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Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis

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ABSTRACT

Objectives To evaluate the ability of tacrolimus to reduce the corticosteroid dose in patients with myasthenia gravis (MG) and the drug's safety in a double-blind, placebo-controlled, parallel group study.

Methods Patients being treated with oral prednisolone at doses equivalent to 10–20 mg/day, and with stable symptoms, were randomised to tacrolimus or placebo in a 28-week double-blind study. The dose of corticosteroid was tapered with the procedures specified in the protocol. The primary efficacy endpoint was the mean daily prednisolone dose given in the last 12 weeks of the study.

Results Eighty patients received the study drug (40 patients in each group) and were included in the full analysis set. In the full analysis set, there was no significant difference in the primary efficacy endpoint between the two groups (p=0.078). However, some secondary analyses suggested the steroid-sparing effect of tacrolimus. Tacrolimus was well tolerated, and no safety concerns were noted.

Conclusions This study suggests that tacrolimus has a potential advantage as a steroid-sparing agent in the treatment of MG patients.

Clinical trial registration number NCT00309088. Name of the trial registry: FK506 Phase 3 Study: A Study for Steroid Non-Resistant MG Patients.

INTRODUCTION

Myasthenia gravis (MG) is a disease of the neuromuscular junction. It is mainly caused by an autoimmune response to the nicotinic acetylcholine receptor (AChR) and less frequently to muscle-specific tyrosine kinase in a subset of anti-AChR-negative MG.[1] In Japan, the estimated number of MG patients is 15 000 to 20 000 according to an epidemiological study conducted in 2006.[2] Corticosteroids are the main therapeutic option to control MG symptoms.[3] However, the long-term use of corticosteroids is associated with several AEs, leading to a lowered quality of life.[4–8] To prevent these AEs, there is an overwhelming need to reduce or avoid the long-term use of corticosteroids.

Tacrolimus (Prograf[R], Astellas Pharma, Tokyo, Japan) has been used as a non-steroidal immunosuppressant. This drug specifically inhibits T-cell activation via disruption of calcineurin signalling and suppresses the antigen-specific proliferation of T cells. Its inhibitory effect on autoantibody production leading to electrophysiological improvement was shown in experimental autoimmune MG in rats.[9] We reported the efficacy and safety of low-dose tacrolimus in MG patients whose symptoms cannot be controlled by corticosteroids.[10 11] Based on the study results, the use of tacrolimus has been approved in Japan in thymectomised MG patients who have not responded well to prednisolone or experienced substantial adverse effects when continuous therapy with prednisolone was administered. Recently, several studies reported that tacrolimus was administered in MG patients who had responded to corticosteroids and that it could reduce the dose of corticosteroids in this population.[12–14] If true, this could prevent or reduce AEs caused by corticosteroid therapy, resulting in an improvement in quality of life. However, these studies were not placebo-controlled, and further investigation based on a well-designed, randomised, controlled trial is recommended. We designed this double-blind, placebo-controlled study to evaluate the steroid-sparing effect and safety of tacrolimus in MG patients who have stable symptoms on maintenance doses of prednisolone.

METHODS

Study design

The study was executed as a multicentre, double-blind, placebo-controlled parallel group study. Fifty centres in Japan participated in the study (see appendices), which was conducted in accordance with Good Clinical Practice. Enrolment started in April 2006, and study-drug administration for the last patient was completed in February 2008. When oral corticosteroids other than prednisolone were used in the study, their doses were expressed as an equivalent of prednisolone.

Standard protocol approvals, registrations and patient consents

The study (http://ClinicalTrials.gov number NCT00309088) was approved by each site's local institutional review board, and written informed consent was obtained from all subjects enrolled.

Patients

We included MG patients diagnosed according to the criteria provided by the Survey and Study Group of Specified Diseases of the Autoimmune Nervous System, the Specified Disease Treatment Research Program of the Ministry of Health and Welfare (available from http://www.nanbyou.or.jp/sikkan/049_i.htm#, in Japanese). These criteria comprise histories (essential), symptoms/signs (essential) and at least one laboratory finding that supports a diagnosis of MG (Harvey–Masland test, an edrophonium test and AChR antibody assay). Patients were also clinically classified at onset using the MG Foundation of America (MGFA) Clinical Classification,[15] had to be receiving corticosteroid treatment and had to meet our definition criteria: (1) aged ≥16 and <65 years, (2) receiving oral

prednisolone at doses of 10–20 mg/day for a period of 4 weeks prior to enrolment with dose variation of prednisolone limited to 2.5 mg/day in the 12 weeks prior to the start of the study, (3) receiving pyridostigmine at doses of ≤180 mg/day or ambenonium at doses of ≤15 mg/day, and (4) being maintained, according to the above-mentioned criteria, in a state of 'minimal manifestations' (MM) based on the MGFA postintervention status classification.[15] Excluded were patients who had received intravenous steroid pulse therapy, plasmaphaeresis, intravenous immunoglobulin, radiation exposure or new administration of immunosuppressants other than corticosteroids within 12 weeks prior to the start of administration of the study drug. Any patient who had previously received tacrolimus was excluded. Patients who had undergone thymectomy (whether for thymoma or not) within 24 weeks prior to the start of administration of the study drug were excluded as well as those with thymoma requiring an operation. Pregnant and lactating women or those contemplating pregnancy were excluded from the study. Any patients who were considered by the treating physicians to be inappropriate to participate in the study were excluded.

