498	p
Age, yrs	58.3 ± 13.2	61.4 ± 12.8	< 0.001
Women, %	82.0	83.3	0.568
Disease duration, yrs	9.5 ± 8.6	9.2 ± 9.2	0.654
Steinbrocker stage (III or IV), %	55.1	43.8	< 0.001
DAS28 (3/CRP)	3.9 ± 1.0, n = 642	2.8 ± 1.0, n = 495	< 0.001
MTX use, %	69.0	60.2	0.002
MTX dose, mg/wk	7.6 ± 2.2	6.4 ± 2.0	< 0.001
MTX dose > 8 mg/wk, %	11.1	5.0	< 0.001
Use of immunosuppressive drugs, except for MTX, %*	3.7	20.5	< 0.001
Corticosteroid use, %**	71.4	62.0	0.001
Prednisolone dose, mg/day	5.7 ± 3.0	4.6 ± 2.1	< 0.001
> 7.5 mg prednisolone/day, %	13.6	3.1	< 0.001
No of failed DMARD	1.6 ± 1.1	1.3 ± 1.1	< 0.001
Chronic pulmonary disease, %***	21.4	16.7	0.046
Diabetes, %	10.7	6.8	0.024

* Including tacrolimus, leflunomide, mizoribine, and cyclosporine. ** Converted to corresponding prednisolone dosage. *** Including interstitial pneumonia, chronic obstructive pulmonary disease, bronchial asthma, prior pulmonary tuberculosis, and bronchiectasis. DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; MTX: methotrexate; DMARD: disease-modifying antirheumatic drug. † Number of DMARD that were tried but did not bring about a response.

Statistical analyses. Serious infections observed within the first year of the observation period were analyzed for each patient. The observation period for the present analysis was defined as follows: for patients who initiated treatment with the TNF inhibitors infliximab or etanercept or nonbiological DMARD at the time of study entry, the start of the observation period was the date these agents were first administered; for patients receiving the treatment with nonbiological DMARD at the time of study entry, the start of the observation period was the date of their enrollment in the REAL database. The observations ended 1 year after the start of the observation period, or on the day a patient died or met the exclusion criteria, or for the exposed group, no longer received either infliximab or etanercept, or for the unexposed group, started biologics, whichever came first. Patients were not removed even after the development of SAE as long as they did not meet the above criteria for censoring a patient. Considering the time it takes for pharmacokinetic/pharmacodynamic effects and data to appear from previous studies of at-risk periods[6], we considered any SAE occurring within 90 days after the last administration of infliximab or etanercept that was within the first year of the observation period to be attributable to the effects of the TNF inhibitors. Because the length of the at-risk period (90 days) after the date of discontinuation of treatment is more than 10 times as long as the half-lives of the 2 TNF inhibitors (i.e., 8.1 days for infliximab and 4.8 days for etanercept), we defined the date of drug discontinuation as the date of last administration, instead of the date of the first missed dose, which was the method used by another study[6]. The same number of SAE was found in the exposed group of our study using either definition for the date of drug discontinuation (data not shown). The date of the last administration of infliximab or etanercept was retrieved from medical records and reported by the participating physicians.

The incidence rates (IR) per 100 PY and incidence rate ratios (IRR) with their 95% CI were calculated. For univariate analysis, the chi-squared

test for categorical variables and the Student t-test or Mann-Whitney U tests for continuous variables were used for comparisons among groups. For multivariate analysis, Poisson regression analyses were used to estimate the risk of serious infection with the TNF inhibitors infliximab and etanercept, and to identify any variable having a significant and independent influence on the development of serious infections. Variables that were included in the multivariate analysis were chosen using the results of univariate analysis. The analyses were conducted using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) and R statistical language software (version 2.8.1, R Foundation for Statistical Computing, Vienna, Austria). All p values were 2-tailed and $p < 0.05$ was considered statistically significant.

