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Myasthenia gravis

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CONTENTS

Abstract

Myasthenia gravis is an autoimmune disorder that affects neuromuscular transmission and leads to recurrent weakness and fatigue. Circulating autoantibodies block acetylcholine (ACh) receptors at the neuromuscular junction of skeletal muscle, thus impairing neuromuscular signaling and causing muscle weakness. Although myasthenia gravis can be managed with currently available medication, this is not devoid of adverse effects. Research efforts aimed at understanding the disease process in myasthenia gravis should help to develop more targeted treatments.

Introduction

Myasthenia gravis is a common autoimmune disorder with an estimated prevalence ranging from 5 to 15 per 100,000 and an annual incidence of approximately 1 per 100,000 individuals. However, myasthenia gravis has been suggested to be underdiagnosed in the elderly population, where it may represent an incidence of 1 per 60,000 (1). Myasthenia gravis is associated with the existence of one maternal relative with the disorder or another autoimmune disorder in 30% of patients. There is also an increased frequency of concomitant autoimmune disease in myasthenia gravis patients. There are no apparent gender differences, although early-onset myasthenia gravis, which develops before the age of 50 years (see below), is more frequent in females (male/female ratio: 1:4). Late-onset myasthenia gravis is equally frequent in women and men and mainly develops around 70-80 years of age. Ocular myasthenia gravis may affect all age groups, but children and late-onset male patients appear to be more susceptible. Myasthenia gravis is characterized in the majority of patients by the presence of autoantibodies against the acetylcholine receptor (AChR), which impair neuromuscular transmission, causing muscle weakness (2).

Pathogenesis

The neuromuscular junction is the synapse between a motor neuron axon terminal and a muscle fiber at the motor endplate. Axon terminals from cholinergic motor neurons in the ventral horns of the spinal cord and brain stem split into many terminals that innervate individual skeletal muscle fibers. At the muscle fiber, the axons loose their myelin sheath and subdivide into presynaptic buttons containing ACh synaptic vesicles. Nerve action potentials stimulate the release of ACh into the synaptic cleft, which binds postsynaptic AChR and triggers the opening of the associated cation channel, allowing sodium ions into the muscle fiber, which initiates the process of muscle contraction (Fig. 1). In myasthenia gravis, circulating antibodies to AChR disrupt normal neuromuscular transmission by three mechanisms: 1) AChR antibody binding and activation of the complement cascade, prompting the destruction of the neuromuscular junction; 2) increased internalization rate of the AChR and subsequent degradation by antigenic modulation of AChR antibodies; and 3) functional blockade of the AChR, thus interfering with ACh binding (3).

A pathogenic role for the thymus has been proposed, as around 75% of myasthenia gravis patients present with thymic abnormalities and intrathymic expression of the AChR has been detected. The disorder has been associated with impaired thymic deletion of $CD4^+$ AChR-reactive T-cells as a result of a dysfunction in thymic regulatory ($CD2^+CD25^+$) T-cells, which are essential for the development and maintenance of peripheral tolerance (4). Thus, interaction between anti-AChR $CD4^+$ T-cells and B-lymphocytes is critical for the synthesis of AChR autoantibodies. In this context, major histocompatibility complex (MHC) (or human leukocyte antigen [HLA]) molecules, which present antigen epitopes to $CD4^+$ T-cells, are important for the pathogenesis of myasthenia gravis, as patients express certain HLA alleles more frequently than the normal population. Genetic susceptibility to myasthenia gravis frequently involves polymorphisms at type I and II MHC (5-7), α and δ subunits of the human AChR (8, 9)

