The emerging role of tacrolimus in myasthenia gravis

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Abstract

Objective: To describe and evaluate the available evidence assessing the role of tacrolimus in the management of patients with myasthenia gravis (MG).

Data sources: A literature search of MEDLINE (1946 to September 2014) and EMBASE (1947 to September 2014) was performed using the terms ‘tacrolimus’ and ‘myasthenia gravis’. Citations of retrieved articles were examined for relevance.

Study selection and data extraction: The search was limited to prospective clinical trials focused on clinical outcomes in patients with generalized MG. Case reports, retrospective evaluations and non-English articles were excluded.

Data synthesis: A total of 12 studies met inclusion criteria, of which seven articles evaluated tacrolimus in steroid-dependent patients and two examined the utility of tacrolimus in patients failing corticosteroids and cyclosporine. Other studies evaluated early initiation of tacrolimus after thymectomy, effectiveness of tacrolimus in *de novo* MG and the effectiveness of tacrolimus post-thymectomy in thymoma patients *versus* nonthymoma. A total of eight trials showed statistically significant improvements in quantitative MG score (QMGs) and postintervention status criteria – Myasthenia Gravis Foundation of America (PSC-MGFA). Of the trials examining steroid reduction with tacrolimus, two reported high rates of complete withdrawal; however, the most robust trial was unable to detect a difference in mean steroid dose. Long-term effects of tacrolimus (up to 5 years) were assessed in eight trials, which consistently showed positive effects on QMGs or reduction in adjunct therapies.

Conclusions: There is limited yet promising information to suggest a beneficial role for tacrolimus in reducing QMGs and corticosteroid burden in patients with refractory symptoms or new-onset MG. Long-term use appears to be safe in this population.

Keywords: disease management, drug information, immunosuppressants, myasthenia gravis, neurology

Introduction

Myasthenia gravis (MG) is an autoimmune disorder in which antibodies are produced that target and destroy nicotinic acetylcholine (ACh) receptors at the neuromuscular junction of striated muscle cells [Drachman, 1994]. Although the disease is rare, its prevalence has continued to rise over the past 50 years. Approximately 20.4 out of every 100,000 individuals or about 60,000 Americans are afflicted with the disease [Phillips, 2004]. There is a bimodal distribution for age of onset, more commonly affecting females in their 20s and 30s whereas males face a typical onset

after age 50 [Howard, 2008]. The average annual healthcare plan cost of treating the disease may approach \$25,000 per patient per year, and it has been estimated that MG is comparatively more expensive to treat than other neurological disorders such as multiple sclerosis, Alzheimer’s disease and migraine headache [Guptill *et al.* 2011].

Symptoms are often fluctuating in nature and may include muscle weakness or easy fatigability, ptosis, and trouble with chewing and swallowing. [Howard, 2008] A standard classification system for the disease may be used to define prognosis

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and possible response to treatment; this includes five classes with increasing levels of symptom severity (Table 1). Class I is mild in nature, whereas Class V is the most severe form requiring intubation [Jaretzki *et al.* 2000]. Classes II, III and IV may be further subdivided into category 'a', which primarily impacts the limbs, or 'b', which primarily affects the respiratory muscles or those of the tongue or mouth.

Treatment strategies usually involve an individualized approach based on patient-specific characteristics such as age, sex, disease severity, functional impairment and organ function [Howard, 2008; Jani-Acsadi and Lisak, 2002]. Treatment goals include achieving disease remission and normal functionality, while minimizing the risk for adverse effects of medications [Nicolle, 2002; Juel and Massey, 2005]. In 2010, the European Federation of Neurological Societies developed evidence-based consensus recommendations for MG [Skeie *et al.* 2010]. There are no consensus guidelines in the United States for the optimal management of patients with MG; however, several review articles have been published on the subject [Drachman, 1994; Jani-Acsadi and Lisak, 2010; Nicolle, 2002; Juel and Massey, 2005; Richman and Agius, 2003; Silvestri and Wolfe, 2012; Sanders and Evoli, 2010; Diaz-Manera *et al.* 2009].

The four main modalities in the management of MG patients include augmenting neuromuscular transmission with the use of anticholinesterase inhibitors, surgical removal of the thymus gland, immunosuppression with corticosteroids or other agents, and acute immune-modulating therapies including intravenous immunoglobulin (IVIG) or plasma exchange (PLEX) [Drachman, 1994]. Anticholinesterase inhibitors (e.g. pyridostigmine) have been a mainstay in the symptomatic management of weakness associated with MG for many years [Nicolle, 2002; Richman and Agius, 2003; Silvestri and Wolfe, 2012]. These agents act by inhibiting the action of the cholinesterase enzyme at the neuromuscular junction, which increases available ACh for binding, thus enhancing transmission of nerve impulses [Valeant Pharmaceuticals, 2013]. When rapid symptom control is necessary, such as during an exacerbation of the disease, IVIG or PLEX may be indicated to provide temporary improvement. These therapies may also be utilized in a prophylactic manner, such as before undergoing surgery [Ahmed *et al.* 2005].

Table 1. Myasthenia Gravis Foundation of America clinical classification.