RESULTS

Types and incidence rates of SAE. One hundred thirty-six SAE were reported during the observation period, 41 in the unexposed group and 95 in the group exposed to infliximab or etanercept. Based on the SAE categories classified using the SOC, infections and infestations were the most common, followed by injury, poisoning, and procedural complications, in which fractures accounted for 76% (Table 2). In the exposed group, there were 38 serious infections including 25 bacterial, 11 opportunistic (6 cases of herpes zoster, 3 PCP, 1 pulmonary cryptococcosis, and 1 pulmonary nontuberculous mycobacterial infection), and 2 other infections. In the unexposed group, 12 serious infections occurred, including 8 bacterial, 3 opportunistic (1 each PCP, pulmonary tuberculosis, and pulmonary nontuberculous mycobacterial infection), and 1 viral infection. The respiratory system was the most frequent infection site (23 for the exposed group and 9 for the unexposed group), followed by skin and subcutaneous tissue (9 for the exposed and 1 for the unexposed), urinary tract (1 for each group), and bone and joints (1 for each group). The rates of treatment discontinuation after serious infections were 2.19/100 PY in the exposed group and 0.22/100 PY in the unexposed group. The rate ratio comparing the exposed group with the unexposed group was 9.98 (95% CI 1.31–76.29), a significant elevation. On the other hand, the rates of treatment discontinuation after SAE other than serious infections were not statistically different between the 2 groups [1.86/100 PY in the exposed group and 0.66/100 PY in the unexposed group; the rate ratio was 2.81 (95% CI 0.79–10.09)].

Table 2. Categories of serious adverse events (SAE) using the system organ class (SOC).

System Organ Class Allocation	No. SAE in Study Patients: Exposed Group, n = 646	Unexposed Group, n = 498	Total
Cardiac disorders	2	1	3
Endocrine disorders	1	0	1
Eye disorders	1	1	2
Gastrointestinal disorders	6	4	10
General disorders and administration site conditions	2	1	3
Hepatobiliary disorders	4	4	8
Infections and infestations	38	12	50
Injury, poisoning, and procedural complications	12	5	17
Metabolism and nutrition disorders	0	1	1
Musculoskeletal and connective tissue disorders	1	1	2
Neoplasms benign, malignant, and unspecified	4	5	9
Nervous system disorders	1	1	2
Renal and urinary disorders	3	2	5
Reproductive system and breast disorders	1	0	1
Respiratory, thoracic, and mediastinal disorders	14	2	16
Skin and subcutaneous tissue disorders	2	1	3
Vascular disorders	3	0	
Total	95	41	136

In the exposed group, the IR of SAE was 16.04/100 PY and the IR of serious infection was 6.42/100 PY. The crude IRR comparing the exposed group with the unexposed group for SAE was 1.78 (95% CI 1.23–2.57) and for serious infections was 2.43 (95% CI 1.27–4.65); both of these IRR were significantly elevated (Table 3).

Contribution of TNF inhibitors to the development of serious infections. Because the background data of the patients differed considerably between the exposed and unexposed groups (Table 1), we performed univariate analysis to identify candidate risk factors for the development of serious infections (data not shown) and selected age, chronic pulmonary diseases, Steinbrocker stage[19], disease activity, corticosteroid dosage, and MTX dosage as covariates for multivariate analyses. We used the Poisson regression model to evaluate the risk for development of serious infection from the use of TNF inhibitors. The use of TNF inhibitors was found to constitute a significant risk factor for serious infection. The relative risk (RR) was 2.37 (95% CI 1.11–5.05, $p = 0.026$; Table 4).

Among the confounding factors, we found that these factors were independently associated with development of serious infection (Table 4): increasing age (RR 1.82 per 10-year increment; 95% CI 1.32–2.52; $p = 0.00031$), chronic pulmonary diseases (RR 2.61; 95% CI 1.38–4.94; $p = 0.0031$), advanced disease (Steinbrocker stage III or IV; RR 2.07; 95% CI 1.07–3.97; $p = 0.03$), and dosage of MTX > 8 mg/week (RR 2.61; 95% CI 1.40–4.86; $p = 0.0024$). When the dosages of MTX and prednisolone (PSL) were recategorized as MTX use (yes/no), MTX dosage > 6 mg/week (yes/no), PSL use (yes/no), and PSL dosage > 5 mg/day (yes/no), or were used as continuous variables, the analyses gave essentially the same results (data not shown).

Risk factors for infection during treatment with the TNF inhibitors infliximab or etanercept. To identify the risk factors contributing to the development of serious infections during treatment with infliximab or etanercept, we compared the background data of those patients who did or did not develop serious infections, using univariate analyses (Table 5). The patients who developed serious infections

Table 3. Number and incidence of serious adverse events (SAE) in patients with rheumatoid arthritis who were treated with (exposed) and without (unexposed) the tumor necrosis factor-α inhibitors infliximab or etanercept.

