Efficacy and Safety of Tacrolimus in Myasthenia Gravis: A Systematic Review and Meta-analysis

Zuojie Zhang[1,2,3,*], Chunsong Yang[1,2,*], Lingli Zhang[1,2], Qiusha Yi[3], Zilong Hao[4]

[1]Department of Pharmacy, Evidence-based Pharmacy Center, West China Second Hospital, Sichuan University, [2]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Sichuan University, [3]West China School of Pharmacy, Sichuan University, [4]Department of Neurology, West China Hospital, Sichuan University, Sichuan, P.R China

[*]Zuojie Zhang and Chunsong Yang contributed equally to this study

Abstract

Aims: This study was designed to determine whether treatments with tacrolimus would provide benefit for patients with myasthenia gravis (MG). **Materials and Methods:** The databases of Medline, EMBASE, the Cochrane Library, and four Chinese databases were searched for eligible studies. Weighted mean differences and standardized mean differences (SMD) with corresponding 95% confidence intervals (CIs) were used to summarize the primary outcome, namely, steroid-sparing effect of tacrolimus in maintaining minimal manifestations, and the secondary outcome, namely, the effect of tacrolimus in reducing the severity of MG, respectively. **Results:** After systematic retrieval, 13 researches with two randomized controlled trials (RCTs) and 11 prospective open-label single-arm clinical trials were included in the study. For the primary outcome of two RCTs, one RCT which was followed up for 1 year showed a positive effect and the other RCT which was associated with treatment duration of 28 weeks showed a negative result. For the secondary outcome, meta-analyses of other 11 trials showed a benefit effect, overall. For the quantitative MG (QMG) score, there were significant differences with high heterogeneity (SMD: 2.93; 95% CI: 1.14–4.73; I^2 = 86%). In contrast, for MG activities of daily living (MGADL) score, it was reduced by tacrolimus with significant SMD and less heterogeneity (SMD: 0.59; 95% CI: 0.33–0.85; I^2 = 7%). Adverse effects were mentioned as mild. **Discussion:** The opposite results of two RCTs showed that tacrolimus with enough treatment duration might have positive steroid-sparing effect. The most possible cause of heterogeneity in the outcome of QMG score between trials was the baseline severity of MG. **Conclusion:** The above finding suggests that there might be a potential beneficial role with no serious side effects of tacrolimus, and additional better RCTs including larger sample sizes and long-term study are needed to confirm or refute the results.

Keywords: Efficacy, meta-analysis, myasthenia gravis, safety, tacrolimus

Introduction

Myasthenia gravis (MG) is an autoimmune disorder in which antibodies reduce the available functional nicotinic acetylcholine receptors, thereby impairing neuromuscular transmission,[1] and prevalence rate of which is approximately 1 in 10,000–50,000 every year.[2]

Over the past 40 years, changing treatment modalities have obviously reduced mortality and severity of MG. Thymectomy may be useful in selected people. Treatment options include steroids, azathioprine, cyclosporin, cyclophosphamide, methotrexate, and intravenous immunoglobulin.[3] Moreover, corticosteroids might be the relative effective intervention to slow down the development of MG.[4] However, corticosteroids would bring many side effects if patients received them for a long time.[5] Recently, to overcome this condition, tacrolimus (FK506) has been available for MG.

Tacrolimus is a macrolide immunosuppressant,[6] which inhibits the production of interleukin-2 (a T-cell activation factor).[7] Recently, it has been successfully used for preventing organ rejection in organ transplantation.[8] Furthermore, some

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Address for correspondence: Prof. Lingli Zhang, West China Second University Hospital, Sichuan University, No. 20, Third Section, Renmin Nan Lu, Chengdu, Sichuan - 610041, P.R.China. E-mail: zhlingli@sina.com

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researchers observed that low-dose tacrolimus could be used as an effective treatment for *de novo* MG with no significant side effects.[9-11] However, several other studies found that tacrolimus yielded no significant effects.[12,13] The overall efficacy and safety of tacrolimus in patients suffering from MG is unknown. Although one review has described the role of tacrolimus,[14] no meta-analysis has been performed. Meta-analysis could quantitatively pool all the available evidences and evaluate whether the efficacy of tacrolimus in some trials was stochastic or systematic. Based on these truths, we designed this first meta-analysis to assess whether tacrolimus could successfully control the development of MG with safety.