Class	Description
I	Weakness of ocular muscles only
IIa	Mild body weakness (mainly limb and/or axial weakness), may also include eye muscles
IIb	Mild body weakness (mainly oropharyngeal and/or respiratory muscle weakness), may also include eye muscles
IIIa	Moderate body weakness (mainly limb and/or axial weakness), may also include eye muscles
IIIb	Moderate body weakness (mainly oropharyngeal and/or respiratory muscle weakness), may also include eye muscles
IVa	Severe body weakness (mainly limb and/or axial weakness), may also include eye muscles
IVb	Severe body weakness (mainly oropharyngeal and/or respiratory muscle weakness), may also include eye muscles
V	Intubation (mechanical ventilation may or may not be required)
Adapted from Jaretzki <i>et al.</i> [2000].	

Long-term suppression of the immune system may be achieved with use of corticosteroids, or other agents such as azathioprine, cyclophosphamide, cyclosporine, mycophenolate and methotrexate [Howard, 2008; Cahoon and Kockler, 2006]. While effective for immunosuppression, there are numerous untoward effects of continued corticosteroid use that have been reported in patients with MG, such as Cushingoid appearance, osteoporosis, hyperglycemia, weight gain, elevated blood pressure and increased risk for infection [Pascussi *et al.* 1984]. In 2007, a Cochrane review found that cyclosporine as monotherapy or in addition to corticosteroids, and dual therapy with cyclophosphamide plus corticosteroids, demonstrated significant disease improvement, whereas azathioprine, mycophenolate and tacrolimus were not associated with significant benefit in MG; however, only one trial examining the use of tacrolimus was included in the analysis [Hart *et al.* 2007].

Tacrolimus may be a favorable option in the management of patients with MG. It is a calcineurin inhibitor indicated for the prevention of rejection

after heart, kidney and liver transplant. Unlike cyclosporine, tacrolimus has greater potency and may result in less nephrotoxicity when used at low doses [Sanders and Evoli, 2010]. Depending on the indication, the initial dosage in transplant patients may range from 0.075 to 0.2 mg/kg/day, which may achieve whole blood trough concentrations of 4–20 ng/ml. Its immunosuppressive action is suggested to be due to the inhibition of T lymphocyte activation through its intracellular binding to the protein FKBP-12. This complex interferes with the activity of calcineurin, which in turn prevents nuclear factor of activated T-cells (NF-AT) from translocation and initiation of gene transcription for lymphokines such as interleukin-2 (IL-2). In addition to causing T-cell inhibition, tacrolimus may play a role in the suppression of humoral immunity [Astellas Pharma, 2013]. Preliminary data suggests that tacrolimus may also improve excitation-contraction coupling early after treatment initiation, perhaps due to potentiation of the ryanodine receptor, which is responsible for release of calcium ions from the sarcoplasmic reticulum in muscle cells [Imai *et al.* 2012].

Tacrolimus exhibits a variable rate of absorption and bioavailability, and oral doses of 3 to 4 times the intravenous dose may be required to achieve similar therapeutic plasma concentrations. The drug is approximately 99% protein bound and is extensively metabolized in the liver by cytochrome P450 system to several metabolites. It is predominately excreted in the feces and less than 1% is eliminated in the urine as unchanged drug [Astellas Pharma, 2013; Venkataramanan *et al.* 1995]. Notable adverse effects include hypertension, headache, tremor, renal impairment, new-onset diabetes mellitus, diarrhea, malignancy (e.g. lymphoma and dermatologic) and increased risk of infection. Additionally, the potential for drug interactions involving the CYP 3A4 pathway should be taken into consideration [Astellas Pharma, 2013].

Case reports and retrospective analyses have suggested tacrolimus may be utilized in the treatment of MG and that it may be useful in a variety of patient types, including those with immunosuppressant-dependency, recent thymectomy, or those in which thymectomy is contraindicated [Ponseti *et al.* 2007, 2008; Chung *et al.* 2008; Tsukaguchi *et al.* 2005; Shimojima *et al.* 2004]. The objective of this review is to describe and evaluate the available evidence that assesses the

role of tacrolimus in the management of patients with MG in order to determine in which settings it may be safe and effective.

Data sources

A search of MEDLINE (1946–September 2014) and EMBASE (1947–September 2014) was performed using the terms *tacrolimus* and *myasthenia gravis*. The search was limited to prospective clinical trials with a focus on clinical outcomes in patients with generalized MG written in the English language. Case reports and retrospective evaluations were excluded from this review. Citations of retrieved articles were also examined for relevance and included if applicable. A total of 12 studies met inclusion criteria [Shimojima *et al.* 2006; Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2003, 2005; Zhao *et al.* 2011; Yoshikawa *et al.* 2011; Ponseti *et al.* 2005a, 2005b, 2006; Nagane *et al.* 2005; Mitsui *et al.* 2007]. Table 2 provides a summary of the pertinent findings from each trial.

Literature evaluation

Steroid-sparing effects

The review covered seven articles which evaluated the use of tacrolimus in steroid-dependent patients [Shimojima *et al.* 2006; Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2003, 2005; Zhao *et al.* 2011; Yoshikawa *et al.* 2011].