Study period

In this study, a treatment period after starting administration of the study drug was set at 28 weeks. An improvement in muscle strength (MG score[16]) 12 to 16 weeks after treatment was demonstrated in a previous study.[10 11] Accordingly, it seemed reasonable to assume that a treatment period of 28 weeks would be sufficient to detect any difference in the corticosteroid-sparing effect between tacrolimus and placebo.

Treatment

Investigational drug

The efficacy and safety of tacrolimus had been confirmed at a dose of 3 mg once a day in a previous study.[10 11] Accordingly, three 1 mg capsules of tacrolimus or placebo, in identical capsules, were orally administered once a day after dinner for 28 weeks. Dose alterations were not permitted.

Tapering of prednisolone

In each subject, the prednisolone dose was gradually reduced by 2.5 mg/day every 4 weeks from week 4 (4 weeks after the start of tacrolimus or placebo). The dose was reduced after confirming maintenance of MM status based on the judgement of the treating physician. The prednisolone dose was tapered to 2.5 mg/day, and subsequent doses were maintained at the same level or further reduced at the treating physician's discretion. In this study, we used MM status as defined in the MGFA[15] as a basis for allowing dose reduction of prednisolone. In order to standardise symptom assessment, the following reference criteria for MM status were used: (1) no finding worse than Grade 1 for the quantitative myasthenia gravis (QMG) score in the assessment of ocular, facial, bulbar and respiratory muscles; (2) no finding worse than Grade 1 for the MG activities of daily living (MG-ADL) score; and (3) no significant increase in the dose of cholinesterase inhibitor within 1 week prior to each observation. However, the MM status was judged at the discretion of investigators, based on the MGFA Postintervention Status.[15] If the symptoms worsened, and MM status was not maintained, the prednisolone dose was immediately increased until the symptoms were judged to have recovered and stabilised at MM status. After MM status was judged to have been regained, the prednisolone dose was once again reduced.

Cholinesterase inhibitor

Cholinesterase inhibitors should have been reduced or stopped as far as possible before study entry. However, if needed, concurrent use of cholinesterase inhibitors within the usual dosage range (pyridostigmine ≤180 mg/day or ambenonium ≤15 mg/day) was permitted during the study, but the doses of these agents were not permitted to be changed so as not to influence the evaluation of the clinical trial. In exceptional circumstances, it was permissible to increase the dose temporarily in the interests of patient safety if the patient's MG symptoms worsened. QMG scores were to be measured at least 8 h after administration of a cholinesterase inhibitor to prevent any influence of cholinesterase inhibitors on the QMG assessment.

Other therapies

MG therapies were not allowed during the study including thymectomy, radiotherapy, administration of immunosuppressive agents other than corticosteroids (eg, azathioprine, cyclosporin A and mycophenolate mofetil), steroid pulse therapy, plasmaphaeresis and intravenous immunoglobulin.

Assessment

The dose of prednisolone was recorded by patients on each day. The MG-ADL score and the MGFA postintervention status were assessed by the treating physician at baseline, and at weeks 2, 4, 8, 12, 16, 20, 24 and 28. The QMG score was measured at baseline and week 28. Serum AChR antibody titres and IL-2 production were tested at baseline and weeks 4, 16 and 28. IL-2 was measured by ELISA in the cell-cultured supernatants of lymphocytes stimulated with pokeweed mitogen. The blood concentration of tacrolimus was measured at patient visits (weeks 2, 4, 8, 12, 16, 20, 24 and 28). Blood samples for the evaluation of blood concentration were taken from patients approximately 12 h after the last dose of tacrolimus. Safety was evaluated by clinical observation, 12-lead ECG, vital signs and laboratory tests. The 12-lead electrocardiogram was conducted at baseline and weeks 4, 12, 20 and 28. Vital signs were recorded and laboratory tests were performed at each visit (weeks 2, 4, 8, 12, 16, 20, 24 and 28).

Outcome measures

The prednisolone dose required to maintain MM status was periodically assessed to ascertain the steroid-sparing effect of tacrolimus. The primary endpoint was the mean daily prednisolone dose given in the last 12 weeks of the study. Regarding patients whose duration of dosing was less than 12 weeks (four patients excluded from the per protocol set (PPS)), the mean of the prednisolone doses administered in the study up to the day of discontinuation was used to calculate data (figure 1). The secondary endpoints were the mean daily prednisolone dose at each observation, total prednisolone dose during the study, the percentage of patients who achieved 75% or more dose reduction compared with the initial doses, QMG scores and MG-ADL scores. Adverse reactions were monitored for the safety evaluation.