Event	Exposed Group, n = 646 592.35 PY	Unexposed Group, n = 498 454.74 PY	Crude IRR (95% CI)
All SAE, no. events	95	41	0
IR (/100 PY)	16.04 (12.81–19.26)	9.02 (6.26–11.78)	1.78 (1.23–2.57)
Serious infection, no. events	38	12	
IR (/100 PY)	6.42 (4.38–8.46)	2.64 (1.15–4.13)	2.43 (1.27–4.65)
Serious respiratory tract infection, no. events	23	9	
IR (/100 PY)	3.88 (2.30–5.47)	1.98 (0.69–3.28)	1.96 (0.91–4.24)

PY: patient-years; IR: incidence rate; IRR: incidence rate ratio.

Table 4. Multivariate analysis of independent risk factors for serious infections in the Registry of Japanese Rheumatoid Arthritis Patients for Longterm Safety (REAL) database. The relative risk (RR) of biologics for development of serious infection for up to 1 year of the observation period was calculated using the Poisson regression model after adjusting for the confounding factors of age, chronic pulmonary disease, Steinbrocker stage, disease activity, corticosteroid dosage, and methotrexate dosage.

	RR (95% CI)	p
TNF inhibitor* (yes)	2.37 (1.11–5.05)	0.026
Age, by decade	1.82 (1.32–2.52)	0.00031
Chronic pulmonary disease (yes)	2.61 (1.38–4.94)	0.0031
Stage III or IV (vs Stage I or II)**	2.07 (1.07–3.97)	0.030
MTX dose > 8.0 mg/wk	2.61 (1.40–4.86)	0.0024
DAS28 (3/CRP)	0.87 (0.66–1.14)	0.31
Prednisolone dose > 7.5 mg/day	1.21 (0.58–2.55)	0.61

* Infliximab or etanercept. ** Steinbrocker classification[19] was used to define RA disease stages. TNF: tumor necrosis factor-α; DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; MTX: methotrexate.

Table 5. Comparison of background data for patients with rheumatoid arthritis (RA) who were treated with the tumor necrosis factor inhibitors infliximab or etanercept. Values are mean ± SD, unless otherwise stated.

Factors	Infection, n = 612	Without Infection, n = 34	p
Age, yrs	57.9 ± 13.3	67.1 ± 8.1	< 0.001
Women, %	82.0	82.4	0.961
RA disease duration, yrs	9.3 ± 8.5	13.0 ± 10.2	0.008
Steinbrocker stage (III or IV), %*	53.9	76.4	0.010
DAS28 (3/CRP)	3.9 ± 1.0	3.7 ± 1.2	0.356
MTX dose mg/wk	5.2 ± 3.9	5.6 ± 4.2	0.387
Use of immunosuppressive drugs except for MTX, %**	3.8	2.9	0.636
Corticosteroid use, %	62.0	71.4	0.001
Prednisolone dose, mg/day***	4.0 ± 3.6	4.7 ± 3.4	0.214
Chronic pulmonary disease, %†	20.4	38.2	0.014
Diabetes, %	10.3	17.6	0.143

* Steinbrocker classification[19] was used to define RA disease stages. ** Including tacrolimus, leflunomide, mizoribine, and cyclosporine. *** Converted to corresponding prednisolone dosage. † Including interstitial pneumonia, chronic obstructive pulmonary disease, bronchial asthma, prior pulmonary tuberculosis, and bronchiectasis. DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; MTX: methotrexate.

were significantly older ($p < 0.001$) and had longer disease duration ($p = 0.008$) as well as more advanced disease (Steinbrocker stage III or IV; $p = 0.01$). The percentages of patients given corticosteroids and having chronic pulmonary diseases were higher for patients who developed serious infections. The contributions of age, disease duration, corticosteroid use, and chronic pulmonary disease to the development of serious infections in the exposed group were analyzed using the Poisson regression model. This multivariate analysis showed increasing age per 10-year increment (RR 1.97; 95% CI 1.34–2.84) and the use of corticosteroids (RR 2.97; 95% CI 1.04–8.50) to be significantly associated ($p = 0.00058$ and $p = 0.042$, respectively) with the development of serious infection during TNF inhibitor therapy.