Materials and Methods

Data sources

A systematic literature review was conducted from 1976 to May 2016, with the help of Medline, EMBASE, Cochrane Library, Chinese Biomedical Literature Database, China Knowledge Resource Integrated Database, VIP, and Wangfang using key words: tacrolimus and MG. all relevant references cited in eligible articles were also retrieved.

Inclusion/exclusion criteria

There were some inclusion standards with regard to choosing eligible studies: the study (1) focused on clinical efficacy and safety of tacrolimus in MG and published in English or Chinese; (2) were prospective studies; (3) reported any of the primary and secondary outcomes. Primary outcome was defined as steroid-sparing effect of tacrolimus in maintaining "Minimal Manifestations (MM)" of Myasthenia Gravis Foundation of America (MGFA) postintervention status,[15] measured as dose of steroid. Secondary outcome was defined as the efficacy of tacrolimus in reducing the severity of MG, which was measured by any of the MG severity scales (a validated quantitative MG [QMG] score for disease severity;[15] MG activities of daily living score [MGADL];[16] a test to evaluate muscular strength [TEMS];[17] and clinical absolute evaluation method [CAEM][18]). The reporting of secondary outcome should include sufficient data for estimating the change means and standard deviations from with tacrolimus to without.

Studies were excluded from the analysis if they used overlapping data published by the same author.

Data extraction

The following informations were extracted from each study independently by two trained investigators using a predefined data extraction form: the first author's name, the year of publication, study design, clinical situation, age of participants, sample size, disease duration, interventions, outcome definition, and adverse events. The methodological quality of included randomized controlled trials (RCTs) was independently assessed by two trained investigators according to Cochrane Handbook for Systematic Reviews of Interventions.[19]

A third investigator coordinated the disagreement between the above two investigators.

Statistical analyses

Meta-analyses were conducted with the help of Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration). For the primary outcome measure, the doses of same kinds of steroid administered at the end point to maintain MM were used to compute the weighted mean differences (WMDs) and corresponding 95% confidence intervals (CIs). The changes in score (QMG score, MGADL, TEMS, and CAEM scores) were used to compute the standardized mean differences (SMDs) and corresponding 95% CIs. Statistical heterogeneity was assessed with the help of the Q statistic and I^2 statistic. If $I^2 < 50\%$, the WMD and SMD were pooled according to the fixed effects model, otherwise random effects model will be applied. Sensitivity analyses were conducted as well. Besides, if any of the outcomes included over ten studies, funnel plots were used visually to demonstrate publication bias.[19]

Results

Study description

The search yielded 18 studies. Finally, 13 researches[11,12,20-30] were brought into analyses [Figure 1], of which only two were RCTs and others were prospective open-label single-arm clinical trials. The clinical characteristics of the included studies are registered in Tables 1 and 2. Five studies were not included because of either inapposite study design or incomplete data to calculate WMD or SMD of outcome measures.[31-35]

A total of 495 patients were included. The median age was 44.58 years and median treatment period was 13.6 months. There was no generally accepted schedule for tacrolimus application, and a fixed daily dose of 3 mg was most frequently used.

Study quality of randomized controlled trials

Overall, for the only two RCTs, the quality of trial conducted by Yoshikawa *et al*. was better than that of Nagane *et al*. The trial conducted by Yoshikawa *et al*. had been registered in the network of ClinicalTrial.gov (Clinical trial registration number: NCT00309088), and it was a double-blind, placebo-CT. The section of random sequence generation, allocation concealment and binding was rated as low. However, the trial by Nagane *et al*. was an unblinded and nonplacebo CT without registration in advance and the participants were randomly and reciprocally selected to receive treatment with or without FK506. Hence, it was judged to be prone to a high risk of bias in the section of random sequence generation and blinding. Other risk biases of included RCTs were displayed in Table 3.