Randomisation and blinding

The external appearance of the study drugs (tacrolimus and placebo) was identical, and the study-drug allocation and randomisation were conducted at an independent facility. At randomisation, the prednisolone dose at baseline (allotted to <15 mg/day or ≥15 mg/day), the past history of thymectomy or non-thymectomy, time elapsed after thymectomy (allotted to <1 year or ≥1 year) and histology of the thymus were

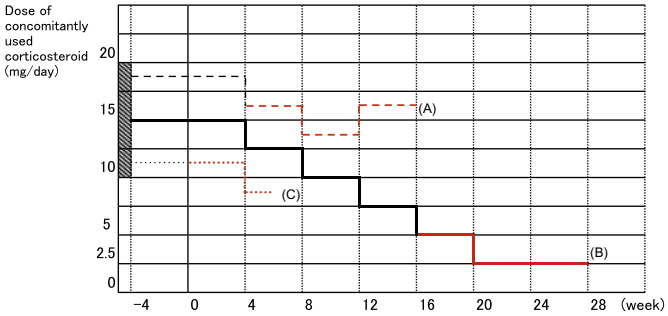

Figure 1 Examples of prednisolone tapering schemes. The primary endpoint is the mean daily prednisolone dose given in the last 12 weeks of the study. The mean daily prednisolone dose of the primary endpoint was calculated for each model case as follows: (A) the mean daily prednisolone dose during the period from week 4 to week 16 (shown in red); (B) the mean daily prednisolone dose during the period from week 16 to week 28 (shown in red); (C) the mean daily prednisolone dose during the period from week 0 (day 1) to week 6 (shown in red).

considered as adjustment factors for dynamic allocation. In addition, blood concentration of tacrolimus, anti-AChR antibody titre and IL-2 production were measured in an independent facility. Until the randomisation code was broken, the results of blood analyses were not revealed to study personnel or patients.

Statistical methods

Efficacy analyses were performed using the full analysis set (FAS), which was defined as patients who were randomised to the study and took at least one dose of either tacrolimus or placebo, and data from 80 patients were analysed. As a secondary analysis, the primary endpoint (the mean daily prednisolone dose given in the last 12 weeks of the study) was analysed in the PPS, which was defined as patients fulfilled the inclusion/exclusion criteria and took either tacrolimus or placebo for 12 weeks or more, and data from 76 patients were analysed. For the endpoint of mean daily prednisolone dose, analysis of covariates (ANCOVA) was conducted using the mean daily prednisolone dose at baseline and the presence or absence of thymectomy as covariates. Logistic regression analysis was used to evaluate categorical variables. All statistical tests were two-sided, and p values of <0.05 were considered significant. The planned enrolment of 40 patients per group gave approximately 90% power to detect a difference between groups of 3.5 mg/day in the mean daily prednisolone dose in the last 12 weeks of this 28-week study based on the assumption that the difference between the two groups would be 5 mg/day on week 28, at a 5% significance level using a two-sided test, and assuming an SD of 5.0 mg/day.

RESULTS

Patient disposition

A total of 80 patients were enrolled: 40 were allocated to the tacrolimus group and 40 to the placebo group at randomisation. Of the 80 patients who received either the study drug or placebo and were included in the FAS, 73 patients (38 tacrolimus and 35 placebo) completed the 28-week study, and seven patients (two tacrolimus and five placebo) discontinued the study (figure 2). Seventy-six patients (38 for tacrolimus and 38 for placebo) were included in the PPS by excluding the patients whose treatment duration was shorter than 12 weeks. Of the two patients excluded from the PPS in the tacrolimus group, one required hospitalisation for surgery of appendicitis (withdrawn on day 39), and the other withdrew consent due to insomnia (withdrawn on day 36). Of the two patients excluded from the PPS in the placebo group, one did not take the correct number of capsules (withdrawn on day 8) and the other withdrew consent (withdrawn on day 57).

We excluded these four patients (two in the tacrolimus group, two in the placebo group, as mentioned above) from the PPS because they were not eligible for the assessment of steroid-sparing effect. The reasons are as follows: they had stable disease, there was no possibility that the prednisolone dose could be reduced, or the degree of dose reduction was insufficient.

The patients' backgrounds were similar in the tacrolimus group and the placebo group, as were the mean daily prednisolone dose, the mean of the QMG scores and the mean of the

Figure 2 Patient flow chart and reasons for withdrawal from the study. FAS, full analysis set; †Excluded from the per protocol set. ‡One of the two was excluded from the per protocol set. MG, myasthenia gravis.

