Efficacy and Safety of Tacrolimus (FK506) in Treatment of Rheumatoid Arthritis: A Randomized, Double Blind, Placebo Controlled Dose-Finding Study

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ABSTRACT. ***Objective.*** To evaluate the efficacy and safety of tacrolimus (FK506) in patients with active rheumatoid arthritis (RA) exhibiting resistance to disease modifying antirheumatic drug (DMARD) therapy, and to determine the optimal dosage.

Methods. A total of 212 patients with DMARD-resistant RA were enrolled in this double blind, multicenter, randomized, placebo controlled study and allocated to 3 groups. Patients were administered tacrolimus at a dosage of 1.5 mg/day (68 patients) or 3 mg/day (70 patients), or placebo (74 patients), for 16 weeks. They were allowed to continue taking prednisolone (≤ 5 mg/day) and/or one nonsteroidal antiinflammatory drug (NSAID) during the study. Clinical assessment was based on the American College of Rheumatology (ACR) 20% criteria.

Results. ACR 20% response rates were higher in both tacrolimus groups (3 mg: 48.3%; 1.5 mg: 24.6%) than in the placebo group (14.1%), with the rate in the 3 mg group significantly higher. There were no significant differences between the tacrolimus groups and placebo group in the incidence of adverse events. The main adverse events in the tacrolimus groups, especially in the 3 mg group, were renal function abnormalities and gastrointestinal symptoms. However, no significant differences were observed among the 3 groups in the incidence of any adverse event except decrease in serum Mg level.

Conclusion. Our findings demonstrate excellent dose-dependent efficacy of tacrolimus in patients with DMARD-resistant RA and strongly suggest the usefulness of tacrolimus for treatment of RA. The optimal dosage appears to be 3 mg/day in terms of efficacy and safety. (J Rheumatol 2004; 31:243–51)

Key Indexing Terms:
RHEUMATOID ARTHRITIS FK506 TACROLIMUS CLINICAL TRIAL

Tacrolimus (FK506) is an immunosuppressive macrolide isolated from fermentation broth of *Streptomyces tsukubaensis*. It potently inhibits helper T-lymphocyte activation. Although its mode of action is similar to that of cyclosporine, the immunosuppressive effect of this macrolide is 30 to 100 times greater *in vitro* and 10 to 20 times greater *in vivo* than that of cyclosporine[1]. Tacrolimus has been approved in more than 70 countries including the United States, European countries, and Japan for prevention of allograft rejection in solid organ transplant. It is also approved for prevention of graft-versus-host disease after allogenic bone marrow transplant and for myasthenia gravis in Japan. A topical formulation of tacrolimus has recently been approved for treatment of atopic dermatitis in countries where it is prevalent. In addition, a role for tacrolimus in the treatment of other various autoimmune diseases such as systemic lupus erythematosus, psoriasis, and uveitis is supported by several studies[2-5]. Tacrolimus has been shown to inhibit transcription of the early activation genes for cytokines such as interleukin 2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ in T cells). Its immunosuppressive effect appears to be mediated, in part, through inhibition of IL-2 synthesis and release, as well as decrease in number of IL-2 receptors on activated lymphocytes[6,7].

Rheumatoid arthritis (RA) is believed to be a T cell mediated autoimmune disease characterized by an activated T cell system[8]. In addition, inflammatory cytokines such as TNF-α, IL-1ß, and IL-6 are reported to play roles in the pathogenesis of RA[9]. Anticytokine therapy in clinical studies has also provided supporting evidence of the involvement of these cytokines in RA[10,11]. Tacrolimus potently suppresses TNF-α, IL-1ß, and IL-6 production

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through T cell activation in human peripheral blood mononuclear cells, without affecting the proliferation or differentiation of normal cells, such as bone marrow cells[12,13]. Thus, specific inhibition of T cell activation and subsequent inflammatory cytokine production is considered the primary mode of action of tacrolimus in treatment of RA. In an animal model of RA, tacrolimus decreased the incidence and severity of passive arthritis induced by anti-type II collagen in rats[14]. Further, a recent study showed that tacrolimus was more effective and less toxic than methotrexate (MTX) in the treatment of rat adjuvant-induced arthritis[15], suggesting the potential usefulness of tacrolimus as an antirheumatic drug. Preliminary studies of tacrolimus in Japan and in the US indicated that tacrolimus treatment of RA patients experiencing MTX or other DMARD failure/intolerance resulted in favorable clinical and laboratory improvements[16]. In addition, a Phase II randomized double blind placebo controlled dose-finding study in the US showed tacrolimus to be effective even in the treatment of patients with RA who had failed MTX.

We report the results of a late Phase II double blind, multicenter, randomized, placebo controlled study in Japan to evaluate the efficacy and safety of tacrolimus in patients with active RA resistant to DMARD therapy and to determine the optimal dosage.