Shimojima and colleagues prospectively evaluated seven patients with resistant MG symptoms despite corticosteroid therapy [Shimojima *et al.* 2006]. Tacrolimus was initiated at a dose of 3 mg/day, divided every 12 hours and adjusted to maintain a trough concentration of 5–10 ng/ml. Patients were observed for changes in quantitative MG score (QMGS) and MG-related activities of daily living (MG-ADL) score. The mean age was 49.5 years with disease duration of 10.3 years; all but one had undergone thymectomy. At study entry, six of the seven patients were treated with prednisolone and were taking a mean daily dose of 20.8 mg (range 5–30 mg). One patient was unable to take corticosteroids due to adverse effects. Disease severity was classified as follows: grade I, three patients; grade IIb, two patients; grade IIa, one patient; and IIIa, one patient. At the 3 and 6 month time points, there was a statistically significant reduction in both the QMGS and MG-ADL ($p < 0.05$). Although reduction in steroid dose

Table 2. Prospective trials of tacrolimus in myasthenia gravis.

Clinical situation	Study	Design	Population	Dosage	Duration	Efficacy measures	Results [†]	Adverse effects (% of patients)
Steroid-sparing effects	Shimojima <i>et al.</i> [2006]	Open-label, single center	<i>n</i> = 7	3 mg/day	6–32 months	Mean Δ QMGS at 3, 6 months Mean Δ MG-ADL at 3, 6 months Δ Anti-AChR AB	$\downarrow 4.2^*$, $\downarrow 5.0^*$ $\downarrow 1.3^*$, $\downarrow 2.0^*$ $\downarrow \uparrow$	No notable adverse effects
	Nagaishi <i>et al.</i> [2008]	Open-label, single center	<i>n</i> = 10	3 mg/day	1–5 years	Mean Δ MG-ADL at 1, 6 months PSC-MGFA	$\downarrow 3$ (1–4), $\downarrow 5.8$ (4–12) [§] 1 PR, 4 MM, 2 exacerbations [§]	No serious adverse effects 2 discontinuations after 1 month due to new diabetes mellitus and diarrhea
	Tada <i>et al.</i> [2006]	Open-label, single center	<i>n</i> = 9	3 mg/day	24–46 months	QMGS Δ at 3, 6, 12 months 3 point $\downarrow$ in QMGS at 3, 6 months Mean Δ Anti-AChR AB at 6 months	$\downarrow 2.6^*$, $\downarrow 3.0^*$, $\downarrow 3.2^*$ 66.7%, 77.8% of patients [§] $\downarrow 51.6$ nM [*]	No serious adverse effects Hemoglobin A1C increase and lymphocyte reduction (33%)
	Konishi <i>et al.</i> [2003]	Open-label, multicenter	<i>n</i> = 19	3 mg/day	16 weeks	Median Δ in MG score at 16 weeks MG-ADL score Δ at 12, 16 weeks 3 point $\downarrow$ in MG score 1 point $\downarrow$ in ADL score Median Δ Anti-AChR AB at 12, 16 weeks Median Δ IL-2 production at 16 weeks	$\downarrow 4^*$ $\downarrow \uparrow$ 37% of patients [§] 42% of patients [§] $\downarrow 2.8$ nM [*] , $\downarrow 2.1$ nM [*] $\downarrow 8.5$ U/ml [§]	No serious adverse effects Increased neutrophil count and decreased lymphocyte count (37%)
	Konishi <i>et al.</i> [2005]	Open-label extension	<i>n</i> = 12	2–4.5 mg/day	2 years	3 point $\downarrow$ in MG score 1 point $\downarrow$ in MG-ADL score Mean Δ Anti-AChR AB Mean Δ prednisolone dose	41.7% of patients [§] 50% of patients [§] $\downarrow 7.5$ nM [§] $\downarrow 37\%$ (8.3–57.0%) [§]	Minor (66.7%), increased neutrophil count and decreased lymphocyte count (33%) Drug held for headache/eye pain in 1 patient, later resumed
	Zhao <i>et al.</i> [2011]	Open-label, multicenter pilot study	<i>n</i> = 47	3 mg/day	24 weeks	Median Δ QMGS Median Δ MMT score Median Δ MG-ADL Median Δ prednisone dose	$\downarrow 5.34 \pm 4.79^*$ $\downarrow 14.7 \pm 14.02^*$ $\downarrow 4.75 \pm 4.30^*$ $\downarrow 11.8$ mg/day [§]	Hyperlipidemia (18%), hyperglycemia (12%), diarrhea (12%), respiratory infection (12%) 1 death due to MG exacerbation 9 days into study, not attributed to tacrolimus

(Continued)

Table 2. (Continued)