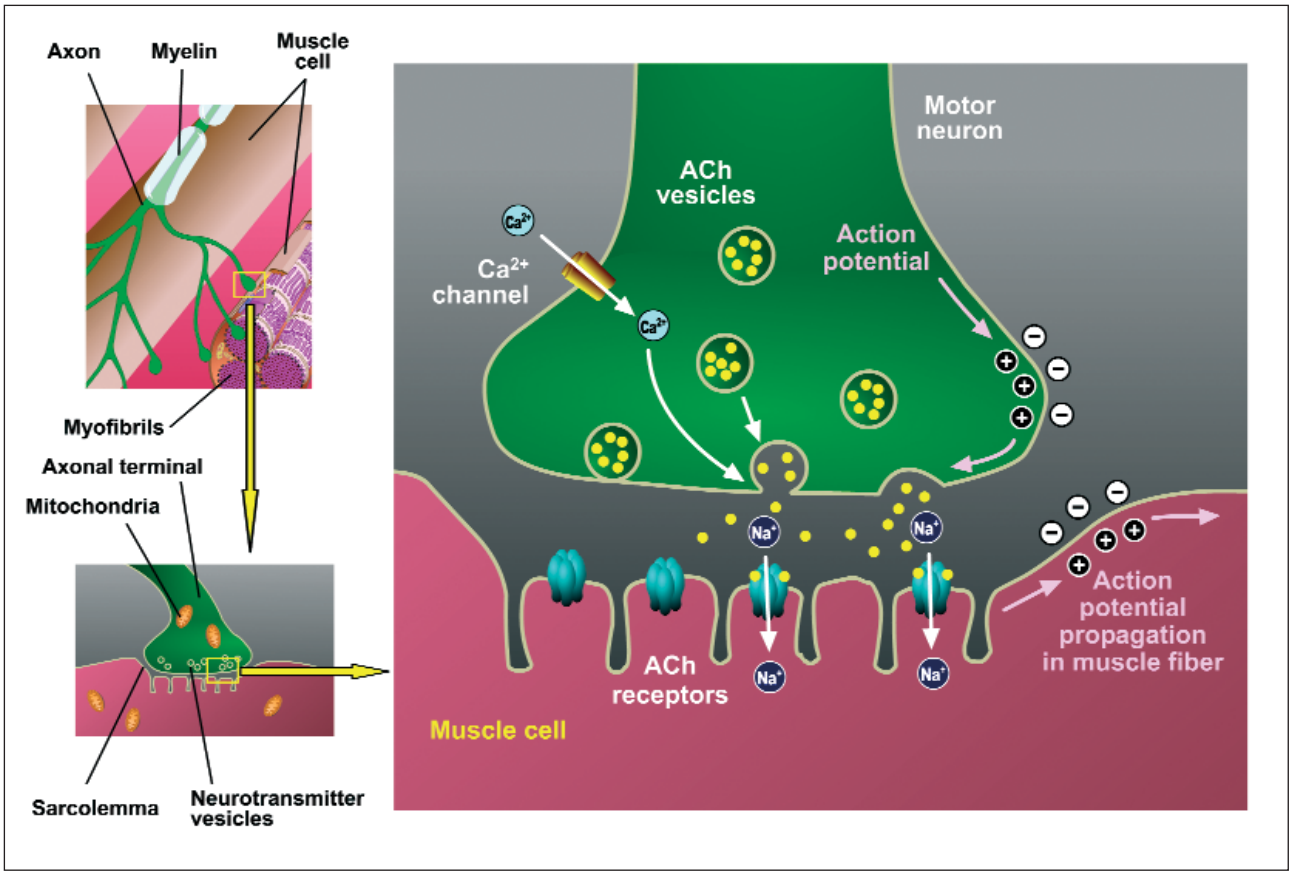

Fig. 1. The neuromuscular junction is the synapse between the presynaptic motor neuron and the postsynaptic skeletal muscle membrane. The motor neuron axon divides into terminal buttons that innervate individual skeletal muscle fibers. Acetylcholine (ACh) is stored in synaptic vesicles in the presynaptic neuron. The arrival of a nerve action potential opens presynaptic voltage-gated calcium channels, thereby allowing calcium to enter the motor neuron cytoplasm, causing synaptic vesicle fusion to the plasma membrane and subsequent ACh release into the synaptic cleft. ACh then binds to postsynaptic nicotinic ACh receptors (AChR) on the motor endplate, thereby activating sodium channels. Sodium influx results in membrane depolarization, leading to muscle contraction. After muscle contraction, ACh is metabolized by acetylcholinesterase (AChE) present at the synaptic cleft.

and receptors for the constant fraction of immunoglobulin G (IgG), or Fcγ receptors (10). In particular, there is a strong correlation between the different clinical forms of myasthenia gravis and some HLA genotypes. Hence, early-onset disease has been linked to HLA-A1, -B8, -DQB1, -DR3, -DR52a and -DR14-DQ5 (2, 5) in Caucasian patients, whereas HLA-A3, -B7, -DR2, -DR4 and -DR7 are all weakly associated with late-onset myasthenia gravis (2). Furthermore, about 15% of myasthenia gravis patients are seronegative for AChR autoantibodies, and instead may develop antibodies against the postsynaptic protein muscle-specific kinase (MuSK). MuSK is important for normal synaptic function, as it mediates clustering of AChRs at the neuromuscular junction (3).