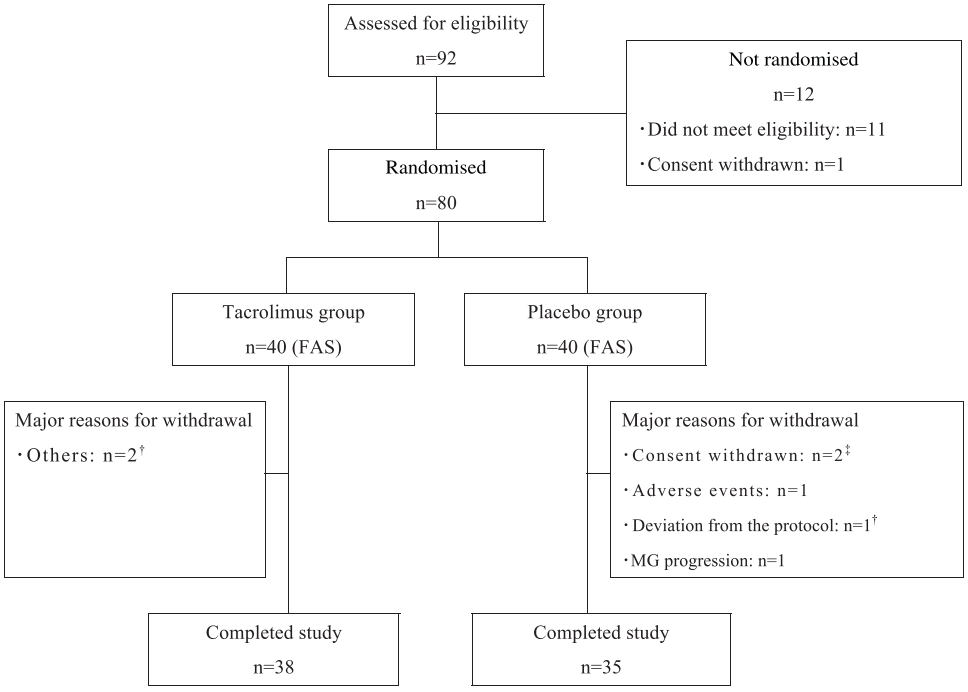

Table 1 Patients enrolled in the study (full analysis set)

	Tacrolimus (n=40)	Placebo (n=40)
Gender		
Male	17 (42.5%)	13 (32.5%)
Female	23 (57.5%)	27 (67.5%)
Age, years, mean (SD)	45.9 (11.50)	44.4 (12.36)
Duration from myasthenia gravis onset to the entry, years, mean (SD)	7.41 (9.016)	7.94 (9.540)
Myasthenia Gravis Foundation of America classification at the onset		
I	14 (35.0%)	11 (27.5%)
II	21 (52.5%)	22 (55.0%)
III	4 (10.0%)	4 (10.0%)
IV	0 (0.0%)	2 (5.0%)
V	1 (2.5%)	1 (2.5%)
Thymectomy	28 (70.0%)	30 (75.0%)
Years since thymectomy, mean (SD)	6.56 (7.474)	6.73 (8.484)
Thymoma	12 (30.0%)	8 (20.0%)
Acetylcholine receptor antibody-positive (>0.3 nmol/l)	28 (70.0%)	29 (72.5%)
Prednisolone dose at the entry, mg/day, mean (SD)	13.78 (3.958)	13.88 (3.545)
Coadministration of cholinesterase inhibitor	24 (60.0%)	23 (57.5%)
Total quantitative myasthenia gravis score at the entry, mean (SD)	4.7 (3.74)	4.8 (3.42)
Total myasthenia gravis activities of daily living score at the entry, mean (SD)	1.8 (1.53)	1.6 (2.31)

Values are n (%) unless otherwise indicated.

MG-ADL scores at baseline (table 1). The mean duration of dosing was 185.5±34.79 days in the tacrolimus group and 182.2±36.83 days in the placebo group (mean±SD).

Efficacy

The efficacy results are shown in table 2. The primary endpoint could not be achieved. In the FAS, the mean daily prednisolone doses (mg/day) for the last 12 weeks of the study were 4.91 mg/day in the tacrolimus group and 6.51 mg/day in the placebo group at the primary endpoint, showing no significant difference between the two groups (difference; 95% CI −1.58; −3.342 to 0.184, p=0.078). However, in the PPS (38 patients in each group: reduction in number of patients was due to the exclusion of four patients with an administration period of less than 12 weeks; defined as early withdrawal in the protocol), the mean daily prednisolone dose was 4.45 mg/day in the tacrolimus group and 6.19 mg/day in the placebo group (difference; 95% CI −1.68; −3.323 to −0.033, p=0.046).

Figure 3 shows the changes in prednisolone dose by patient. Doses decreased with time in many patients in both the placebo group and the tacrolimus group until week 20. After week 20, doses tended to increase in many patients in the placebo group, while they continued to decrease in many patients in the tacrolimus group until weeks 24 to 28.