Clinical situation	Study	Design	Population	Dosage	Duration	Efficacy measures	Results [‡]	Adverse effects (% of patients)
Corticosteroid with cyclosporine failure	Yoshikawa <i>et al.</i> [2011]	Randomized, double-blind, placebo-controlled, multicenter	<i>n</i> = 80	3 mg/day	28 weeks	Mean prednisolone dose, ITT	4.91 ± 4.04 <i>versus</i> 6.51 ± 4.89 mg/day	Nasopharyngitis (25 <i>versus</i> 30%), WBC elevation (12.5 <i>versus</i> 5%), URI (12.5 <i>versus</i> 5%), A1C increase (10 <i>versus</i> 2.5%), muscle spasm (10 <i>versus</i> 0%)
						Mean prednisolone dose, PP	4.45 ± 3.44 <i>versus</i> 6.19 ± 4.77 mg/day*	
						QMGS	4.4 ± 3.62 <i>versus</i> 5.8 ± 5.09	
						MG-ADL	1.2 ± 1.33 <i>versus</i> 2.3 ± 3.0	2 serious events: 1 appendicitis and 1 hearing loss which resolved with treatment
						Mean Δ Anti-AChR AB	No Δ [¶]	2 discontinuations for appendicitis and insomnia
						Mean Δ IL-2 production	No Δ [¶]	No notable adverse effects
Postoperative thymectomy	Ponseti <i>et al.</i> 2005a	Open-label, single center	<i>n</i> = 13	0.1 mg/kg/day	1 year	Median Δ QMGS	↓20.93 [§]	
						Mean Δ Anti-AChR AB	↓6.6 nM*	
						PSC-MGFA	13 PR [§]	
						Mean Δ TEMS	↑26.53 [§]	
	Ponseti <i>et al.</i> 2005b	Open-label, single center	<i>n</i> = 79	0.1 mg/kg/day	2.5 years	Mean Δ prednisolone dose	↓56.5 mg/day. [§]	3 new malignancies, included 2 patients with lung cancer and 1 with renal cancer (after 4 to 6 months of treatment)
						Withdrawal of steroids	77 patients. [§]	
De novo diagnosis						Mean Δ QMGS	↓20.2 [§]	
						Mean Δ Anti-AChR AB	↓34.8 nM. [§]	
						PSC-MGFA, % of patients	CSR 5.1%, PR 88.6%, MM 6.4%	
						Mean Δ TEMS	↑26.8 [§]	
	Ponseti <i>et al.</i> [2006]	Open-label, single center	<i>n</i> = 49	0.1 mg/kg/day	6–60 months	Withdrawal of steroids at 1, 2 years	93.7%, 100% of patients [§]	Hypomagnesemia (23.7%), paresthesia (5.3%), tremor (5.3%)
						Mean Δ prednisone dose	↓80.7 mg/day [§]	
						Mean Δ QMGS	↓20.7 [§]	
						Mean Δ Anti-AChR AB	↓20.8 nM [§]	
						PSC-MGFA, % of patients	CSR 33%, PR 62.6%, MM 4% [§]	
						Mean Δ TEMS	↑24 [§]	
	Nagane <i>et al.</i> [2005]	Randomized, controlled, single center	<i>n</i> = 34	3 mg/day	1 year	Early phase:		No significant adverse effects
						Number of PLEX + HMP treatments	1.3 ± 1.5 <i>versus</i> 3.1 ± 2.3*	Serum creatine increased from 0.4–1.1 mg/dl to 1.4–1.5 mg/dl in 1 patient
						Mean oral prednisolone dose	5.1 ± 4.1 <i>versus</i> 6.7 ± 3.0 mg/day	
						Follow-up phase:		
						Number of PLEX + HMP treatments	0.2 ± 0.5 <i>versus</i> 1.1 ± 1.6*	
						HMP	0.4 ± 0.9 <i>versus</i> 1.8 ± 2.7*	
						Mean oral prednisolone dose	4.6 ± 4.1 <i>versus</i> 8.1 ± 2.6 mg/day*	

Table 2. (Continued)

Clinical situation	Study	Design	Population	Dosage	Duration	Efficacy measures	Results [‡]	Adverse effects [% of patients]
Presence of thymoma	Mitsui <i>et al.</i> [2007]	Open-label, controlled, single center	<i>n</i> = 10	3 mg/day	3 months	Δ QMGS at 1, 3 months Baseline anti-AChR AB Anti-AChR AB at 1mo Anti-AChR AB at 3 months	↓* 68.16 ± 36.4 <i>versus</i> 16.5 ± 7.8 nM 53.8 ± 30.9 <i>versus</i> 16.5 ± 8.3 nM 79.2 ± 53.9 <i>versus</i> 15.25 ± 5.2 nM	No notable adverse effects
Anti-AChR AB= anti-nicotinic acetylcholine receptor antibody titer; CSR= complete stable remission; HMP= high dose intravenous methylprednisolone; IL-2= interleukin-2; ITT= intention to treat; MG= myasthenia gravis; MG-ADL= Myasthenia Gravis Activities of Daily Living; MM= minimal manifestation; MMT= Manual Muscle Testing; PLEX= plasma exchange; PP= per protocol; PR= pharmacologic remission; PSC-MGFA= Post-Intervention Status Criteria, Myasthenia Gravis Foundation of America; QMGS= Quantitative Myasthenia Gravis Score; TEMS= Test to Evaluate Muscle Strength; URI= upper respiratory inflammation. * <i>p</i> < 0.05 § <i>p</i> ≤ 0.01 ‡For comparisons of tacrolimus <i>versus</i> placebo or, if no comparator used, for final results <i>versus</i> baseline. § <i>p</i> values not reported. Thymoma <i>versus</i> nonthymoma group †Specific values not reported.								

was not an endpoint of this investigation, dosing changes were reported. Four patients had the dose of prednisolone tapered at their own request; two of these patients were tapered rapidly 1 month after starting tacrolimus, from 20 and 30 mg per day to 5 mg per day. After several months, symptoms worsened and the steroid dose was increased to 20 mg daily in both patients. The other two patients were tapered more slowly (starting doses of 20 and 30 mg per day reduced by 2.5 mg every 3 months) after a much longer tacrolimus treatment duration (6–9 months) and did not require a dose re-escalation. No notable adverse effects were observed [Shimajima *et al.* 2006].