Meta-analysis

Steroid-sparing effects

The only two RCTs (Numbers of patient = 114) investigated the steroid-sparing effect of tacrolimus; however, we could not pool the primary outcome since the duration of these two studies was very different from each other. The study by Yoshikawa *et al*. was a 28-week double-blind trial, while the study by Nagane

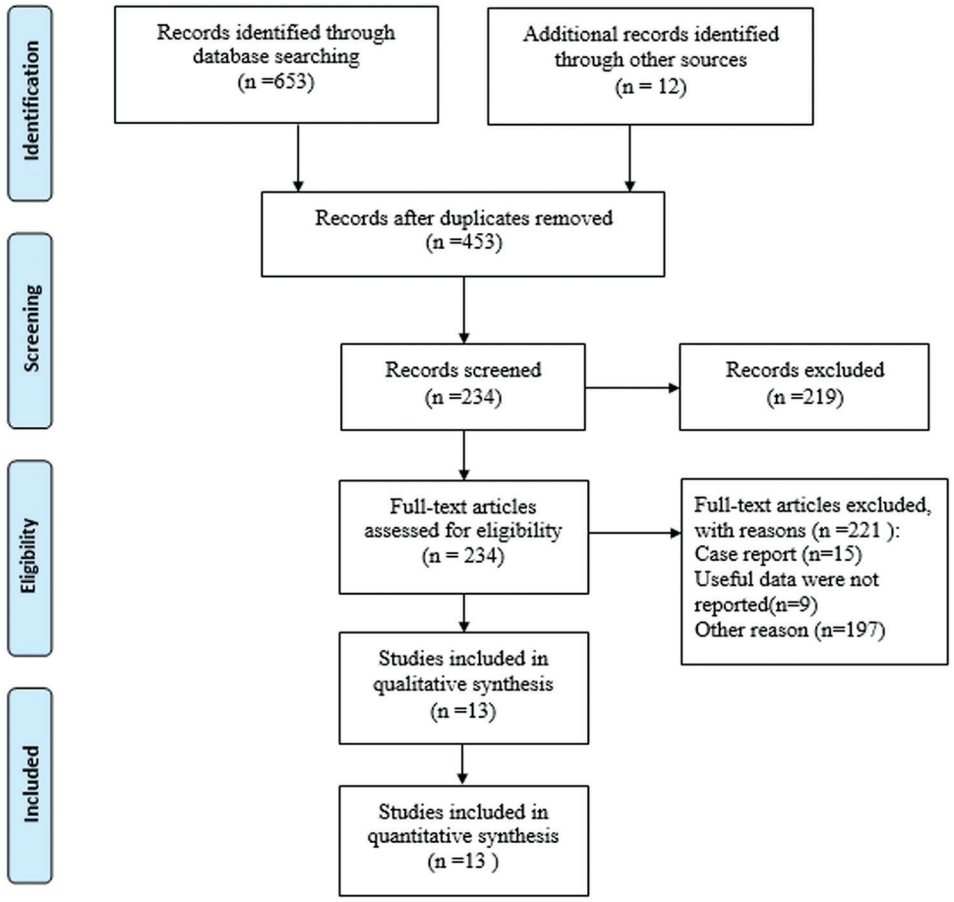

Figure 1: Flow chart of meta-analysis

Table 1: General characteristics of included randomized controlled trials

References	Characteristics of participants						Interventions		Treatment period	Adverse effects (percentage of patients)
	Age (years)	Sample (male)	Comparability of baseline	Disease duration	Clinical situation	AChR+ (%)	Treatment group	Control group		
Yoshikawa, *et al*. 2011[12]	T: 45.9±11.5 C: 44.4±12.36	80 (30) T: 40 C: 40	Yes	T: 7.41±9.02 C: 7.94±9.54 (years)	Steroid-dependent MG	57 (71.3)	Tacrolimus + CT Tacrolimus: 3 mg/d	Placebo + CT	28 weeks	Nasopharyngitis (25 vs. 30%); URI (12.5 vs. 5%); WBC elevation (12.5 vs. 2.5%); glucose urine present (10 vs. 7.5%); 2 serious events: 1 appendicitis and 1 hearing loss
Nagane, *et al*. 2005[11]	T: 56.6±17.0 C: 54.2±16.1	34 (9) T: 18 C: 16	Yes	T: 11.5±7.7 C: 10.5±13.5 (months)	*de novo* diagnosis	21 (61.8)	Tacrolimus + CT Tacrolimus: 3 mg/d	CT	1 year	No significant side effects. Serum creatine increased from 0.4-1.1 mg/dl to 1.4-1.5 mg/dl in one patient