DISCUSSION

In our prospective study of a Japanese hospital-based cohort of patients with RA, the multivariate analysis demonstrated that treatment with the biologic TNF inhibitors infliximab or etanercept was associated with an increased risk for serious infections. Increasing age, chronic pulmonary diseases, an advanced disease stage of RA, and dosage of MTX were also identified as independent risk factors for serious infections in this population.

The IR of serious infection in the exposed group (6.4/100 PY; 95% CI 4.4–8.5) is comparable to those reported previously [6.2–6.4/100 PY from a German RA registry and 6.1/100 PY (95% CI 5.7–6.5) from a British RA registry][5,6]. Our data were also consistent with the results of the post-marketing surveillance programs in Japan, which found the IR of serious infection during the first 6 months of anti-TNF therapy was 8.1/100 PY in patients treated with infliximab and 7.7/100 PY for those treated with etanercept[16,17,20]. Schneeweiss, *et al*[8] reported a lower IR for serious infection, 4.8/100 PY (95% CI 3.1–6.6), in patients receiving TNF inhibitors. This difference from our results can probably be

explained by variations in such methodologies as inclusion criteria or definition of infectious events. Schneeweiss, *et al*[8] focused on hospitalizations of elderly patients due to serious bacterial infections while being treated with TNF inhibitors. The IR of SAE and serious infections in the unexposed group of our study were similar to those of other clinical trials conducted in Japan[21,22,23], as well as to those reported from 4 European registries (IR 2.3–3.9/100 PY)[5,6,8,9]. Thus, we postulate that our results did not underestimate the risk of serious infections during treatment with nonbiological DMARD. Examining the infection sites in our study, the respiratory system was the most frequent site for both exposed and unexposed groups, followed by skin and subcutaneous tissue, which is consistent with other epidemiological studies of patients with RA[7,24].

Evaluating patients with RA for predisposing factors for infection prior to initiating TNF inhibitor therapy is important. The independent risk factors identified in our study were in overall agreement with previous reports of predictors of infection among patients with RA[25]. First, the association of corticosteroid use with serious infection, as shown by the multivariate analysis of the exposed group, is consistent with several reports describing corticosteroid use as an important risk factor for infection[8,9]. The relatively low number and rate of serious infections in the unexposed group probably resulted in a lack of enough power to detect the risk from corticosteroid in the analysis of the total population of our study. Second, finding an association between Steinbrocker stage and increased risk for serious infection is also supported by the results of the postmarketing surveillance of infliximab in Japanese patients with RA, which found that Steinbrocker stage III or IV was a predictor for bacterial pneumonia by multiple logistic regression analysis[16]. It has been reported that the Health Assessment Questionnaire (HAQ) score is associated with serious infection in patients with RA[7,11]. Because the HAQ comprises disease activity-related and joint damage-related components[26], it is plausible that joint damage can be a risk factor for serious infection. The results of our study and those of postmarketing surveillance of infliximab in Japan[16] support this concept. Third, we found that MTX dosage was associated with increased risk of serious infection; however, this association disappeared when the unexposed and exposed groups were analyzed separately. According to some reports using cohorts much larger than ours, the immunosuppressive DMARD, such as leflunomide, cyclosporine, and azathioprine, were associated with an increased risk of infection, but MTX was not[8,27]. Others have found the use of MTX to be a risk factor for infection in patients with RA[28]. Further studies are needed to assess any association between MTX dosage and serious infection in a larger number of Japanese patients with RA.

Our study provides the first pharmacoepidemiological evidence of the safety of treatment with the TNF inhibitors infliximab or etanercept in Japanese patients with RA, compared to nonbiological DMARD. In our study cohort, treatment with infliximab or etanercept was associated with increased risk for serious infections when compared to treatment with nonbiological DMARD. The results of our study suggest that careful pharmacovigilance procedures are essential to insure safe use of TNF inhibitors in patients with RA.