Nagaishi and colleagues published the results of a small, uncontrolled trial which enrolled 10 patients with generalized, steroid-dependent MG classified as grade IIb to IIIb. A total of nine patients had undergone thymectomy and were receiving prednisolone at a mean dose of 18.5 mg per day (range 5–60 mg) [Nagaishi *et al.* 2008]. Tacrolimus was administered as 3 mg/day after the evening meal. Pyridostigmine doses remained constant during the study period. Patients were followed for a mean of 3.1 years (range 1–5.1 years). Long-term tacrolimus was tolerated in seven out of 10 patients. Within 1 month, two patients discontinued due to adverse effects (diarrhea and diabetes mellitus) and one withdrew voluntarily. Improvements in

MG-ADL were observed for five patients at 1 and 6 months, respectively. Of the five subjects who achieved pharmacologic remission (PR) or minimal manifestation (MM) of the disease according to the Post-Intervention Status Criteria, Myasthenia Gravis Foundation of America (PSC-MGFA), four were slowly titrated off prednisolone after a mean 13.2 months [Nagaishi *et al.* 2008].

Tada and colleagues evaluated the long-term safety and efficacy of tacrolimus in steroid-dependent or unresponsive patients [Tada *et al.* 2006]. A total of nine subjects with disease severity class IIa to IVb were administered 3 mg of tacrolimus every evening. Significant improvement, defined as a decrease in total QMGS by 3 points or more was achieved in a majority of patients after 6 months and 1 year. In addition, total QMGS significantly decreased after 3, 6 and 12 months of treatment with tacrolimus. Adverse effects were noted to be mild in nature and included an increase in hemoglobin A1c (Hb_{A1c}) and a reduction in lymphocytes in 33.3% of subjects. Of the patients on prednisolone doses of 10 mg/day or more at study initiation, half were able to achieve a dose reduction after one year (mean dose 18.2 *versus* 10.4 mg/day) [Tada *et al.* 2006].

Konishi and colleagues conducted a 16 week open-label trial at 10 centers in Japan [Konishi

et al. 2003]. Patients were included if they were on corticosteroids and required further immunosuppression or dose reduction due to adverse effects. Those with cardiac, renal, hepatic or pancreatic dysfunction were excluded. Tacrolimus was initiated at a dose of 3 mg by mouth once daily after the night-time meal. The primary endpoint was a reduction in the modified MG score of 3 points or more or any improvement in the MG-ADL score. A total of 19 thymectomized patients on corticosteroids (dosage range 5–35 mg/day) with a median age of 47 and disease duration of 12 years were evaluated. Statistically significant findings included a reduction of total MG score at weeks 8, 12 and 16, and improvement in MG-ADL scores at weeks 12 and 16. When evaluating total daily corticosteroid dose, dose decreases of 21%, 9% and 40% were achieved in three patients. One patient required a 33% increase in dose. No other dosage adjustments were reported. Adverse effects occurred in seven of 19 patients, which included nonserious hematological abnormalities. Serum creatinine and Hb_{A1c} were unchanged from baseline [Konishi *et al.* 2003].

A total of 12 patients from the above trial entered into a long-term follow-up study, which extended to 88–104 weeks [Konishi *et al.* 2005]. Of note, two patients who did not respond in the original trial showed improvement in total MG score by 3 or more points at the end of the long-term follow-up evaluation, although one patient exhibited worsening symptoms. Overall, five patients had a significant improvement in total MG score and MG-ADL scores improved in half of the subjects. Adverse effects occurred in eight patients, although most were noted to be mild in nature. One patient required tacrolimus administration be held due to headache and eye pain; however, the drug was later restarted [Konishi *et al.* 2005].

Zhao and colleagues conducted a 24-week open-label pilot study at four centers in China [Zhao *et al.* 2011]. Subjects were included based on a diagnosis of generalized MG and steroid treatment associated with a poor response, lack of symptom control, adverse effects or intolerance. Tacrolimus was administered as a 1 mg dose in the morning and a 2 mg dose in the evening. A total of 47 patients with a mean age of 44.87 years were evaluated for changes in clinical outcomes. At each time point (4, 8, 12, 16, 20 and 24 weeks), the change from baseline QMGs, manual muscle testing (MMT) and MG-ADL was significantly improved ($p < 0.0001$). Of the 31 patients taking

prednisone at study entry, 74.2% tolerated a dose reduction at 24 weeks. Adverse effects occurred in 66.0% of patients and included hyperlipidemia, hyperglycemia, diarrhea and respiratory infection. One patient died from a MG exacerbation during the study [Zhao *et al.* 2011].