AChR+ = Acetylcholinereceptor (>0.3 nmol/l), CT = Conventional treatment including prednisone, cyclosporine, cholinesterase inhibitors, plasmapheresis, pyridostigmine, corticosteroids, URI = Upper respiratory inflammation, WBC = White blood cell

et al. investigating the steroid-sparing effects of tacrolimus to maintain MM was followed up for 1 year. In the former study, a total of eighty patients who received prednisolone (PSL) and were maintained in the state of MM were randomized to 3 mg tacrolimus (n = 40) or placebo (n = 40) nightly. The authors failed to find a significant difference between tacrolimus and placebo in the full analysis set (WDM: –1.6; 95% CI: –3.57, 0.37). Nagane *et al*. investigated the efficacy of tacrolimus for *de novo* MG patients. In the early-phase therapy, these individuals were randomized to interventions with (n = 18) tacrolimus or without (n = 16), and they discharged from early-phase therapy in hospital until they reached MM. After that, authors investigated the steroid-sparing effects of tacrolimus to maintain MM for 1 year. In this therapy phase, immunoabsorption (IA) plus high-dose intravenous methylprednisolone (HMP) (IA + HMP), HMP alone, oral PSL,

Table 2: General characteristics of included prospective clinical studies

References	Clinical situation	Age (years)	Sample (male)	Disease duration (years)	AChR+ (%)	Dosage of tacrolimus	Treatment period (mean)	Outcome measures indicators	Adverse effects
Ponseti *et al*., 2006[20]	Postoperative thymectomy	40.5±14.7	48 (16)	9.02±10.4	40 (83.3)	0.1 mg/kg/d, bid	6-60 (24.4) months	1. QMG score 2. TEMS	Hypomagnesemia (23.7%); hypercholesterolemia (7.9%); cushingnoid syndrome, tremor, paresthesias (5.3%)
Ponseti *et al*., 2005a[21]	Cyclosporine- and prednisone-dependent MG	47.9±15.8	79 (30)	Unclear	Unclear	0.1 mg/kg/d, bid	0.13-3.72 (2.5) years	QMG score	Renal insufficiency (3.8%); ataxia and neuropathy in 1 patient
Ponseti *et al*., 2005b[22]	Cyclosporine- and prednisone-dependent MG	45.6	13 (6)	Unclear	Unclear	0.1 mg/kg/d, bid	12 months	QMG score	Weight loss (4.4%); new malignancies in 3 patients
Konishi *et al*., 2003[23]	Steroid-dependent MG	18-59 (median: 47)	19 (6)	2.8-31.5	Unclear	3-5 mg/d	16 weeks	1. QMG score 2. MGADL	Increase in neutrophil count and decrease in lymphocyte count (37%)
Kawaguchi *et al*., 2004[24]	Steroid-dependent MG	28-72 (mean: 44)	17 (8)	1-30 (mean: 8.5)	15 (88.2)	3 mg/d	4-58 (19.2) months	MGADL	Deterioration of DM in 1 patient
Konishi *et al*., 2005[25]	Steroid-dependent MG	28-59	12 (3)	4-31	Unclear	2-4.5 mg/d	88-104 weeks	1. QMG score 2. MGADL	Increase in neutrophil count and decrease in lymphocyte count (33.3%)
Zhao *et al*., 2005[26]	Steroid-dependent MG	23-42 (mean: 34.6)	5 (1)	2-7 (mean: 3.4)	3 (60)	3 mg/d	20 weeks	MGADL	No significant side effects
Tada *et al*., 2006[27]	Steroid-dependent MG	35-83 (mean: 51.1)	9 (0)	0.2-29.9	9 (100)	3 mg/d	24-46 (34.6) months	QMG score	Increase in HbA1c level and decrease in lymphocyte count (33.3%)
Zhao *et al*., 2011[28]	Steroid-dependent MG	44.8±14.72	47 (19)	Unclear	Unclear	3 mg/d	24 weeks	1. QMG score 2. MGADL	Hyperlipidemia (18%); hyperglycemia, diarrhea, respiratory infection (12%)
Wang, 2014[29]	Steroid-dependent MG	46.1±8.2	40 (22)	10.1±3.4	Unclear	2-6 mg/d	12 months	CAEM score	Decrease in lymphocyte count (5%); hyperglycemia (10%)
Chen and Li, 2015[30]	Steroid-dependent MG	47.2±8.2	82	10.2±3.5	Unclear	2-6 mg/d	12 months	CAEM score	Decrease in lymphocyte count in 1 patient

AChR+ = Acetylcholinereceptor (>0.3 nmol/l), DM = Diabetes mellitus, HbA1c = Hemoglobin A1c, CAEM = Clinical absolute evaluation method, MG = Myasthenia gravis, QMG = Quantitative myasthenia gravis, MGADL = Myasthenia gravis activities of daily living, TEMS = Test to evaluate muscular strength

or pyridostigmine bromide were administered as needed to maintain MM. The 1-year follow-up indicated that tacrolimus significantly decreased the number of treatments with steroids (WDM: –3.5; 95% CI: –5.73, –1.27).