Clinical symptoms

Myasthenia gravis is diagnosed based on the occurrence of weakness and muscle fatigue. Typically, weakness increases after muscle use and improves following rest. Extrinsic ocular muscles are commonly affected in about two-thirds of patients, resulting in ptosis (eyelid drooping) and diplopia (double vision). Weakness may spread to facial, neck, bulbar and limb muscles, leading to generalized myasthenia gravis in around 85% of patients (2). Myasthenia gravis can be classified into different subgroups: 1) ocular disease, which is AChR antibody-positive and features ocular symptoms only; 2) early-onset disease, presenting with AChR antibodies, generalized symptoms and often with concomitant autoimmune disorders, and disease onset before the age of 50 years; 3) late-onset myasthenia gravis, with the same features as early-onset disease but with an onset after 50 years of age; 4) thymoma myasthenia gravis, in which, in addition to muscular affectation, patients have a concurrent thymus tumor (thymoma), as well as the presence of serum AChR antibodies; and 5) seronegative myasthenia gravis, which occurs in 15% of patients and is characterized by the absence of anti-AChR antibodies (2).

Myasthenia gravis is associated with thymic abnormalities, hyperthyroidism and other autoimmune diseases, including rheumatoid arthritis, pernicious anemia,

systemic lupus erythematosus (SLE), sarcoidosis, Sjögren's disease, polymyositis, ulcerative colitis and pemphigus (11, 12). Current treatment strategies for myasthenia gravis have decreased mortality from 20-30% to < 4%. However, if patients are not appropriately treated, they may develop myasthenic crises that cause an acute exacerbation of weakness of the diaphragm and intercostal muscles, resulting in respiratory failure (13).

Lambert-Eaton myasthenic syndrome, botulism and hyperthyroidism are the most common disorders mimicking myasthenia gravis symptoms. Lambert-Eaton myasthenic syndrome is an autoimmune disorder of the neuromuscular junction that can be ruled out by the presence of antibodies to P/Q-type calcium channels. Botulism can also cause generalized weakness and ophthalmoplegia, but pupil dilatation and enhanced response to repetitive nerve stimulation can differentiate it from myasthenia gravis. Hyperthyroidism can be discarded with routine thyroid function tests (12).

Anti-AChR antibodies are detected in approximately 85% of patients with generalized myasthenia gravis. Besides AChR antibodies, antibodies to striated muscle proteins, such as ryanodine receptors (RyR) and titin, are found in patients with thymoma and late-onset myasthenia gravis. In general, disease severity correlates with the presence of AChR antibodies and RyR and/or titin antibodies in the case of late-onset disease (11). In approximately 31-41% of patients seronegative for AChR antibodies, antibodies to MuSK are relatively common and have been associated with more prominent affectation of bulbar and respiratory muscles (3, 14).

Current treatment options

Therapeutic strategies for the management of myasthenia gravis are aimed at increasing neuromuscular transmission to improve fatigue and increase muscle strength, and involve nonpharmacological and drug therapies.

Nonpharmacological therapies

Regardless of the presence of a thymoma, therapeutic ablation of the thymus or thymectomy is a common therapeutic option in myasthenia gravis. Although solid evidence from randomized clinical trials is lacking, an evidence-based review by the American Academy of Neurology established that thymectomy is recommended as an option to increase the probability of remission or improvement in patients with generalized nonthymomatous autoimmune myasthenia gravis, in particular those positive for AChR antibodies (15). In AChR antibody-negative patients, the indication for thymectomy remains to be determined. In patients with a thymoma, surgical removal of the tumor is advised to avoid the possibility of tumor spread (16). The National Institute of Neurological Disorders and Stroke (NINDS) is currently investigating whether thymectomy in combination with prednisone therapy is more effective in treating myasthenia gravis without thymoma than prednisone treatment alone (17).

Another treatment option is plasmapheresis, a procedure involving removal of autoantibodies from the patient's plasma by membrane filtration or centrifugation. Blood cells are then reconstituted with saline, plasma substitute or donor plasma and returned to the patient. This causes a transient improvement in symptoms and is recommended as short-term treatment to manage acute disease exacerbations (myasthenic crises) and to prepare patients for thymectomy. There is no evidence from randomized clinical trials supporting the use of recurrent plasmapheresis as a long-term treatment in patients with myasthenia gravis (16).