Yoshikawa and colleagues conducted a 28 week randomized, double blind, placebo-controlled trial to assess the steroid-sparing effect of tacrolimus in patients with MG class I–V [Yoshikawa *et al.* 2011]. Patients were included if they were on a stable dose of prednisolone 10–20 mg/day in the month preceding enrollment and had MM of the disease as per the PSC-MGFA. Use of pyridostigmine was permitted; however, the dosage had to remain unchanged throughout the study. No acute therapies such as IVIG or PLEX were allowed during the study period, nor was the use of additional immunosuppressant agents. There were 80 patients randomized to receive tacrolimus 3 mg nightly ($n = 40$) or placebo ($n = 40$). The study protocol specified that prednisolone dosage would be reduced by 2.5 mg every 4 weeks as tolerated based on patient MM status. A total of seven patients withdrew (two in the active arm and five in placebo), leaving 38 and 35 subjects per group, respectively. Baseline characteristics such as age, disease duration and severity, thymectomy status, corticosteroid doses, acetylcholinesterase (AChE) use, QMGs and MG-ADL score were well matched between the study groups. The primary outcome measure of mean daily prednisolone dose in the final 12 weeks was not found to be statistically significant in the full analysis set; however, the per protocol analysis reached statistical significance. Other endpoints evaluated also lacked statistical significance including QMGs, MG-ADL, anti-AChR antibody titer and IL-2 production. Adverse effects were experienced by 87.5% and 80% of subjects in the tacrolimus and placebo groups, respectively. Tacrolimus was discontinued in one patient after development of appendicitis and in another patient due to insomnia [Yoshikawa *et al.* 2011].

Corticosteroid with cyclosporine failure

Two trials were identified which examined the utility of tacrolimus in patients failing both corticosteroids and cyclosporine [Ponseti *et al.* 2005a, 2005b]. In a small open-label investigation, Ponseti and colleagues enrolled 13 patients with generalized MG classes IIIa–IVb who had previously undergone thymectomy and were currently

treated with prednisone (mean dose 33.1 mg/day) plus cyclosporine (2–3 mg/kg/day). Patients were suffering from long-term adverse effects of drug therapy such as obesity, hypertension and renal insufficiency [Ponseti *et al.* 2005a]. All subjects were switched from cyclosporine to tacrolimus at a dose of 0.1 mg/kg/day given in two divided doses. Doses were adjusted to target plasma concentrations of 7–8 ng/ml. Patients were followed for 1 year and were evaluated based on their ability to titrate off prednisone. Other parameters such as PSC-MGFA, QMGs, anti-AChR antibody titer and test of muscle strength (TEMS) were reviewed. After 1 year, all patients were in pharmacologic remission per the PSC-MGFA and were able to be completely weaned off the corticosteroid. Statistically significant results *versus* baseline were obtained for QMGs, anti-AChR antibody titers and TEMS. Tacrolimus was tolerated well without evidence of adverse effects [Ponseti *et al.* 2005a].

Ponseti and colleagues also conducted a larger and longer study [Ponseti *et al.* 2005b]. A total of 79 thymectomized patients with disease severity class IIB–V on high-dose prednisone (mean 58.9 mg/day) and cyclosporine (mean 205.9 mg/day) were included in the study. All subjects were similarly switched from cyclosporine to tacrolimus 0.1 mg/kg/day with doses adjusted to target plasma concentrations of 7–8 ng/ml. Patients were followed for a mean of 2.5 years. Clinical status, as per the PSC-MGFA, improved at study conclusion, with most patients achieving pharmacologic remission. Mean prednisone dose was reduced to an average of 2.4 mg and all but two patients were able to have their corticosteroid discontinued. QMGs was significantly reduced, as was the titer of circulating anti-AChR antibodies. Muscle strength significantly improved from baseline. In this study there was a statistically significant decrease in the percentage of patients experiencing cyclosporine and prednisone attributable adverse effects (e.g. hypertrichosis, acne, paresthesias, gingival hyperplasia, etc.) from a baseline value of 96.2% to final visit 35.4% ($p < 0.001$). New malignancies were found in 3 patients after taking tacrolimus for 4–6 months. These malignancies included one patient diagnosed with metastatic lung cancer who expired within 1 month, another case of lung cancer which was treated with lobectomy, and a third patient with renal cancer treated with nephrectomy [Ponseti *et al.* 2005b].

Post-operative thymectomy

Early initiation of tacrolimus in patients undergoing thymectomy was evaluated in an open-label study [Ponseti *et al.* 2006]. Patients with new-onset disease (mean 9 months from diagnosis), severity class IIIa–V underwent thymectomy with initiation of tacrolimus 0.1 mg/kg/day with doses adjusted to target plasma concentrations of 7–8 ng/ml within 24 hours post-operatively. Patients were followed for a mean of 24.4 months and clinical outcomes were compared between baseline and final visits. Prednisone was discontinued in all patients after 2 years. TEMS, QMGs and AChR antibody titers all significantly improved from baseline. The time to achieve complete stable remission (CSR) was 37.9 months from initiation of tacrolimus. Adverse effects included hypomagnesemia (with tremors and paresthesias); however, drug discontinuation was not required in any patient [Ponseti *et al.* 2006].