Quantitative myasthenia gravis score

For seven prospective clinical trials with 227 patients, there were significant differences with high heterogeneity, so we used the random effects model to pool the estimate (SMD: 2.93; 95% CI: 1.14, 4.73; I^2 = 86%) [Table 4].

Myasthenia gravis activities of daily living

Six prospective clinical trials yielding 63 patients involved the outcomes of MGADL. The difference was statistically significant with SMD 0.59 (95% CI: 0.33, 0.85; I^2 = 7%) [Table 4].

Test to evaluate muscular strength

Three prospective clinical trials yielding 140 patients involved the outcomes of TEMS, which was reduced by tacrolimus with SMD - 4.12 (95% CI: 5.46, –2.79; I^2 = 0%) [Table 4].

Clinical absolute evaluation method score

Four prospective clinical trials yielding 122 patients involved the outcomes of CAEM score, which was reduced by tacrolimus with SMD 1.35 (95% CI: 0.14, 2.56; I^2 = 0%) [Table 4].

Heterogeneity and bias

No significant heterogeneity was apparent for all these outcomes except QMG score. As for the sensitivity analysis, the results of pooled SMD did not change greatly through removing any one research. In addition, because none of

Table 3: Risk bias of included randomized controlled trials

Study ID	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Yoshikawa *et al*., 2011	L	L	L	L	L	U	U
Nagane *et al*., 2005	H	U	H	H	U	U	U

H = High risk, L = Low risk, U = Unclear

our outcomes included ten or more studies, we did not evaluate publication bias in our meta-analysis based on the recommendations of Cochrane Handbook.[19]

Adverse events

Adverse effects were mentioned as mild in many trials. The most common event was increase in hemoglobin A1C level and neutrophil count [Table 5]. This symptom often settled if researchers stop the drug or reduce the dose. Some reported problems such as abnormality in liver and renal function were transient. Of note, the data revealed here are not comprehensive, because the trials included in this meta-analysis were mostly small and often short lasting.

Discussion

MG is a chronic autoimmune disease which might need long-term immunization therapy; therefore, therapies must not only be efficacious but should also cause less side effect. Clinical guideline indicated that corticosteroids might be an effective medicine for the management of MG,[4] but even the dose of <7.5 mg/day might increase the risk of side effect,[36] which made researchers to add other immunosuppressive regimens to decrease the dose of it.[4] Immunosuppression is effective in controlling the progression of MG,[37,23] which can be sorted into three categories: inhibition of the cell cycle (such as azathioprine, cyclophosphamide, and mycophenolate mofetil), immunosuppression of T-cells (such as tacrolimus), and B-cell depletion (such as rituximab).[38] Tacrolimus is a macrolide and binds to FK506-binding protein 12 (FKBP12) to form a complex of tacrolimus–FKBP12 which can inhibit T-lymphocyte, and then inhibit the phosphatase activity of calcineurin.[39]

Until now, one review, several RCTs, prospective clinical trials, and case reports have addressed the efficacy and safety of tacrolimus in MG. However, none of these studies pooled all the relevant data into meta-analysis to provide comprehensive information for clinic workers. Therefore, we conducted this first meta-analysis investigating the efficacy and safety of tacrolimus for the management of MG.

Our research only found 2 RCTs (Numbers of patient = 114) and 11 prospective clinical trials (N = 381). Various clinical situations have been included, such as *de novo* diagnosis, cyclosporine- and prednisone-dependent MG, steroid-dependent MG, and postoperative thymectomy. However, we could not find any other kind of properly designed studies relevant to this topic.