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APPENDIX

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ORIGINAL ARTICLE

Pneumocystis jirovecii pneumonia associated with etanercept treatment in patients with rheumatoid arthritis: a retrospective review of 15 cases and analysis of risk factors

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Abstract
Objectives The association of anti-tumor necrosis factor therapy with opportunistic infections in rheumatoid arthritis (RA) patients has been reported. The goal of this study was to clarify the clinical characteristics and the risk factors of RA patients who developed *Pneumocystis jirovecii* pneumonia (PCP) during etanercept therapy.
Methods We conducted a multicenter, case–control study in which 15 RA patients who developed PCP were compared with 74 RA patients who did not develop PCP during etanercept therapy.
Results PCP developed within 26 weeks following the first injection of etanercept in 86.7% of the patients. All PCP patients presented with a rapid and severe clinical course and the overall mortality was 6.7%. Independent risk factors were identified using multivariate analysis and included age ≥65 years [hazard ratio (HR) 3.35, $p = 0.037$], coexisting lung disease (HR 4.48, $p = 0.009$), and concomitant methotrexate treatment (HR 4.68, $p = 0.005$).

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In patients having a larger number of risk factors, the cumulative probability of developing PCP was significantly higher ($p < 0.001$ for patients with two or more risk factors vs. those with no risk factor, and $p = 0.001$ for patients with one risk factor vs. those with no risk factor).
Conclusion Physicians must consider the possibility of PCP developing during etanercept therapy in RA patients, particularly if one or more risk factors are present.

Keywords *Pneumocystis jirovecii* pneumonia · Rheumatoid arthritis · Etanercept · Anti-TNF therapy · Opportunistic infection

Introduction

Tumor necrosis factor (TNF) plays an important role in the pathological mechanism of rheumatoid arthritis (RA) [1]. The excellent efficacy of TNF inhibitors for RA seen in various clinical trials has established TNF as a major pathogenic cytokine in RA [2–4]. TNF is one of the key molecules protecting the human body against microorganisms in vivo. The blockade of TNF with TNF inhibitors in RA patients has been associated with increased risks of opportunistic and serious infections [5–8].

In Japan, mandatory post-marketing surveillance (PMS) programs have been implemented, requiring registration and 6-month tracking of all RA patients who have received TNF inhibitors. Of 5,000 patients treated with infliximab, 13,894 patients treated with etanercept, and 3,000 patients treated with adalimumab tracked by these programs, 22 patients (0.4%) receiving infliximab, 25 patients (0.18%) treated with etanercept, and 9 patients (0.3%) treated with adalimumab developed *Pneumocystis jirovecii* pneumonia (PCP) [9–11], a rare opportunistic infectious disease seen in patients with human immunodeficiency virus (HIV) infection [12] and other immune-compromised states [13]. The incidence rate of PCP in the PMS programs in Japan was notably higher than that found in corresponding studies in the United States [14, 15].

The diagnosis of PCP in immunosuppressed patients without acquired immunodeficiency syndrome (AIDS) is challenging because of lower numbers of the organism in the lung [13]. In order to overcome this problem and to achieve prompt clinical diagnosis of PCP in non-AIDS patients who have a lower burden of *P. jirovecii* [16], several molecular techniques, such as the polymerase chain reaction (PCR) [17] and the use of serum markers, such as 1,3-β-D-glucan (BDG) [18, 19], have been developed. We, and other investigators, have performed several clinical studies of PCP using diagnostic criteria that involved these new diagnostic tools [9, 20–22].

Although the etanercept PMS program in Japan identified 25 patients with PCP, the diagnoses were based on reports from attending physicians and detailed analyses have not been implemented. Independent from the etanercept PMS program, we conducted a multicenter case–control study of PCP in RA patients treated with etanercept to delineate the clinical characteristics of PCP and identify risk factors in this population.

Patients, materials, and methods

Patients

In this study, we collected data from 21 hospitals on 28 RA patients suspected of having PCP; data were collected either through the PMS etanercept program or from voluntary case reports at scientific meetings, or from the relevant pharmaceutical company. Among these 28 patients, we identified one definitive PCP patient (patient 14) and 14 presumptive PCP patients (patients 1–13 and 15) based on the predefined criteria presented below. These 15 patients did not have other risk factors for PCP, such as malignancy, post-transplantation status, or other immunodeficiency states. We did not examine antibody for HIV because this laboratory test was not routinely conducted in clinical practice in Japan. These 15 patients were classified as the 'PCP group' in this study. The other 13 patients were not diagnosed with PCP because their data were incompatible with the diagnostic criteria for PCP and diagnoses including other infectious diseases or rheumatoid lung were considered more appropriate, and these patients were

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