Cholinesterase inhibitors

Inhibitors of the ACh-degrading enzyme acetylcholinesterase (AChE) increase ACh concentrations in the synaptic cleft, thus enhancing cholinergic transmission at the neuromuscular junction and improving symptoms of fatigue. They are usually the first agents prescribed after diagnosis and may be enough to adequately control symptoms of mild myasthenia gravis. AChE inhibitors are usually well tolerated, although adverse effects may arise from excessive ACh concentrations, resulting in muscarinic effects such as hypermotility of the digestive tract, sweating and respiratory and gastrointestinal hypersecretion, or nicotinic adverse effects including muscle fasciculations and cramps (16). A representative and widely used agent of this class is pyridostigmine bromide (Mestinon®; Valeant), which has been prescribed for more than 50 years.

Immunomodulating agents

Targeting the immune system helps to reduce the production of pathogenic autoantibodies. Corticosteroids may be first-line therapy in mild to moderate cases of myasthenia gravis and they can be used alone or in combination with other immunosuppressant agents. In particular, oral prednisolone is considered the first-choice drug and should be initiated at 10-25 mg on alternate days, progressively increasing to 60-80 mg in order to avoid transient worsening of symptoms. Clinical response usually occurs within 4-16 weeks of treatment. Prednisolone should be gradually tapered to the lowest possible dose (16).

Azathioprine is widely used in the management of myasthenia gravis along with corticosteroids when long-term immunosuppression is needed. Clinical evidence has shown that azathioprine reduces the need for corticosteroid treatment (16).

The alkylating agent cyclophosphamide has been shown to reduce the need for systemic corticosteroids and improve muscle strength in myasthenia gravis patients in controlled clinical trials. However, its use is restricted to patients unresponsive/intolerant to the above-mentioned medications, due to its potential to cause myelotoxicity and other severe adverse effects (16).

Investigational therapies

Different strategies have been approached in order to develop more effective myasthenia gravis therapies. Here we present a summary of experimental therapies currently under investigation for the treatment of myasthenia gravis. Detailed information on these studies is depicted in Table I.

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG), which binds to pathogenic autoantibodies, has shown comparable benefit to plasma exchange therapy in the treatment of myasthenia gravis exacerbations (16). The efficacy of IVIG therapy has been associated with reduced humoral and cellular responses in experimental autoimmune myasthenia gravis in rats. Thus, IVIG administration led to decreased levels of AChR-specific IgG isotypes, reduced lymphocyte proliferation and a downregulation of Th1-type cytokines and factors involved in B-cell function and survival, indicating a potential reduction in antibody synthesis (18). A recent clinical study showed that IVIG therapy (2 g/kg) reduced disease severity scores (Quantitative Myasthenia Gravis [QMG] score for disease severity) 14 days after treatment compared to placebo, especially in patients with moderate to severe disease (19). A previous investigation demonstrated that acute exacerbations of myasthenia gravis were effectively treated with IVIG. This study also found comparable efficacy between a single dose and two consecutive doses of 1 g/kg (20).

Debio-0513

In myasthenia gravis, the production of anti-AChR antibodies is modulated by AChR-specific T-cells. The α subunit of the AChR contains T-cell epitopes whose sequences have been identified as highly immunogenic epitopes for the development of myasthenia gravis, namely p195-212 and p259-271. Debio-0513 (formerly PTR-262), discovered at the Weizmann Institute of Science in Israel and under development by Debiopharm under a licensing agreement with DeveloGen, is a dual altered peptide ligand (APL) composed of two single amino acid-substituted analogues of these AChR α subunit myasthenogenic epitopes. In preclinical models of myasthenia gravis, treatment with Debio-0513 created a shift in the immune system from the harmful $CD4^+$ autoreactive T-cell population to regulatory T-cells ($CD4^+CD25^+$), which downregulates the autoimmune response of myasthenia gravis, resulting in significant clinical improvement (21). Furthermore, in peripheral blood serum derived from myasthenia gravis patients, Debio-0513 inhibited the proliferation of T-cell populations that are responsible for myasthenogenic autoimmune-mediated reactions, and thus the development of disease. Recent findings have demonstrated that Debio-0513 exerts its reversing effects on experimental autoimmune myasthenia gravis symptoms via upregulation of the Fas death pathway in autoreactive T-lymphocytes, but not in the regulatory T-cell subset, as well as via downregulation of antiapoptotic molecules (22). Debiopharm received European orphan drug designation for Debio-0513 for the treatment of myasthenia gravis in September 2006 (23).

rEV-576

Inhibition of complement cascade activation has been proposed as another strategy for treating myasthenia gravis. The complement system is part of the innate immune system and essential for recognition and opsonization of pathogenic organisms, initiating a cascade of events that culminates in pathogen destruction by phagocytes. In myasthenia gravis, antibody binding to the AChR triggers the activation of the complement cascade, with subsequent formation of the membrane attack complex (MAC) and destruction of the neuromuscular junction (3). Clinical improvement in myasthenia gravis has been observed after blockade of complement activation in rodents with experimental autoimmune myasthenia gravis, suggesting the potential clinical utility of this approach (24).