MATERIALS AND METHODS

Patient population and study design. Patients with RA of more than 6 months' duration that met the American College of Rheumatology (ACR) 1987 revised criteria for disease classification were included in the study after giving written informed consent. The inclusion criteria were (1) resistance to at least one DMARD with at least 6-month administration; (2) "active disease" (defined by the following 3 criteria: (i) erythrocyte sedimentation rate (ESR) of at least 30 mm/h or C-reactive protein (CRP) concentration of at least 1.0 mg/dl, (ii) at least 6 of 48 joints assessed as tender or painful with pressure, and (iii) at least 3 of 46 joints assessed as swollen; and (3) age between 20 and 64 years.

Patients were excluded if they had impaired renal function [serum creatinine ≥ 1.0 mg/dl or more than the upper limit of normal (ULN)] or impaired liver function [aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > ULN], hyperkalemia (serum K > ULN), heart disease or abnormal electrocardiogram (ECG) (in particular arrhythmia and change in ST/T) or a previous history of these, pancreatitis or diabetes mellitus (fasting blood sugar ≥ 110 mg/dl or postprandial blood sugar ≥ 160 mg/dl) or a history of these, malignancies or a previous history of them, severe infections, or serious drug hypersensitivity. Patients who had received 2 or more NSAID except for external application, received intraarticular, intramuscular or intravenous corticosteroids, started oral corticosteroid therapy, or received oral corticosteroids or prednisolone exceeding 5 mg/day within 4 weeks prior to baseline were also excluded.

The trial was a multicenter randomized, double blind, placebo controlled, dose-finding study. An institutional review board approved the protocol at all sites. Patients were randomly assigned to 3 treatment groups — tacrolimus 1.5 mg once daily (tacrolimus 0.5 mg capsule × 3), 3 mg UID (tacrolimus 1.0 mg capsule × 3), or placebo UID (placebo capsule × 3) — for a 16-week period. They switched their DMARD to the study medication with no DMARD washout period, and were to take the study medication after supper.

Patients were allowed to take daily fixed-dose prednisolone not exceeding 5 mg and/or one NSAID they had been taking at study entry during the entire period of the study. DMARD, additional NSAID, additional physiotherapy, and surgical treatment were prohibited.

Clinical assessments. The following clinical disease variables were measured at baseline and after 4, 8, 12, and 16 weeks: tender/swollen joint count, ESR, CRP, patient assessment of pain using the 10-cm visual analog scale (VAS), and patient/physician global assessment of disease activity by VAS. Patient's assessment of physical function (quality of well-being)[17] was measured at baseline and at Week 16.

The ECG was obtained at baseline and after 2, 4, 8, 12, and 16 weeks of study. Blood pressure and body weight were measured at baseline and at Week 16.

The following variables were measured at baseline and after 2, 4, 8, 12, and 16 weeks: complete blood cell count; serum concentrations of AST, ALT, alkaline phosphatase, γ-glutamyl transpeptidase, lactate dehydrogenase, amylase, bilirubin, creatinine (Cr), uric acid, urea nitrogen (BUN), sugar, hemoglobin A_{1c}, fructosamine, microglobulin, cholesterol, triglyceride, Mg, K, Na, Cl, Ca; and urine concentrations of protein, sugar, urobilinogen, N-acetyl-ß-glucosaminidase (NAG), and sediment.

Rheumatoid factors were measured at baseline and after 4, 8, 12, and 16 weeks. IgG, IgM, IgA, IgE, circulating immune complexes, and complement were measured at baseline and at Week 16.

If patients dropped out prior to Week 16, all assessments settled at Week 16 were measured at that time.

At each visit, the patient was asked whether adverse events including infections had occurred in the interim. All adverse events were followed up until restoration in principle. Patients who had persistent serum-Cr elevations ≥ 0.3 mg/dl above baseline levels or > 1.2 mg/dl were discontinued from the study. Similarly, patients who had persistent fasting blood sugar elevations ≥ 140 mg/dl or persistent blood sugar ≥ 200 mg/dl were discontinued. In addition to routine medical monitoring, an independent data safety monitoring board, consisting of 4 physicians, periodically reviewed trial safety data.

Statistical analysis. Continuous data are presented as the mean ± SD. For demographic and other baseline variables, the chi-square test or Kruskal-Wallis test was used to assess comparability among the 3 treatment groups. Variables that exhibited differences ($p < 0.15$) among treatments were to be incorporated as covariates in secondary analysis (logistic regression) of the primary variable to assess the effect of the imbalance. Dunnett-type multiple comparison (2 sided) was used to compare the tacrolimus groups (1.5 mg or 3 mg) and the placebo group for ACR 20% response, ACR efficacy measures, and adverse events. The Wilcoxon signed-rank test was used for comparison between baseline and each time point within groups. Statistical tests were 2 sided and findings of $p < 0.05$ were statistically significant unless otherwise specified.