Figure 4 shows the mean daily prednisolone dose recorded every 4 weeks in both groups. The doses decreased with time until week 20 in both groups. After week 20 (eg, weeks 20–24 and weeks 24–28), however, the mean daily prednisolone dose increased with time in the placebo group, whereas it kept decreasing in the tacrolimus group. The mean prednisolone dose (mg/kg) in the last 4 weeks was 3.81 mg/day in the tacrolimus group and 7.23 mg/day in the placebo group (difference; 95% CI −3.48; −6.010 to −0.953, p=0.008) (table 2). The total prednisolone dose given during the trial in the tacrolimus group was slightly smaller than that of the placebo group (difference; 95% CI −143.96; −303.497 to 15.576, p=0.076) (table 2). With regard

Table 2 Results of efficacy endpoints

Endpoints	Tacrolimus	Placebo	(Tacrolimus−placebo) or OR	95% CI	p Value
Mean prednisolone dose (mg/day)					
Last 12 weeks*	4.91 (4.041)	6.51 (4.889)	−1.58‡ (−3.342 to 0.184)		0.078¶
Last 12 weeks†	4.45 (3.441)	6.19 (4.770)	−1.68‡ (−3.323 to −0.033)		0.046¶
Last 4 weeks*	3.81 (4.066)	7.23 (7.319)	−3.48‡ (−6.010 to −0.953)		0.008¶
Percentage of patients who achieved a prednisolone dose reduction of 75% or more, % (n)					
Last 12 weeks*	50.0 (20)	37.5 (15)	1.93§ (0.651 to 5.751)		0.235††
Last 4 weeks*	67.5 (27)	45.0 (18)	2.85§ (1.082 to 7.518)		0.034††
Total prednisolone dose* (mg)	1457.63 (677.948)	1590.79 (746.004)	−143.96‡ (−303.497 to 15.576)		0.076**
Quantitative myasthenia gravis score* (end of study)	4.4 (3.62)	5.8 (5.09)	−1.3‡ (−3.26 to 0.65)		0.187¶
Myasthenia gravis activities of daily living score* (end of study)	1.2 (1.33)	2.3 (3.00)	−1.0‡ (−2.07 to 0.02)		0.054¶

Values are mean (SD).
*Full analysis set (Tacrolimus: n=40; placebo: n=40).
†Per protocol set (Tacrolimus: n=38; placebo: n=38).
‡Difference in adjusted means.
§OR.
¶Covariate analysis using the prednisolone dose at baseline and thymectomy as covariates.
**Covariate analysis using the prednisolone dose at baseline, thymectomy and the duration of study drug administration as covariates.
††Logistic regression analysis using the prednisolone dose at baseline and thymectomy as covariates.

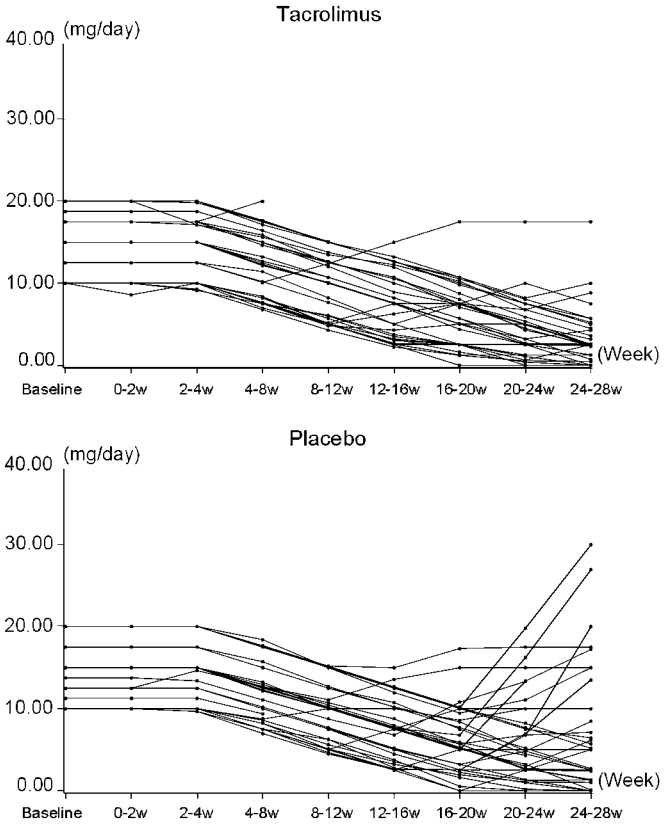

Figure 3 Prednisolone dose at each time point by patient. Each patient is indicated as a single line.

to the mean daily prednisolone dose in the last 4 weeks, the percentage of patients who achieved 75% or more dose reduction compared with their initial doses was 67.5% in the tacrolimus group and 45.0% in the placebo group (OR; 95% CI 2.85; 1.082 to 7.518, p=0.034), while 50.0% of the tacrolimus group and 37.5% of the placebo group achieved 75% or more dose reduction in the last 12 weeks (OR; 95% CI 1.93; 0.651 to 5.751, p=0.235) (table 2). In the present study, patients whose symptoms had been stable in the MM state were included, so the mean QMG and MG-ADL scores at baseline were low. The QMG and MG-ADL scores at final observation in the tacrolimus group were slightly lower than that in the placebo group (table 2).