Evolutec has developed a novel complement inhibitor protein, rEV-576, which is a recombinant version of a natural C5 inhibitor identified in the salivary gland of the soft tick *Ornithodoros moubata* (25). In preclinical studies, a single rEV-576 injection completely prevented paralysis and weight loss for at least 7 days in mice with experimental autoimmune myasthenia gravis. Interestingly, animals treated with rEV-576 presented higher AChR density than untreated controls, indicating reduced receptor loss or internalization. In addition, rEV-576's low immunogenic profile favors its long-term use.

rEV-576 interferes with the late steps of the complement activation pathway by binding C5 protein and preventing its cleavage, thus blocking the formation of key inflammatory molecules (C5a) and MAC (25, 26). The anaphylatoxin C5a is an extremely potent proinflammatory peptide that promotes neutrophil and eosinophil chemotaxis and enhances capillary permeability, among other functions. The advantage of targeting C5 and not the early steps of the complement system cascade is that this reduces the risk of concomitant infection, especially during long-term complement inhibition. Evolutec plans to progress rEV-576 to the clinic in 2008 (27).

Monarsen

A specific variant of the ACh-degrading enzyme AChE has been associated with the onset and progression of myasthenic disease (28). Acute stress or excessive AChE inhibitor use is able to trigger the formation of an AChE variant formed by alternative splicing of its primary gene transcript (pre-mRNA). This readthrough AChE variant (AChE-R) differs from the predominant

membrane-associated AChE (or synaptic) variant in the *C*-terminal domain, as AChE-R exhibits no splicing after the last exon encoding the catalytic domain (29).

AChE-R accumulation in response to acute stress has been suggested to operate as a protective system in the brain to prevent stress-induced cholinergic hyperactivation. In contrast, excess AChE-R accumulation in muscle has been correlated with impaired cholinergic neurotransmission in mice subjected to prolonged cholinesterase inhibition (30). AChE-R was hypothesized to have a role in the pathogenesis of myasthenia gravis, as patients with the disease and experimental autoimmune myasthenia gravis rats presented with elevated AChE-R serum levels. Moreover, AChE-R was found to be overexpressed in the muscle of experimental autoimmune myasthenia gravis rats (28).

Researchers at Ester Neurosciences have developed the antisense oligonucleotide monarsen (formerly EN-101), a short chain of 20 nucleic acids that inhibits the translation of AChE by interfering with the correct reading of the protein from mRNA. In preclinical studies, intravenous injection of monarsen reduced AChE-R levels in muscle and serum, thereby normalizing the compound muscle action potentials in rats with experimental autoimmune myasthenia gravis. Daily oral administration of monarsen enhanced survival and improved clinical status compared to rats treated with pyridostigmine (28). Further studies in rats with experimental autoimmune myasthenia gravis reported benefit with monarsen at the electrophysiological level, as demonstrated by improvement in the mean consecutive difference (MCD) values upon stimulated single-fiber electromyography, as well as in clinical symptoms (31).

A phase Ib trial of monarsen was the first to demonstrate the safe and effective use of an orally administered antisense therapy for a neurological disease. Sixteen patients with stable myasthenia gravis discontinued all previous AChE inhibitor treatments during a washout period of 12-18 h and were given increasing oral doses of monarsen for 1 day, followed by a single daily oral dose of 500 μg/kg of monarsen for 3 days. Fourteen patients had better scores on the QMG scale on the last day of dosing as compared to baseline. Improvement in total QMG score for these days ranged from 27.8% to 53.4%. The benefits induced by monarsen were maintained for up to 72 h after the last dose. After monarsen treatment, patients also experienced a significant and clinically relevant improvement in performance. The drug was well tolerated, as no serious adverse events and no cholinergic side effects were reported (32). In November 2003, the FDA granted monarsen orphan drug designation for the treatment of myasthenia gravis, and the EMEA followed suit in May 2004. Ester Neurosciences commenced a double-blind phase IIb trial in 2004 that enrolled 18 myasthenia gravis patients who were assigned to three different doses of monarsen given once daily for 1 week. The study is designed to evaluate the safety and quality of life following 1-month administration of monarsen (33).