The data were gathered and monitored according to Ministry of Health and Welfare of Japan regulations. Data were analyzed by statisticians at Fujisawa Pharmaceutical Co., Ltd. according to prespecified criteria.

RESULTS

Two hundred twelve patients with active RA who had been found to be resistant to DMARD therapy were enrolled in this study and randomly allocated to 3 groups. In total, 64 patients discontinued the study prematurely (Table 1). Most discontinued due to inefficacy or adverse events. The discontinuation rates due to inefficacy in the placebo, 1.5 mg, and 3 mg groups were 28.4%, 16.2%, and 14.3%, respectively. The discontinuation rates due to adverse events in the placebo, 1.5 mg, and 3 mg groups were 5.4%, 10.3%, and 4.3%, respectively.

Efficacy was evaluated for 179 patients (placebo, n = 64, 1.5 mg 57, 3 mg 58) who conformed to the protocol (per-protocol set, PPS). Table 2 shows the demographic and clin-

Table 1. Reasons for discontinuation during the study.

	Placebo, n = 74	Tacrolimus 1.5 mg, n = 68	Tacrolimus 3 mg, n = 70	Total, n = 212
No. of discontinuations (%)	30 (40.5)	18 (26.5)	16 (22.9)	64 (30.2)
Reason				
Inefficacy	21	11	10	42
Adverse events	4	7	3	14
Other	5	0	3	8

Table 2. Characteristics of the 179 study patients.

Characteristic	Placebo, n = 64	Tacrolimus 1.5 mg, n = 57	Tacrolimus 3 mg, n = 58	p*
Female/male	60/4	48/9	55/3	0.088
Age, yrs	52.3 ± 8.2	51.8 ± 6.6	49.8 ± 10.0	0.330
Body weight, kg	50.9 ± 8.2	54.2 ± 7.5	51.7 ± 8.4	0.032
Disease duration, yrs	11.0 ± 10.1	10.1 ± 6.4	8.7 ± 6.8	0.276
Tender joint count	11.8 ± 5.8	13.1 ± 8.3	13.7 ± 9.2	0.936
Swollen joint count	8.6 ± 5.6	10.6 ± 6.6	8.8 ± 6.1	0.025
ESR, mm/h	79.7 ± 28.5	69.4 ± 31.8	67.7 ± 26.2	0.019
CRP, mg/dl	5.23 ± 3.47	4.05 ± 2.94	4.40 ± 3.17	0.357
Corticosteroid use, present/absent	44/20	44/13	45/13	
No. of previous DMARD	2.1 ± 1.3	2.1 ± 1.4	2.2 ± 1.1	
Previous DMARD**				
Sodium aurothiomalate	18 (6)	14 (3)	16 (8)	
Auranofin	8 (4)	11 (1)	14 (8)	
D-penicillamine	8 (3)	9 (5)	6 (3)	
Bucillamine	33 (23)	17 (12)	23 (11)	
Lobenzarit disodium	2 (0)	1 (0)	1 (0)	
Actarit	13 (9)	12 (8)	13 (12)	
Salazosulfapyridine	20 (16)	25 (20)	23 (19)	
Methotrexate	23 (14)	21 (13)	20 (15)	
Mizoribine	8 (6)	8 (7)	9 (8)	
Cyclosporine	1 (1)	1 (1)	0 (0)	
Azathioprine	0 (0)	1 (1)	0 (0)	

* Comparison of proportion in each group. ** Values in parentheses are number of patients that used the specific DMARD most recently.

ical characteristics of the 179 patients. Although 4 variables (sex, body weight, swollen joint count, and ESR) exhibited differences among the 3 groups ($p < 0.15$), their imbalances were found to have no effect on the primary endpoint by logistic regression analysis.

The primary endpoint of efficacy was ACR 20% response rate at end of treatment. The ACR 20% response rates were 14.1%, 24.6%, and 48.3% in the placebo, 1.5 mg, and 3 mg groups, respectively (Figure 1). A statistically significant difference was observed between the 3 mg group and the placebo group ($p < 0.001$), but not between the 1.5 mg group and the placebo group ($p = 0.246$). ACR 20% response rates in the completer subset were identical to those obtained in PPS, because all ACR 20% responders completed the study. The ACR 50% response rates were 6.3%, 10.5%, and 12.1% in the placebo, 1.5 mg, and 3 mg groups, respectively, and no significant differences were observed among the 3 groups (Figure 1).

The changes from baseline in ACR efficacy measures at the end of the treatment period are shown in Table 3. In both tacrolimus groups, all variables except patient assessment of physical function and CRP were significantly improved compared with the placebo group. CRP in the 3 mg group was significantly improved compared with the placebo group.

Numbers of tender/swollen joints were significantly decreased in all 3 groups after 4 weeks of the study. The changes in CRP and ESR over the 16-week study period are shown in Figure 2. Both CRP and ESR values decreased significantly from baseline in the tacrolimus groups, but not in the placebo group. Statistically significant differences in these variables were observed at Week 16 between the placebo group and the 3 mg group.