Table 4: Summary of different secondary outcome results of included prospective clinical trial

Outcome	Number of studies	SMD	95% CI	I^2 (%)	Model
QMG score	7	2.93	1.14, 4.73	86	Random
MGADL	6	0.59	0.33, 0.85	7	Fix
TEMS	3	−4.12	−5.46, −2.79	0	Fix
CAME score	2	1.35	0.14, 2.56	0	Fix

SMD = Standardized mean differences, CI = Confidence interval, QMG = Quantitative myasthenia gravis, MGADL = Myasthenia gravis activities of daily living, CAEM = Clinical absolute evaluation method, TEMS = Test to evaluate muscular strength

Table 5: Summary of adverse event

Adverse event	Rate (%)
Increase in HbA1c level	33.3
Increase in neutrophil count	33.3-37
Nasopharyngitis	25
Hypomagnesaemia	23.7
Hyperlipidemia	18
WBC elevation	12.5
Respiratory infection	12-12.5
Diarrhea, hyperglycemia	10-12
Glucose urine present	10
Hypercholesterolemia	7.9
Deterioration of diabetes mellitus	5.9-10
Cushingnoid syndrome	5.3
Tremor	5.3
Paresthesias	5.3
Decrease in lymphocyte count	5-37
Weight loss	4.4
Renal insufficiency	3.8

HbA1c = Hemoglobin A1c, WBC = White blood cell

Overall, our meta-analysis suggested that tacrolimus might benefit the development of MG.

In the primary outcome, since steroids might bring many side effects if patients received them for a long time, we investigated the steroid-sparing effects of tacrolimus to maintain MM. The only two RCTs showed contradictory results, a possible explanation for which might be that the duration of these two trials was different. Participants of the study by Nagane *et al*. were followed up and were treated to maintain MM for 1 year, investigators of which found that tacrolimus significantly reduced the use of steroids; however, the study by Yoshikawa *et al*. which only lasted for 28 weeks yielded the opposite result.

The above two RCTs showed that tacrolimus with enough treatment duration might have positive steroid-sparing effects.

In the secondary outcome, namely the efficacy of tacrolimus in reducing the severity of MG, the meta-analyses of prospective clinical trials showed a benefit effect. To be specific, QMG score was reduced by tacrolimus with high heterogeneity, in which we found that the most possible cause of heterogeneity between trials was the baseline severity of MG. Three studies conducted by Ponseti *et al*. were associated with high baseline severity of MG. Since we failed to find value stratification of MG baseline severity, we did not conduct subgroup analysis. In contrast, other outcomes including MGADL, TEMS, and CAEM scores all suggested that tacrolimus could significantly improve the development of MG.

To date, there is only one review conducted to assess the efficacy of tacrolimus by Cruz *et al*.,[14] which only described prospective clinical trials focusing on clinical outcomes in patients with generalized MG without quantitative analysis. We used meta-analytic method to provide more comprehensive information and our conclusions aligned with their review. Differing from this review, we included four more trials[24,26,29,30] and excluded two trials from their eligible studies due to insufficient data for outcome calculation.[32,33]

Limitations of this meta-analysis

The most important limitation which largely discounted the reliability of our findings might be the poor quality of included evidences and limited study sample sizes with low statistical power. What is more, it is possible that corticosteroids or other combined interventions might influence our outcomes. However, we could not access the efficacy of tacrolimus as a monotherapy because tacrolimus in all included studies was combined with other immunotherapies.

What is more, the estimates of overall side effects might be imprecise, which could be attributed to the fact that researches have reported details of side effects but not all. It might be also because that the duration of follow-up in some trials might be insufficient enough to detect comprehensive adverse events.

Suggestion for future research

Since our meta-analysis was based only on two RCTs and several prospective clinical trials, more RCTs with larger sample size and long-term duration will be necessary in investigating the efficacy and safety of tacrolimus for the therapy of MG, as well as to help reduce random error and identify publication bias. Especially, in the field of steroid-sparing effect of tacrolimus to maintain MM, the enough treatment duration might be particularly crucial.

Conclusion

Despite the above limitations, this meta-analysis suggests that there might be a potential beneficial role with safety for tacrolimus in the management of MG; additional high-quality researches are needed to confirm or refute these results. However, in applying these evidences to MG patients, the choice of tacrolimus for an individual patient is neither automatic nor straightforward, as risks of drawbacks and benefits of this drug must be balanced.

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Conflicts of interest

There are no conflicts of interest.

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