Anti-AChR antibody titre and IL-2 production were similar between the tacrolimus and placebo groups throughout the study (data not shown). The mean tacrolimus level in whole blood of the patients receiving tacrolimus was stable throughout the study, with the mean level in the entire period being 3.406±2.064 ng/ml. We also compared the efficacy between the group with relatively low tacrolimus blood levels (lower than 5 ng/ml) and the group with high tacrolimus blood levels (5 ng/ml or higher). No correlation between blood level and drug efficacy was found.

Safety

Thirty-five patients (87.5%) in the tacrolimus group and 32 patients (80.0%) in the placebo group had adverse drug reactions during the study. No deaths occurred in either group. The commonly observed adverse drug reactions in this study are shown in table 3. For tacrolimus, the most frequent adverse drug reactions were nasopharyngitis, increased white-blood-cell count, upper-respiratory-tract inflammation, glucose urine, increased glycosylated haemoglobin and muscle spasms. Adverse drug reactions that led to study discontinuation in the tacrolimus group were appendicitis and insomnia in one patient each. Serious adverse drug reactions were appendicitis and sudden hearing loss in one patient each in the tacrolimus group, and herpes zoster in one patient in the placebo group. All serious adverse drug reactions resolved with treatment.

Two patients in the placebo group experienced MG progression, which led to the patients' withdrawal. Of the two, one was admitted after withdrawal to receive plasmaphaeresis (withdrawn on day 182), and the other was withdrawn due to consent withdrawal after MG progression (withdrawn on day 150).

DISCUSSION

We now consider MG to be a chronic autoimmune condition that requires long-term immunotherapy. Therapy should therefore both be effective and cause few adverse events. Corticosteroid administration, even at doses lower than 7.5 mg/day, may increase the frequency of adverse drug reactions.[8] Determination to decrease steroid-related complications has led us to combine other means of immunosuppression with prednisolone.[3] The effects of immunosuppressants can largely be classified into three categories: inhibition of the cell cycle (such as azathioprine, cyclophosphamide and mycophenolate mofetil),

Figure 4 Mean prednisolone doses of the tacrolimus and placebo groups at each time point. The black squares indicate the mean prednisolone doses of patients receiving tacrolimus. The white diamonds indicate the mean prednisolone doses of patients receiving placebo. The vertical bars indicate the range of SD. The doses of prednisolone decreased with time until week 20 in both groups. After week 20, however, the mean daily prednisolone dose increased with time in the placebo group, whereas it kept decreasing in the tacrolimus group (analysis of covariates, ANCOVA).

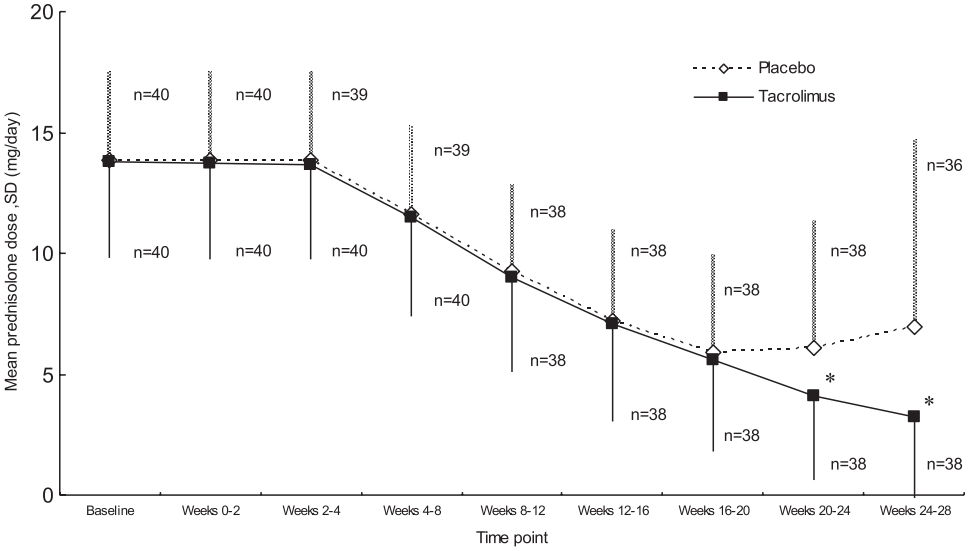

Table 3 List of adverse drug reactions occurring in 5% or more patients in each group and serious adverse drug reactions occurring in both groups