Rheumatoid factor (RA test) and immunological variables (IgG, IgA, IgM, circulating immune complexes, and complements) were also investigated. In the 3 mg group, all

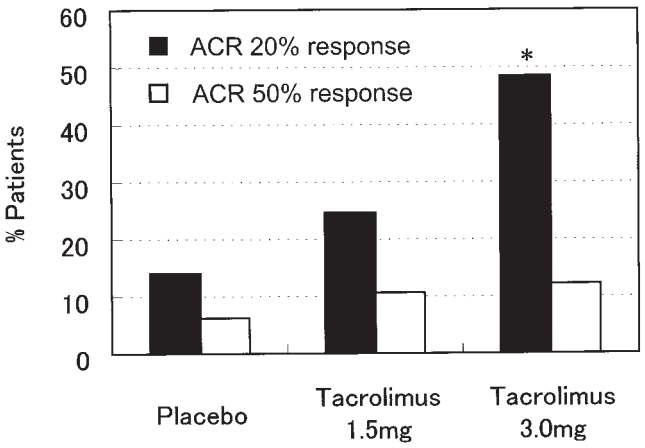

Figure 1. ACR 20% and 50% response rates at the end of treatment. *p < 0.001 vs placebo.

variables except IgM and complements were significantly improved compared with the placebo group (data not shown).

ACR 20% response rates in patients exhibiting resistance to MTX (placebo, n = 23, 1.5 mg 21, 3 mg 20) were 4.3%, 33.3%, and 40.0% in the placebo, 1.5 mg, and 3 mg groups, respectively. Statistically significant differences were observed not only between the 3 mg group and the placebo group (p = 0.008) but also between the 1.5 mg group and the placebo group (p = 0.024). ACR 20% response rates in patients exhibiting resistance to bucillamine (placebo, n = 33, 1.5 mg 17, 3 mg 23) were 15.2%, 29.4%, and 52.2% in the placebo, 1.5 mg, and 3 mg groups, respectively. Statistically significant difference was observed between the 3 mg group and the placebo group (p = 0.006), but not between the 1.5 mg group and the placebo group (p = 0.396). ACR 20% response rates in patients exhibiting resistance to sulfasalazine (placebo, n = 20, 1.5 mg 25, 3 mg 23) were 5.0%, 20.0%, and 39.1% in the placebo, 1.5 mg, and 3

Table 3. Mean changes from baseline to end of treatment for ACR efficacy measures.

Variable	Placebo		Tacrolimus 1.5 mg		Tacrolimus 3 mg	
	Baseline	Change from Baseline	Baseline	Change from Baseline	Baseline	Change from Baseline
Tender joint count	11.8 ± 5.8	–0.4 ± 7.2	13.1 ± 8.3	–3.3 ± 6.2*	13.7 ± 9.2	–5.3 ± 6.6**
Swollen joint count	8.6 ± 5.6	–1.3 ± 4.6	10.6 ± 6.6	–3.6 ± 4.5*	8.8 ± 6.1	–4.2 ± 5.5**
CRP, mg/dl	5.23 ± 3.47	0.36 ± 3.37	4.05 ± 2.94	–0.36 ± 2.05	4.40 ± 3.17	–1.77 ± 2.40***
ESR, mm/h	79.7 ± 28.5	7.1 ± 24.0	69.4 ± 31.8	–0.9 ± 19.3**	67.7 ± 26.2	–14.7 ± 19.0***
Pain, cm on VAS	61.7 ± 27.7	2.6 ± 27.9	58.7 ± 20.0	–8.9 ± 24.5**	57.5 ± 23.5	–15.0 ± 25.4***
Global assessment						
Patient	62.7 ± 23.9	3.1 ± 26.0	59.9 ± 21.9	–9.8 ± 25.8*	63.0 ± 22.4	–20.4 ± 24.6***
Physician	66.1 ± 19.0	–3.7 ± 26.4	63.4 ± 17.8	–15.8 ± 21.5**	65.4 ± 16.7	–29.6 ± 22.4***
Physical function	0.583 ± 0.094	0.001 ± 0.068	0.600 ± 0.081	0.023 ± 0.079	0.590 ± 0.099	0.013 ± 0.091

* p < 0.05, ** p < 0.01, *** p < 0.001 vs placebo.