Mycophenolate mofetil

Despite initial observations indicating the potential therapeutic utility of mycophenolate mofetil (CellCept®; Aspreva, Roche) in myasthenia gravis (34-36), final results from two phase III clinical studies cast doubt on its efficacy. A randomized, double-blind, placebo-controlled phase III trial of mycophenolate mofetil carried out by Aspreva failed to meet both primary and secondary endpoints. The trial was designed to evaluate the efficacy and safety of mycophenolate mofetil in maintaining or improving symptom control with reduced doses of corticosteroids in patients with myasthenia gravis over a treatment period of 36 weeks (37, 38). The primary endpoints included minimal disease activity while maintaining designated low steroid and cholinesterase inhibitor doses. Although the trial failed to meet both primary and secondary endpoints, mycophenolate mofetil appeared to be generally well tolerated. The efficacy of mycophenolate mofetil was further evaluated in a continuation study involving patients who responded to mycophenolate treatment (39). An 80-patient investigator-initiated trial conducted by the Duke University Medical Center with The Muscle Study Group and supported by the FDA's Office of Orphan Products Development, Roche and Aspreva evaluated the effect of treatment with prednisone and CellCept® in comparison to prednisone alone on the signs and symptoms of myasthenia gravis in patients who were not on corticosteroids and had not previously received other immunosuppressants. After 12 weeks of treatment, the efficacy of mycophenolate mofetil and prednisone (20 mg) was no different from prednisone alone based on QMG scores (40, 41).

Etanercept

Targeting tumor necrosis factor-α (TNF-α) has also been tested as a potential treatment option for myasthenia gravis. Etanercept is a recombinant fusion protein comprising the soluble human p75 TNF receptor linked to the Fc portion of human IgG_1, which acts by binding and inactivating TNF-α. A small open study showed clinical benefit with increased muscle strength and improved QMG scores in 6 of 11 steroid-dependent patients treated with etanercept twice weekly for 6 months (42). However, some patients developed increased plasma levels of proinflammatory cytokines (*i.e.,* interferon gamma [IFN-γ] and IL-10), C3 complement factor and immune complex levels, which could induce clinical deterioration in myasthenia gravis patients. Nevertheless, etanercept improved clinical QMG scores, especially in patients with lower baseline IL-6 and IFN-γ levels (43).

Tacrolimus

An Immunosuppressant drug commonly used in transplant rejection, tacrolimus, has been reported to be associated with benefits in patients with poorly controlled myasthenia gravis, in particular patients with antibodies

Table I: Summary of clinical studies of experimental therapies for myasthenia gravis (from Prous Science Integrity®).

Drug	Design	Treatments	n	Conclusions/Objectives	Ref.
Intravenous immuno-globulin	Randomized Double-blind	IVIG, 2 g/kg i.v. Placebo	51	IVIG treatment decreased QMG scores after 14 days compared to placebo, effects that persisted at day 28. Greatest improvement occurred in patients with moderate to severe disease	19
	Multicenter Randomized Double-blind	IVIG, 1 g/kg i.v. infusion on d 1 + Placebo on d 2 IVIG, 1 g/kg i.v. infusion o.d. x 2 d	173	IVIG at doses of 1 and 2 g/kg was effective in the treatment of acute exacerbations of myasthenia gravis	20
Monarsen	Open	Monarsen, 500 µg/kg p.o. o.d. x 3 d	16	Monarsen was more effective than conventional acetylcholinesterase inhibitors in improving the QMG score and performance in patients with stable myasthenia gravis	32
Mycophenolate mofetil	Randomized Double-blind	Mycophenolate mofetil, 1 g b.i.d. x 5 mo Placebo	14	Patients receiving mycophenolate mofetil showed a trend towards improvement in QMG and MMT scores, as well as a non-significant reduction in AChR antibody titers	34
	Open	Mycophenolate mofetil, 1g/d [dose escalated] x 38 mo	31	Mycophenolate mofetil at a dose of 1 g/d was well tolerated and effective in patients with ocular myasthenia gravis	35
	Open	Mycophenolate mofetil	10	Mycophenolate mofetil was safe and effectively improved functional status in patients with refractory myasthenia gravis	36
	Multicenter Randomized Double-blind	Mycophenolate mofetil x 36 wks	136	Mycophenolate mofetil was generally well tolerated, but failed to meet the endpoints of the trial: efficacy and safety in maintaining or improving symptom control with reduced doses of corticosteroids in patients with myasthenia gravis	37, 38
	Multicenter Randomized Double-blind	Mycophenolate mofetil, 1g b.i.d. + Prednisone Placebo + Prednisone	NR	This trial will evaluate the safety of continued treatment with mycophenolate mofetil in myasthenia gravis patients who participated in a previous study of mycophenolate mofetil and showed good symptom control with a stable dose of prednisone	39
	Multicenter Randomized Double-blind	Mycophenolate mofetil, 1.25 g b.i.d + Prednisone, 20 mg/d Placebo + Prednisone, 20 mg/d	80	After 12 weeks of treatment, no difference in efficacy could be observed between patients treated with mycophenolate mofetil and prednisone compared to those receiving prednisone alone	40, 41
Etanercept	Open	Etanercept, 25 mg s.c. 2x/wk x 6 mo	11	Etanercept was effective in 6 of 8 patients with steroid-dependent myasthenia gravis who completed the trial. Patients with lower pretreatment plasma IL-6 and IFN-γ levels showed better clinical improvement. Three patients were withdrawn due to disease worsening or adverse events	42, 43
Tacrolimus	Open	Prednisolone, 2.5 → 5 mg + Tacrolimus, 3 mg/d x 1 y	7	Tacrolimus offered substantial benefits in patients with anti-ryanodine receptor-positive myasthenia gravis, and enhanced ryanodine receptor-related calcium release from the sarcoplasmic reticulum	44
	Open Multicenter	Tacrolimus, 3 mg/d p.o. → titrated up to 5 [max.] mg/d x 16 wks	19	Tacrolimus treatment improved either muscle strength score or ADL score in 47% of patients with myasthenia gravis. Two patients were withdrawn due to myasthenic crises	46