	Tacrolimus, n (%)	Placebo, n (%)
Adverse drug reactions		
Nasopharyngitis	10 (25.0)	12 (30.0)
Upper-respiratory-tract inflammation	5 (12.5)	2 (5.0)
White-blood-cell count increased	5 (12.5)	1 (2.5)
Glucose urine present	4 (10.0)	3 (7.5)
Glycosylated haemoglobin increased	4 (10.0)	1 (2.5)
Muscle spasms	4 (10.0)	0
Blood triglycerides increased	3 (7.5)	1 (2.5)
Diarrhoea	2 (5.0)	2 (5.0)
Pollakiuria	2 (5.0)	2 (5.0)
Pharyngitis	2 (5.0)	1 (2.5)
Insomnia	2 (5.0)	1 (2.5)
β-*N*-Acetyl-D-glucosaminidase increased	2 (5.0)	1 (2.5)
Cellulitis	2 (5.0)	0
Herpes simplex	2 (5.0)	0
Blood glucose increased	2 (5.0)	0
Blood uric acid increased	2 (5.0)	0
Protein total decreased	2 (5.0)	0
White-blood-cell count decreased	2 (5.0)	0
Dermatitis	2 (5.0)	0
Headache	1 (2.5)	5 (12.5)
Alanine aminotransferase increased	1 (2.5)	3 (7.5)
Gastroenteritis	1 (2.5)	2 (5.0)
Neutrophil count increased	1 (2.5)	2 (5.0)
Dizziness	1 (2.5)	2 (5.0)
Anaemia	1 (2.5)	2 (5.0)
Thirst	0	2 (5.0)
Pharyngolaryngeal pain	0	2 (5.0)
γ-Glutamyltransferase increased	0	2 (5.0)
Lymphocyte count decreased	0	2 (5.0)
Serious adverse drug reactions		
Appendicitis	1 (2.5)	0
Sudden hearing loss	1 (2.5)	0
Herpes zoster	0	1 (2.5)

immunosuppression of T cells and B-cell depletion (such as rituximab).[17] There is limited clinical evidence of the effectiveness of immunosuppressants, because of difficulties with study design and recruitment of patients in sufficiently large numbers.

In our study, the primary endpoint (the mean prednisolone dose given in the last 12 weeks) in PPS and other secondary endpoints in FAS suggested the steroid-sparing effect of tacrolimus, although analysis of the primary endpoint for the FAS failed to demonstrate a steroid-sparing effect of tacrolimus. In this study, four patients of the FAS were excluded from PPS analyses. They discontinued the study before completing the target 12-week prednisolone administration period for evaluation of the primary endpoint. The prednisolone dose in these patients was calculated using the mean of the prednisolone doses administered in the study up to the day of discontinuation (as shown in figure 1). For this reason, the data for FAS analysis include the data of those who did not sufficiently experience the prednisolone tapering period. The use of PPS may maximise the opportunity for both the tacrolimus and placebo groups to show efficacy, and most closely reflects the model underlying the protocol.

The failure to reach the primary efficacy endpoint can be attributed to the following reasons. First, in the course of prednisolone tapering, the mean daily doses of prednisolone were similar between the tacrolimus and placebo groups in the first 20 weeks of the study (figures 3, 4). This finding reflects the possibility that the prednisolone doses at baseline may have been higher than was necessary to maintain the MM state. This could obscure or delay the detection of the efficacy of tacrolimus. Second, the worsening of MG symptoms developed more slowly than expected. The possible explanation is that the time that needs to elapse from the restimulation of anti-AChR antibody production to the point where the safety margin of synaptic transmission was exceeded may be at least 20 weeks. Third, the heterogeneous study population including ocular MG may have precluded demonstration of benefit to selected patients and obscured the effect on responsive patients. Fourth, the long disease duration (about 7.5 years on average) prior to entry may have resulted in the selection of patients less responsive to prednisolone reduction. These four considerations are supported by the fact that the dose of prednisolone could be reduced in study subjects including placebo group patients, and the mean prednisolone dose was beginning to diverge from that of the tacrolimus group 20 weeks after treatment. This suggests that a longer study with a homogenous study population of generalised MG patients with a short disease duration prior to entry, and who have been administered the minimum required corticosteroid to control symptoms, may be more suitable for detecting a clearer difference between tacrolimus and placebo.

We also conducted subgroup analyses using the presence or absence of thymectomy, positive or negative for anti-AChR antibodies at baseline, presence or absence of thymoma history, gender, age, height, body weight, disease period, severity, complications and presence or absence of cholinesterase inhibitor administration as variables (table 4). Although these analyses are exploratory, and the sample size of each subgroup was small, there were statistically significant differences between tacrolimus and placebo in subgroups of thymoma history (+) and body weight (≥59.35) for the mean dose of prednisolone during the last 12 weeks of treatment.

Serious adverse drug reactions such as nephrotoxicity and hypertension reported with the use of other calcineurin inhibitors did not appear in the present study.[18] The lack of rare adverse drug reactions is possibly attributable to the small sample size in this study. Transient muscle spasms noted in four patients may be due to tacrolimus-related hypomagnesaemia, but this was not confirmed by laboratory testing. According to Tindall *et al*, ciclosporin A should be used carefully in the treatment of MG because long-term administration of ciclosporin for more than 12 months may induce nephrotoxicity.[18] In this 28-week study, incidence of nephrotoxicity was low in the tacrolimus group; however, a further examination of the results of the longer-term study will be necessary.