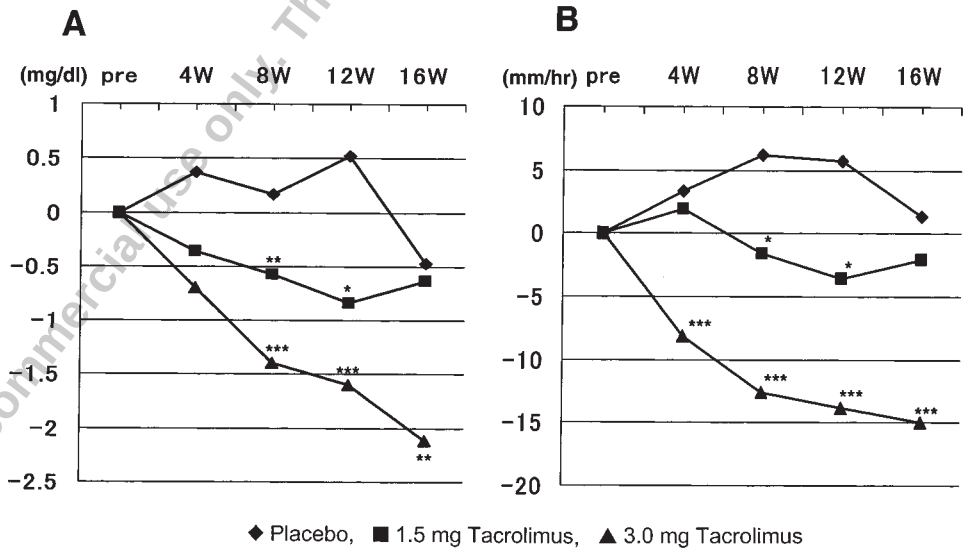

Figure 2. Mean changes in efficacy measures during the study: A. CRP values; B. ESR values. *p < 0.05, **p < 0.01, ***p < 0.001 vs placebo.

mg groups, respectively. Statistically significant difference was observed between the 3 mg group and the placebo group (p = 0.016), but not between the 1.5 mg group and the placebo group (p = 0.240).

On the other hand, ACR 20% response rates in patients exhibiting resistance to all DMARD except MTX (placebo, n = 41, 1.5 mg 36, 3 mg 38) were 19.5%, 19.4%, and 52.6% in the placebo, 1.5 mg, and 3 mg groups, respectively. Statistically significant difference was observed between the 3 mg group and the placebo group (p = 0.004), but not between the 1.5 mg group and the placebo group (p < 1.000).

The ACR 20% response rates of the intent-to-treat set (placebo, n = 66, 1.5 mg 61, 3 mg 61) were 13.6%, 23.0%, and 47.5% in the placebo, 1.5 mg, and 3 mg groups, respectively. A statistically significant difference was observed between the 3 mg group and the placebo group (p < 0.001), but not between the 1.5 mg group and the placebo group (p = 0.295). These results of the intent-to-treat set were consistent with those of PPS.

Safety was evaluated for 192 patients (placebo, n = 67, 1.5 mg 62, 3 mg 63). The incidence of adverse events in the placebo, 1.5 mg, and 3 mg groups was 46.3%, 61.3%, and 44.4%, respectively. The incidence of adverse events in the 3 groups is shown in Table 4. There were no statistically significant differences between the placebo group and the 1.5 mg group or the 3 mg group. The adverse events resulting in discontinuation are listed in detail in Table 5. There was no tendency for adverse events resulting in discontinuation to increase dose-dependently. There were 2 patients who discontinued treatment prematurely for serum-Cr, BUN, and uric acid elevation and those levels normalized within 3 weeks of discontinuing the study medication.

The most frequent adverse events were gastrointestinal symptoms and renal function abnormalities. Renal function measures are shown in Table 6. Although the average values were within the normal ranges, the incidence of abnormal changes in renal function variables in the tacrolimus groups was higher than in the placebo group (Table 7). Patients who experienced serum-Cr elevations ≥ 0.3 mg/dl above baselines (if baseline levels were ≤ 0.5 mg/dl, elevations ≥ 0.2 mg/dl above baseline were picked up) were 0.0%, 3.3% and 16.1% in the placebo, 1.5 mg, and 3 mg groups, respectively. Concerning glucose tolerance abnormality, patients who experienced fasting blood sugar ≥ 110 mg/dl or blood sugar ≥ 160 mg/dl were 41.1%, 16.7% and 19.2% in the placebo, 1.5 mg, and 3 mg groups, respectively. Patients who experienced HbA_{1C} ≥ 5.8% were 22.7%, 23.0%, and 12.9% in the placebo, 1.5 mg, and 3 mg groups, respectively.

The incidence of infections were 13.4%, 4.8%, and 0.0% in the placebo, 1.5 mg, and 3 mg groups, respectively. The infections that occurred in the 1.5 mg group were pharyngitis, upper respiratory infection, and common cold syndrome, and for each a relationship with the study drug was ruled out.

Three patients experienced 4 serious adverse events. Fever, vomiting, and staphylococcal sepsis were observed in 2 (3.0%) patients in the placebo group, and uterine fibromyoma in one (1.6%) patient in the 1.5 mg group. No serious adverse event was observed in the 3 mg group. Since the patient with uterine fibromyoma already had developed hysteromyoma, a causal relationship between uterine fibromyoma and the study drug was ruled out. Therefore, no drug related serious adverse events were observed in the tacrolimus groups. All serious adverse events remitted with appropriate countermeasures.