De novo diagnosis

Nagane and colleagues examined the effectiveness of using tacrolimus in patients with a *de novo* diagnosis of MG [Nagane *et al.* 2005]. A total of 36 patients were randomized to receive usual care with or without tacrolimus 3 mg/day. Mean time from disease onset was 11.5 and 10.5 months in the active and control groups, respectively. Baseline characteristics were well matched in terms of age, thymectomy status and disease severity. Outcomes were evaluated after the early (3.7–5.8 weeks) and follow-up phases (1 year), and consisted of mean number of rescue treatments and corticosteroid dose. There was a statistically significant reduction in the number of PLEX plus high dose intravenous methylprednisolone (HMP) treatments, but no difference in mean oral corticosteroid dose was observed. After a 1 year follow up, there was a significant reduction in the number of treatments with PLEX with HMP, HMP alone, and oral steroid dose for patients treated with tacrolimus compared with those without. Adverse effects after 1 year were minimal but included one notable rise in serum creatinine to 1.4–1.5 mg/dl from baseline 0.4–1.1 mg/dl [Nagane *et al.* 2005].

Presence of thymoma

Mitsui and colleagues conducted a small trial comparing the effects of tacrolimus after thymectomy (within 6 months to 11 years) in patients with thymoma *versus* those without [Mitsui *et al.* 2007]. In

this trial, five patients with thymoma and five patients with nonthymomatous MG classification IIa–IIIb receiving oral corticosteroids, azathioprine, or both were treated with tacrolimus 1 mg 3 times a day for 3 months. Doses of corticosteroids and AChE inhibitors were held constant during the study period; however, azathioprine was discontinued before initiation of tacrolimus. After 1 and 3 months, a statistically significant reduction in QMGs was noted in the thymoma group *versus* baseline values. The nonthymoma group also demonstrated a reduction in QMGs at those time points, but the difference was not statistically significant. The between-group comparison showed a statistically significant improvement in QMGs in the thymoma group *versus* the nonthymoma subjects. Serum AChR antibody titers were not significantly changed in either group compared to baseline. No adverse effects were observed during treatment [Mitsui *et al.* 2007].

Discussion

Several small, prospective trials examining the use of tacrolimus in the management of MG are present in the literature. Various clinical situations have been studied, such as in patients refractory to, or intolerant of, corticosteroids or additional immunosuppression, presence of thymoma, and in patients with a relatively new diagnosis. The most common dosing strategies were: a fixed daily dose of 3 mg with or without assessment of trough drug concentrations; and a weight-based approach of 0.1 mg/kg/day in divided doses with dosage titrated to achieve a trough concentration of 7–8 ng/ml [Shimajima *et al.* 2006; Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2003, 2005; Zhao *et al.* 2011; Yoshikawa *et al.* 2011; Ponseti *et al.* 2005a, 2005b, 2006; Nagane *et al.* 2005; Mitsui *et al.* 2007]. While the weight-based regimen is within the manufacturer's dosing recommendations for prevention of transplant rejection, the 3 mg dose falls below the FDA-approved dosing range [Astellas Pharma, 2013]. With a lower dose, this allows for the potential to minimize or avoid calcineurin inhibitor-induced adverse drug reactions through the use of lower doses while still maintaining efficacy in the treatment of MG.

Eight of these trials [Shimajima *et al.* 2006; Tada *et al.* 2006; Zhao *et al.* 2011; Yoshikawa *et al.* 2011; Ponseti *et al.* 2005a, 2005b, 2006; Mitsui *et al.* 2007], although small and uncontrolled, showed statistically significant improvements in standardized outcome measures such as QMGs

and PSC-MGFA, which are considered to be gold standard tools for assessing patients as well as conducting research on this autoimmune disorder [Jaretzki *et al.* 2000]. Unfortunately, the largest and only randomized, double-blind, placebo-controlled trial failed to show a difference in QMGs in patients receiving tacrolimus *versus* placebo; however, this was not the primary focus of the investigation and it lacked power to detect a difference in this secondary endpoint [Yoshikawa *et al.* 2011]. Although it is unclear why these results are conflicting, it is possible this could be attributed to the relatively stable patient population selected for this trial. MM status was a requirement for study entry, whereas most of the other evaluations included patients based on the need for additional immunosuppression or presence of intolerable side effects to corticosteroids. Similarly, mean baseline QMGs was lower at 4.7 [Yoshikawa *et al.* 2011] compared to some of the other trials which included baseline values of 7.5, 8.7, 11.9, and 13.95 [Konishi *et al.* 2003, 2005; Tada *et al.* 2006; Zhao *et al.* 2011]. Since patients may have started the trial with minimal disease, perhaps there was less room for a positive benefit to be shown in not only the QMGs, but also the MG-ADL, anti-AChR antibody titer and IL-2 production. Additionally, the authors suggest that patients may have begun the study on higher than necessary steroid dosages to control the disease, which was evidenced by similar ability to dose reduce the placebo group within the first 20 weeks of the study. This could certainly impact each of these efficacy parameters and mask the true effect of tacrolimus addition.