Calcineurin inhibitors (tacrolimus and ciclosporin A) have several effects on cellular function besides suppression of interleukin-2 production in T cells.[19] Immunophilin ligands such as tacrolimus, ciclosporin A and rapamycin inhibit the glucocorticoids transporter function of P-glycoprotein, and therefore increase the intracellular concentration of steroids in experimental settings.[20 21] Recently, a similar phenomenon was observed in peripheral blood mononuclear cells of MG patients.[22] In addition, tacrolimus was found to increase the ability of glucocorticoid receptor to bind hormone through the FKBP 51/PP5 interchange.[21] This displacement does not occur with ciclosporin A. Although tacrolimus augments the activation-induced programmed cell death of T cells, ciclosporin A treatment did not enhance this phenomenon in thymocytes and peripheral T cells.[23] A further benefit of tacrolimus has been

Table 4 Prednisolone dose in subgroups

Subgroup	Tacrolimus	Placebo	Tacrolimus−placebo* (95% CI)	p Value
Thymectomy				
+	4.87 (28)	5.82 (30)	−1.07 (−3.106 to 0.970)	0.298‡
−	5.01 (12)	8.57 (10)	−3.03 (−6.899 to 0.843)	0.118‡
Acetylcholine receptor antibody (nmol/l)				
+ (>0.3)	4.61 (28)	6.31 (29)	−1.22 (−3.380 to 0.941)	0.263§
− (≤0.3)	5.63 (12)	7.05 (11)	−2.29 (−6.771 to 2.187)	0.298§
Thymoma history				
+	3.59 (12)	6.79 (8)	−3.03 (−5.548 to −0.508)	0.021§
−	5.48 (28)	6.44 (32)	−1.07 (−3.319 to 1.177)	0.344§
Gender				
Male	5.03 (17)	6.51 (13)	−1.83 (−5.360 to 1.692)	0.295§
Female	4.83 (23)	6.51 (27)	−1.31 (−3.448 to 0.827)	0.223§
Age (years)†				
<46.0	4.81 (19)	7.05 (20)	−2.07 (−4.952 to 0.821)	0.155§
≥46.0	5.01 (21)	5.98 (20)	−0.85 (−3.143 to 1.447)	0.459§
Height (cm)†				
<162.15	4.06 (17)	6.58 (23)	−1.16 (−3.773 to 1.451)	0.373§
<162.15	5.55 (23)	6.42 (17)	−1.85 (−4.571 to 0.875)	0.177§
Body weight (kg)†				
<59.35	4.90 (18)	6.14 (22)	−0.13 (−2.582 to 2.315)	0.913§
≥59.35	4.93 (22)	6.96 (18)	−3.14 (−5.942 to −0.339)	0.029§
Disease period (years)†				
<3.88	5.26 (22)	5.76 (18)	−0.91 (−3.106 to 1.280)	0.404§
≥3.88	4.49 (18)	7.13 (22)	−1.68 (−4.621 to 1.269)	0.256§
Myasthenia Gravis Foundation of America clinical classification at the onset				
Class I	4.10 (14)	4.11 (11)	−0.23 (−2.486 to 2.031)	0.836§
Class II–V	5.35 (26)	7.42 (29)	−2.00 (−4.370 to 0.377)	0.097§
Complications				
+	5.04 (35)	6.48 (36)	−1.71 (−3.605 to 0.175)	0.075§
−	4.05 (5)	6.78 (4)	−2.65 (−13.159 to 7.851)	0.545§
Daily usage of cholinesterase agent				
+	4.82 (24)	7.58 (23)	−2.34 (−5.018 to 0.346)	0.086§
−	5.06 (16)	5.07 (17)	−0.12 (−2.192 to 1.943)	0.903§

Values are the mean prednisolone doses given in the last 12 weeks, mg/day (n).
*Difference in adjusted means.
†Continuous data were categorised using the median at baseline.
‡Covariate analysis using the prednisolone dose at baseline as covariates.
§Covariate analysis using the prednisolone dose at baseline and thymectomy as covariates.

reported in anti-ryanodine receptor antibody-positive patients mostly with associated thymoma, a finding that potentially attributes the improvement of contractile fatigue to enhancement of ryanodine receptor-mediated sarcoplasmic calcium release.[24] Immunologically, peripheral blood mononuclear cells from MG patients treated with prednisolone plus tacrolimus produced a higher concentration of interleukin-10 than the cells from patients treated with prednisolone alone.[25] One of the pharmacological actions of tacrolimus may be to bring about immunomodulating effects on regulatory T cells. The above findings indicate that there may be an as-yet undiscovered mechanism of action of tacrolimus in MG patients.

In summary, this study demonstrated that tacrolimus may have a steroid-sparing effect along and confirmed its safety and tolerability. While there have been several reports on the treatment of MG with tacrolimus, this is the first placebo-controlled randomised study. Further study is required to show long-term effectiveness and safety. We are now conducting an extension study in patients who completed this study to confirm the safety of long-term administration.

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APPENDICES

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