Table 4. Incidence of adverse events. Values in parentheses are percentages.

Characteristic	Placebo, n = 67	Tacrolimus 1.5 mg, n = 62	Tacrolimus 3 mg, n = 63
All systems	31 (46.3)	38 (61.3)	28 (44.4)
Body as a whole	4 (6.0)	5 (8.1)	1 (1.6)
Cardiovascular	1 (1.5)	1 (1.6)	0 (0)
Gastrointestinal	6 (9.0)	3 (4.8)	7 (11.1)
Heart rate and rhythm	0 (0)	1 (1.6)	2 (3.2)
Hematologic	12 (17.9)	5 (8.1)	2 (3.2)
Liver and biliary system	6 (9.0)	6 (9.7)	3 (4.8)
Metabolic and nutritional	7 (10.4)	10 (16.1)	14 (22.2)
Musculoskeletal	1 (1.5)	1 (1.6)	0 (0)
Nervous system	3 (4.5)	0 (0)	2 (3.2)
Platelet, bleeding, clotting	0 (0)	2 (3.2)	0 (0)
Psychiatric	1 (1.5)	1 (1.6)	0 (0)
Resistance mechanism	5 (7.5)	2 (3.2)	1 (1.6)
Respiratory	3 (4.5)	1 (1.6)	0 (0)
Skin and appendages	8 (11.9)	5 (8.1)	3 (4.8)
Reproductive	0 (0)	2 (3.2)	1 (1.6)
Urinary system	9 (13.4)	16 (25.8)	16 (25.4)
Vascular (extracardiac)	2 (3.0)	0 (0)	0 (0)

Table 5. Adverse events resulting in discontinuation.

	Placebo, n = 74	Tacrolimus 1.5 mg, n = 68	Tacrolimus 3 mg, n = 70
No. of Discontinuations (%)	4 (5.4)	7 (10.3)	3 (4.3)
Body as a whole			
Facial edema	1	0	0
Hot flashes	0	1	0
Swelling	0	1	0
Gastrointestinal			
Epigastric pain	0	1	0
Nausea	0	0	1
Heart rate and rhythm			
Atrial premature contraction	0	1	0
T wave inversion (ECG)	0	0	1
Metabolic and nutritional			
Elevation of serum-K	0	0	1
Musculoskeletal			
Arthralgia	0	1	0
Nervous system			
Headache	0	0	1
Light-headedness	1	0	0
Skin and appendages			
Alopecia	2	0	0
Pruritus	1	1	0
Rash	0	1	0
Urinary system			
Elevation of serum-Cr	0	1	1
Elevation of BUN	0	1	1
Elevation of uric acid	0	1	1
Vascular (extracardiac)			
Redness	1	0	0

Table 6. Renal function variables. Values are mean ± SD (n).

		Placebo	Tacrolimus 1.5 mg	Tacrolimus 3 mg
Serum Cr, mg/dl	Pre (n)	0.636 ± 0.174 (67)	0.606 ± 0.174 (62)	0.564 ± 0.155 (63)
	Post (n)	0.636 ± 0.164 (67)	0.645 ± 0.212 (61)	0.637 ± 0.184 (62)
BUN, mg/dl	Pre (n)	17.13 ± 5.39 (66)	15.55 ± 4.50 (61)	15.80 ± 5.03 (63)
	Post (n)	17.30 ± 6.23 (67)	16.90 ± 4.84 (61)	18.61 ± 6.68 (62)
Uric acid, mg/dl	Pre (n)	4.23 ± 1.08 (66)	4.43 ± 1.54 (62)	4.17 ± 1.02 (63)
	Post (n)	4.32 ± 1.16 (67)	4.73 ± 1.72 (61)	4.64 ± 1.23 (62)
Serum ß$_2$-microglobulin mg/l	Pre (n)	2.069 ± 0.753 (65)	2.011 ± 0.872 (59)	1.853 ± 0.610 (58)
	Post (n)	2.118 ± 0.791 (66)	2.021 ± 0.906 (60)	1.974 ± 0.877 (60)
Serum K, mEq/l	Pre (n)	4.20 ± 0.30 (66)	4.17 ± 0.31 (62)	4.15 ± 0.28 (63)
	Post (n)	4.20 ± 0.31 (67)	4.27 ± 0.34 (61)	4.31 ± 0.33 (62)
Serum Mg, mEq/dl	Pre (n)	2.17 ± 0.20 (64)	2.13 ± 0.22 (60)	2.16 ± 0.19 (59)
	Post (n)	2.16 ± 0.23 (65)	2.02 ± 0.25 (61)	1.96 ± 0.17 (62)
Urinary NAG, U/l	Pre (n)	10.1 ± 9.0 (60)	10.7 ± 13.5 (59)	9.7 ± 10.7 (55)
	Post (n)	9.5 ± 7.5 (64)	10.2 ± 11.4 (61)	8.7 ± 11.3 (60)

BUN: blood urea nitrogen, NAG: N-acetyl-ß-glucosaminidase.