Seven of the trials evaluated tacrolimus in patients unresponsive or intolerant to oral corticosteroids [Shimajima *et al.* 2006; Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2003, 2005; Zhao *et al.* 2011; Yoshikawa *et al.* 2011]. Due to the risk for corticosteroid-induced adverse effects, the potential for corticosteroid dose sparing is an alluring option. Of the trials that examined reduction in steroids with the addition of tacrolimus, two studies reported high rates (97.5% and 93.7%) of complete prednisone withdrawal [Ponseti *et al.* 2005b, 2006]. However, the largest and only randomized, double-blind, placebo-controlled trial was unable to detect a statistically significant difference in the primary outcome of mean daily steroid dose over a 12 week period [Yoshikawa *et al.* 2011]. It is worth mentioning that, in a per protocol analysis, four patients were excluded due to early steroid withdrawal, and statistical

significance was achieved. It is possible that the duration of follow up was not sufficient to detect a meaningful difference in the primary endpoint. After week 20, mean corticosteroid doses trended upward in the placebo group and decreased in the treatment group. During the final 4 weeks of the investigation, the mean prednisolone dose was 3.81 mg/day in the tacrolimus group compared with 7.23 mg/day in the placebo group ($p = 0.008$). Also, the percentage of patients who were able to reduce their daily steroid dose by 75% or more during the last 4 weeks was greater among the tacrolimus group (67.5% *versus* 45%, $p = 0.034$). These findings suggest that a longer follow-up period may have been necessary to observe a continued divergence in steroid requirements [Yoshikawa *et al.* 2011]. Although it is disappointing that these findings do not correlate with the other available literature, these data still suggest that tacrolimus is well-tolerated and might have a potential role in reducing steroid burden, although further research is necessary to confirm this.

The long-term effects of the use of tacrolimus for MG management were assessed in several trials [Shimajima *et al.* 2006; Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2005; Ponseti *et al.* 2005a, 2005b, 2006; Nagane *et al.* 2005]. These trials consistently showed positive effects on the QMGs or a reduction in adjunct therapies, and followed patients on tacrolimus for up to a period of 5 years. Positive outcomes were demonstrated as early as 4 months, with subjective improvements noted after only 1–2 months [Shimajima *et al.* 2006, Nagaishi *et al.* 2008].

Although there were varying levels of detail provided regarding adverse effects, all studies addressed the safety profile of tacrolimus, as shown in Table 2. Adverse effects were described as mild or nonserious in many of the trials [Nagaishi *et al.* 2008; Tada *et al.* 2006; Konishi *et al.* 2003, 2005; Nagane *et al.* 2005] and three trials did not identify any at all [Shimajima *et al.* 2006; Ponseti *et al.* 2005a; Mitsui *et al.* 2007]. Collectively, six patients required discontinuation of the drug due to toxicity; these reasons included new-onset diabetes mellitus, diarrhea, appendicitis, insomnia and cancer in two patients [Nagaishi *et al.* 2008; Yoshikawa *et al.* 2011; Ponseti *et al.* 2005b]. One trial showed new-onset malignancy in three patients treated with tacrolimus; yet all patients in that trial received prior therapy with cyclosporine, which can also carry a risk of new-onset malignancy

[Ponseti *et al.* 2005b; Novartis Pharmaceuticals, 2013].

There are limitations to note for all the trials reviewed. A large number of the studies were small, including 20 or fewer subjects total. In addition, all but one were unblinded, open-label investigations, which may introduce the potential for investigator bias; however, this could be minimized due to the objective measurements for efficacy used in many of the trials. Furthermore, because of the lack of a control group in most trials, the results should be interpreted with caution since MG is a disease that is fluctuating in nature and subject to exacerbation from a variety of causes. Aside from these factors, a major limitation of the data contained in this review is the lack of ethnic diversity. All of the trials were conducted in European or Asian populations. This makes it difficult to generalize the results to patients of non-European, non-Asian descent. It would be advantageous to have trials with more rigorous methodology (e.g. randomized, blinded, controlled, and stratified according to disease severity) to not only solidify the role of tacrolimus in controlling the disease process in patients with treatment resistance or new diagnosis, but also to include various nationalities to increase the validity of the results for a broader range of patients. In addition, such high quality trials should include long-term follow up over at least 1 year, and include a spectrum of generalized MG disease manifestations in order to discern if clinical response varies according to severity of disease manifestations. It is unlikely, however, that larger trials with highly robust methodology will be conducted due to the relatively uncommon nature of the disease, which makes recruitment, randomization and blinding of patients a challenge.

Conclusion

MG is a debilitating muscular disorder and tacrolimus may have a potential role in its treatment. There is limited yet promising information to suggest a beneficial role for tacrolimus in reducing QMGs scores as well as corticosteroid burden in patients with refractory symptoms or new-onset disease. Although significant differences were not found in the primary endpoint of the largest randomized controlled trial to date, additional high quality research is needed to confirm or refute these findings. Long-term use of tacrolimus in the management of MG appears to be safe.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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