Continuation

Table I (Cont.): Summary of clinical studies of experimental therapies for myasthenia gravis (from Prous Science Integrity®).

Drug	Design	Treatments	n	Conclusions/Objectives	Ref.
Tacrolimus	Open	Tacrolimus, 2-4.5 mg/d o.d. p.o. x 88-104 wks	12	Long-term treatment of myasthenia gravis patients with tacrolimus appeared to enhance the efficacy of short-term treatment	47
	Randomized Open	Tacrolimus, 3 mg/d p.o. + Prednisolone, 20 [max.] mg/d p.o. + Plasmapheresis + Methylprednisolone, 1 g i.v. x 1 y Prednisolone, 20 [max.] mg/d p.o. + Plasmapheresis + Methylprednisolone, 1 g i.v. x 1 y	34	Administration of low-dose tacrolimus in *de novo* myasthenia gravis patients reduced the number of plasmapheresis plus high-dose intravenous methylprednisolone treatments, as well as the required doses of oral prednisolone	48
	Open	Tacrolimus, 3 mg/d p.o. + Prednisolone	9	Long-term treatment with tacrolimus not only improved myasthenia gravis symptoms, but also allowed a 50% reduction in prednisolone dose in 50% of steroid-dependent myasthenia gravis patients	49
	Open	Tacrolimus, 3 mg/d p.o.	17	Tacrolimus improved performance of activities of daily living and allowed a reduction in the mean prednisolone dose in steroid-dependent thymectomized myasthenia gravis patients	50
	Open	Tacrolimus, 0.1 mg/kg/d p.o. b.i.d → adjusted to C_{ss} 7-8 ng/ml	86	Long-term tacrolimus treatment significantly reduced QMG score, as well as anti-AChR antibody titers, and increased muscle strength in prednisone-dependent myasthenia gravis patients. Prednisone was progressively reduced and withdrawn in all but 2 patients at study end	51
	Open	Tacrolimus, 0.1 mg/kg/d p.o. b.i.d → adjusted to C_{ss} 7-8 ng/ml	49	Starting tacrolimus treatment immediately after trans-sternal extended thymectomy increased complete stable remission rates in myasthenia gravis patients and allowed complete removal of prednisone therapy	52
	Open	Thymectomy → Methylprednisolone [pulsed therapy] + Prednisolone [tapered] + Tacrolimus → [if critical attack] Plasmapheresis → Methylprednisolone [pulsed therapy] → Tacrolimus Thymectomy → Methylprednisolone [pulsed therapy] + Prednisolone [tapered] → [if critical attack] Plasmapheresis → Methylprednisolone [pulsed therapy] → Tacrolimus	11	Tacrolimus reduced ADL score, as well as anti-AChR antibody levels, in patients with severe myasthenia gravis. A reduction in corticosteroid dose was also achieved	53
	Open	Tacrolimus, 3 mg p.o. o.d. x 1 mo	19	Once-daily tacrolimus was effective in reducing disease severity and the production of IL-2 by peripheral blood mononuclear cells in patients with myasthenia gravis	54
	Multicenter Randomized Double-blind	Tacrolimus Placebo	80	This phase III study will determine the safety profile and efficacy of tacrolimus in non-steroid-resistant myasthenia gravis patients	55
	Open Multicenter	Tacrolimus	NR	This phase III study will evaluate the safety and efficacy of tacrolimus in steroid-resistant, nonthymectomized myasthenia gravis patients	56