DISCUSSION

Although several immunosuppressive agents have been approved for use for RA, either alone or in combination with other agents, there is no clear therapeutic choice for many patients[18]. Patients who fail one or more of the approved DMARD are left with a decreasing list of therapeutic options. Consequently, there is a need for newer antirheumatic therapies that can provide more options and potentially more effective treatment for this progressive disease[19]. Thus, this study was performed with patients exhibiting resistance to at least one DMARD. The high dose (3 mg/day) was determined based on the results of previous clinical studies of tacrolimus for RA. The low dose (1.5 mg/day) was set at half the high dose to determine the

Table 7. Normalized laboratory data in renal function variables. Data are mean ± SD to the upper limit of normal by patient by value.

		Placebo (%)	Tacrolimus 1.5 mg (%)	Tacrolimus 3 mg (%)
Serum Cr	Pre (n)	61.0 ± 17.5 (65)	58.4 ± 17.4 (60)	55.2 ± 18.9 (62)
	Post (n)	61.5 ± 16.5 (65)	63.1 ± 21.7 (59)	63.0 ± 22.7 (61)
BUN	Pre (n)	82.3 ± 25.3 (64)	75.6 ± 21.4 (59)	76.6 ± 24.9 (62)
	Post (n)	82.6 ± 28.9 (65)	81.8 ± 23.9 (59)	89.7 ± 32.8 (61)
Uric acid	Pre (n)	72.2 ± 18.6 (64)	73.6 ± 24.4 (60)	70.2 ± 15.3 (62)
	Post (n)	73.1 ± 18.9 (65)	78.5 ± 27.7 (59)	78.2 ± 19.4 (61)
Serum $ß_2$-microglobulin	Pre (n)	108.9 ± 40.6 (61)	104.7 ± 43.6 (56)	97.4 ± 34.8 (57)
	Post (n)	111.0 ± 42.4 (62)	105.1 ± 46.5 (56)	103.2 ± 48.6 (59)
Serum K	Pre (n)	85.7 ± 6.3 (64)	85.4 ± 6.9 (60)	84.6 ± 6.3 (62)
	Post (n)	85.4 ± 7.5 (65)	87.4 ± 7.4 (59)	88.0 ± 7.1 (61)
Serum Mg	Pre (n)	84.3 ± 12.6 (61)	82.2 ± 13.8 (58)	83.7 ± 12.9 (56)
	Post (n)	83.6 ± 13.2 (62)	77.6 ± 13.7 (59)	75.3 ± 11.2 (59)
Urinary NAG	Pre (n)	161.4 ± 154.0 (55)	195.3 ± 329.8 (54)	139.4 ± 150.9 (54)
	Post (n)	149.1 ± 119.5 (59)	170.6 ± 266.9 (56)	122.7 ± 128.7 (59)

BUN: blood urea nitrogen, NAG: N-acetyl-ß-glucosaminidase.

optimal dosage. The administration period (16 weeks) was determined based on the results of previous clinical studies, as well. The data suggested that a 16-week period would be long enough for tacrolimus to exhibit efficacy in treatment of RA.

The ACR 20% response rate was assessed with the per-protocol set as the primary endpoint of efficacy. The ACR 20% response rate increased dose-dependently (placebo: 14.1%, 1.5 mg: 24.6%, 3 mg: 48.3%) and a statistically significant difference was observed between the 3 mg group and the placebo group ($p < 0.001$), but not between the 1.5 mg group and the placebo group ($p = 0.246$). ACR 20% response rates with the intent-to-treat set also showed a statistically significant difference between the 3 mg group and the placebo group ($p < 0.001$), but not between the 1.5 mg group and the placebo group ($p = 0.295$). These results were consistent with those of the PPS. The efficacy of tacrolimus of the 3 mg group was verified, but that of the 1.5 mg group was not.

In the 3 mg group, all ACR efficacy measures except physical function assessment by the patient were significantly improved from baseline levels. Although the quality of well-being criterion is not validated in Japan, it was used in the ACR response criteria. Luckily, it seems responsive in RA and might be considered, although another functional assay would have been better. Compared with the placebo group, CRP and ESR were significantly improved after 4 weeks of this study. Tender and swollen joint counts were significantly improved after Week 12 (data not shown). In general, DMARD exhibit delayed onset of efficacy, and therapeutic effect appears several months after initiation of administration[20]. Thus, our findings suggest that the therapeutic effect of tacrolimus probably appears earlier than those of the traditional DMARD.

We found that IgG and IgA in the 3 mg group were significantly decreased from baseline, and at the end of the study these variables were significantly different from those of the placebo group, indicating that improvement of RA symptoms was due to the immunosuppressive effect of tacrolimus.