QMG: Quantitative Myasthenia Gravis scale; MMT: Manual Muscle Testing scale; ADL: Activities of Daily Living scale; NR: not reported

against RyR (44). The efficacy of tacrolimus therapy has been related to attenuation of the function of the P-glycoprotein drug efflux pump in peripheral blood mononuclear cells (PBMCs) of myasthenia gravis patients, thus increasing local tacrolimus concentrations and PBMC sensitivity to tacrolimus treatment (45).

Results from an open-label study in 16 patients with generalized disease showed that low-dose tacrolimus (3 mg/kg/day) significantly reduced median clinical myasthenia gravis scores by 4 points at 16 weeks after initiation of treatment. The patients' ability to carry out daily activities also improved with tacrolimus therapy. In summary, 47% of patients demonstrated improvement in either activities of daily living (ADL) or myasthenia gravis clinical score (46). Twelve patients from this initial study continued to receive low-dose tacrolimus therapy for up to 2 years. At study end, 67% of patients reported improved ADL or myasthenia gravis scores, supporting long- over short-term tacrolimus treatment. In addition, prednisolone doses were reduced in 7 patients by a mean of 37%. Overall, adverse events were of minor intensity, with only 1 case of headache and eye pain that required temporary discontinuation of tacrolimus therapy (47).

Another open, randomized study in 34 *de novo* myasthenia gravis patients demonstrated that low-dose tacrolimus decreased the need for plasmapheresis and high-dose intravenous methylprednisolone treatment, reduced hospitalization days in the early phase of therapy, as well as the mean dose of prednisolone at 1 year of treatment (48).

Tacrolimus has proven effective in reducing the need for steroid therapy in steroid-dependent myasthenia gravis patients. A small study reported a 50% reduction in prednisolone dose after 1 year of tacrolimus treatment in 3 of 6 steroid-dependent patients (49). Low-dose tacrolimus therapy was also effective in improving ADL scores and reducing the average prednisolone dose in steroid-dependent thymectomized myasthenia gravis patients (50). This effect was further evaluated in a larger study conducted in 79 patients with severe disease unresponsive to combined ciclosporin and prednisone treatment, who received long-term tacrolimus at doses ranging from 6 to 10 mg/day for a mean period of 2.5 years. Patients were switched from ciclosporin to tacrolimus and, at the end of the study, the doses of prednisone were completely withdrawn in all but 2 patients. In addition, tacrolimus significantly reduced QMG score, as well as anti-AChR antibody titers, and increased muscle strength by 39% (51). This research team also investigated the efficacy of tacrolimus after trans-sternal extended thymectomy. Tacrolimus treatment (0.1 mg/kg/day twice daily) increased the efficacy of thymectomy, augmenting the rates of complete stable remission and permitting withdrawal of prednisone therapy in 93.7% and 100% of patients after 1 and 2 years of treatment, respectively (52).

Severe myasthenia gravis cases have been shown to respond to tacrolimus treatment, which was able to improve ADL scores and reduce anti-AChR antibody titers, as well as decrease the corticosteroid dose (53). Another small open study found that high levels of IL-2 were associated with high serum levels of AChR antibodies, severe generalized symptoms and thymic hyperplasia. The administration of tacrolimus once daily for 1 month to 19 myasthenia gravis patients reduced both disease severity and IL-2 production by PBMCs, especially in patients with high IL-2 production (54). Astellas Pharma is currently investigating the safety and efficacy of tacrolimus in myasthenia gravis in two phase III clinical studies: a randomized, double-blind trial in patients who respond to steroid therapy (55) and an open-label study enrolling steroid-resistant patients (56).

Conclusions

In the last few years, much has been learned about the physiology of neuromuscular transmission and the pathogenesis of myasthenia gravis, which has translated into novel treatment modalities. Further knowledge of the autoimmune disease process is key to developing better treatments. Neuromuscular antisense therapeutics, such as monarsen, or immunomodulators directed at destroying autoreactive T-lymphocytes, like Debio-0513, are excellent examples of innovative targeted drugs to fight this debilitating disease. However, classical immunosuppressants, such as tacrolimus, merit further investigation, as results from clinical trials have been particularly encouraging for the management of myasthenia gravis symptoms.

Online links

Subscribers to Prous Science Integrity® can access an on-line animation describing ACh release and nicotinic receptor stimulation.

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