MTX, considered to be the most efficacious DMARD[21–24], has become popular as the first-line drug for RA in Japan, as it is in the US and Europe. However, in many cases MTX is not adequately effective. For patients exhibiting resistance to MTX, statistically significant differences in the ACR 20% response rate were observed not only between the 3 mg group (40.0%) and the placebo group (4.3%) ($p = 0.008$), but also between the 1.5 mg group (33.3%) and the placebo group ($p = 0.024$). In addition, in another clinical study in the US, tacrolimus was found to be effective in patients with RA who exhibited only partial response to MTX[19]. These results indicate the potent antirheumatic effect of tacrolimus in patients with resistance to MTX. This may be due to the difference in mechanisms of action between MTX and tacrolimus[13,15]. While MTX inhibits purine and pyrimidine synthesis as a result of its antifolate activity, and consequently impairs proliferation of immune cells[25], tacrolimus specifically suppresses T cell activation[6,26] and consequently inhibits the production of inflammatory cytokines such as TNF-α and IL-1ß[12].

Regarding safety, no significant differences were seen in the incidence of adverse events between the tacrolimus groups and the placebo group. In any of the adverse events, no significant difference was observed between the placebo group and the tacrolimus groups (1.5 mg group and 3 mg group) in the incidence of total adverse events. No significant difference was observed between the 1.5 mg/day group and the 3 mg/day group either. In addition, there was no

tendency for discontinuation due to adverse events to increase dose-dependently. Only one serious adverse event (uterine fibromyoma) was observed in the tacrolimus group, and a relationship between it and the study drug was ruled out because the patient had already developed hysteromyoma.

Concerning adverse drug reactions specific to tacrolimus, nephrotoxicity is well known from experience in transplant procedures. Therefore, patients who had impaired renal function were excluded from this study. In addition, since concurrent use of tacrolimus and multiple NSAID might be associated with elevated risk of nephrotoxicity, patients were allowed to take only one NSAID with tacrolimus during the study period. The incidence of serum-Cr elevations was higher in the tacrolimus group, especially in the 3 mg group, than in the placebo group. However, the average values of renal function measures including serum-Cr were within the normal ranges (Table 6). There were 2 patients who discontinued prematurely due to renal function abnormalities, and their abnormalities returned to normal speedily.

In addition to renal function abnormalities, glucose tolerance abnormality is one of the adverse events encountered in prophylaxis of rejection of transplants[27-29]. In patients with RA, steroids are frequently administered, and therefore exclusion criteria concerning glucose tolerance were used and clinical laboratory test values of glucose tolerance abilities were carefully monitored during the study. As a result, the incidence of glucose tolerance disorders in the tacrolimus groups was similar to that in the placebo group. Besides, patients who use steroid concomitantly may possibly have complications such as heart disease, and therefore exclusion criteria were established for cardiac function. In this population, 2 abnormal ECG were noted: inverted T-waves in one patient and atrial premature contractions in another. They occurred only in the tacrolimus groups and disappeared after discontinuation of the test drug. This raises the possibility that tacrolimus might be associated with cardiac complications and this should be considered in further testing of this drug.

Since tacrolimus is an immunosuppressant, the occurrence of infectious diseases was a concern. However, the infectious diseases observed in the tacrolimus groups were pharyngitis, upper respiratory tract infection, and common cold syndrome. Since a causal relationship between these infectious diseases and the study drug was ruled out, there appeared to be little possibility of occurrence of opportunistic infection. The dosage used in our study was much lower than that used in transplant procedures, and this appears to be one of the reasons for the low incidence of adverse events in this study.

Although renal function abnormalities were more frequently observed in the tacrolimus groups, most of the events remitted with appropriate countermeasures, indicating that safety of use of tacrolimus in RA therapy can be secured by taking appropriate precautions. However, it is possible that the low incidence of renal function abnormalities in this study was due to the design, by virtue of which all patients had normal renal function and taking multiple NSAID was prohibited. Therefore, in the presence of impaired renal function, tacrolimus should be used very carefully with close monitoring[16]. If serum-Cr elevations were observed, decreasing the dosage might be necessary.

Overall, the optimal dosage of tacrolimus for patients with RA exhibiting resistance to DMARD is estimated to be 3 mg/day; the results of this late Phase II study strongly suggest a significant role for tacrolimus in the treatment of RA.

Many clinical studies have been performed to investigate combination therapy with DMARD for RA[30-33], and several combinations such as MTX/hydroxychloroquine have exhibited more effectiveness than single DMARD therapy[34]. Combination therapy using cyclosporine, the mechanism of action of which is like that of tacrolimus, with MTX or hydroxychloroquine has been reported to be more effective than cyclosporine alone[35]. In addition, new DMARD such as leflunomide and etanercept are also under investigation for combined use with MTX[36,37]. Thus, combined use of tacrolimus and other DMARD may expand the use of tacrolimus.

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