The immunosuppressant fingolimod ameliorates experimental autoimmune myasthenia gravis by regulating T-cell balance and cytokine secretion

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Received March 2, 2020; Accepted May 8, 2020; Epub June 15, 2020; Published June 30, 2020

Abstract: Myasthenia gravis is an autoimmune disease that affects skeletal muscle strength by impeding communication within the neuromuscular junction (NMJ). Research has shown that sphingosine-1-phosphate (S1P)/S1P receptor signaling may be involved in the process of neuromuscular diseases. Fingolimod is structurally similar to S1P, whose immunosuppressive effect has been recognized in many immune diseases. However, the mechanism underlying fingolimod’s action on experimental autoimmune myasthenia gravis is still far from clear. The aim of this study was to investigate the efficacy and possible mechanism of fingolimod on experimental autoimmune myasthenia gravis. Our results showed that pretreatment with fingolimod improved experimental autoimmune myasthenia gravis symptoms in a dose-dependent manner, including decreased anti-acetylcholine receptor-2α autoantibody titer, reduced compound muscle action potential decrement, and increased acetylcholine receptor content. Further investigation indicated that fingolimod inhibited lymphocyte proliferation responses and also regulated the balance of Th1/Th2 cells and Treg/Th17 cells. Moreover, fingolimod suppressed the secretion of pro-inflammatory or inflammatory cytokines IL-17A, IL-6, and INF-γ, but did not noticeably alter the secretion of immunosuppressive cytokines TGF-β1 and IL-4. In conclusion, our results suggest that fingolimod has a preventive effect on experimental autoimmune myasthenia gravis by interfering with lymphocyte function.

Keywords: Myasthenia gravis, fingolimod, Th cells, cytokines, lymphocyte proliferation

Introduction

Myasthenia gravis (MG) is an autoimmune disease that affects skeletal muscle strength by impeding communication within the neuromuscular junction (NMJ). The characteristic symptoms of MG are muscle weakness and fatigue. Antibodies against the muscle nicotinic acetylcholine receptor (anti-AChR Abs) play an important role in the pathogenesis of MG. These antibodies, which are produced by autoreactive B cells, belong to either the IgG1 or IgG3 subclass. The anti-AChR Abs activate the classical complement cascade, resulting in the destruction of neuromuscular architecture and the failure of neuromuscular transmission [1, 2]. In fact, MG patients have abundant anti-AChR Th1 cells, which induce synthesis of complement-fixing IgG subclasses in the peripheral blood [3]. In addition, patients with thymic hyperplasia always display germinal centers (GCs) [4] that exhibit an overexpression of cytokine IL-17 [5, 6]. The frequency of regulatory T (Treg) cells decreases markedly in MG patients [7, 8]. However, the functions of Treg cells are also defective [9].

Current MG therapies mainly target the NMJ and the immune system [10, 11]. Acetylcholinesterase inhibitors (AChEI) increase the activity of acetylcholine (ACh) at the NMJ and compensate for reduced acetylcholine receptor (AChR) densities [12]. General immunosuppression and thymectomy are first-line immunosuppressive treatments for patients who do not adequately respond to AChEI therapy [12]. Additional immunotherapies are emerging, such as eculizumab, which is approved for refractory MG by the FDA, and efgartigimod, which is considered effective in phase II clinical trials [13].
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The existing standard treatments for MG are not guaranteed to keep the disease well-controlled. Furthermore, they require a lifelong regimen and have significant side effects, such as risks of malignancy and infection. Therefore, there is an urgent need to overcome these shortcomings and find more effective treatments.

Fingolimod (FTY-720), a novel immunosuppressant, is structurally similar to sphingosine-1-phosphate (S1P). Current results show that FTY-720 regulates lymphocyte homing to lymphoid organs by binding to sphingosine-1-phosphate receptor 1 (S1PR1) and inducing its internalization and degradation [14-16]. The immunosuppressive effect of FTY-720, which results from its depletion of peripheral lymphocytes, has been recognized in organ transplantation and multiple sclerosis [14]. Moreover, FTY-720 mitigates the severity of chronic colitis and atherosclerosis by interfering with lymphocyte function [17, 18]. In addition to these therapeutic effects, FTY-720 is under consideration for use in treating other autoimmune diseases as well, such as rheumatoid arthritis [19] and systemic lupus erythematosus [20]. Therefore, we speculated that FTY-720 might be a prospective treatment for MG and experimental autoimmune myasthenia gravis (EAMG). Previous studies have shown that FTY-720 ameliorates EAMG [21, 22], although the mechanism of FTY-720 on MG is still far from clear. In this study, we focused on the possible mechanism of FTY-720 on EAMG. Particular attention was paid to the role of FTY-720 in lymphocyte function. The efficacy of FTY-720 against EAMG was determined by measuring autoantibody titer, repetitive nerve stimulation (RNS), and AChR content, followed by the study of possible mechanisms, including lymphocyte proliferation, balance of Th cells, and secretion of cytokines. Our data suggested that FTY-720 pretreatment ameliorated the severity of EAMG, and which was associated with lymphocyte proliferation suppression, regulation of Treg/Th17 and Th1/Th2 balance, and downregulation of proinflammatory or inflammatory cytokines, such as IL-17A, IL-6, and INF-γ. Research has shown that S1P/S1P receptor signaling may be involved in the processes of neuromuscular diseases, including Duchenne muscular dystrophy and MG [23]. FTY-720 is the first reported S1PR agonist with immunosuppressive activity. Since it is a nonspecific S1PR modulator, new similar compounds are expected to develop. Our data indicated that FTY-720 has a therapeutic potential for EAMG via interfering with lymphocyte function. These data may lay the foundation for new drug design in the treatment of autoimmune diseases, including MG.

Materials and methods

Animal

Female Lewis rats (weighing 150-180 g) were provided by the Qinglong Mountain Breeding Center (license no. SCXK (Su) 2017-0001, Nanjing, China) and 8- to 10-week-old male Sprague Dawley (SD) rats were purchased from Shanghai Sippr-BK Laboratory Animal Co. Ltd. The animals were kept in 12-h light/dark cycle conditions with food and water ad libitum. All experiments were performed in accordance with animal ethics rules.

Induction of EAMG and drug administration

Thirty Lewis rats were divided into five groups (six per group). All rats, except for the normal controls, were immunized with 300 μg of AChR2α (Bosgene Biotech, Nanjing, China), a purified recombinant 2 alpha subunit of human AChR, in complete Freund’s adjuvant (CFA) (Sigma-Aldrich, St. Louis, MO, USA) subcutaneously on the back and both feet on day 0. Subsequently, the rats were booster immunized with 300 μg of AChR2α in incomplete Freund’s adjuvant (IFA) (Sigma-Aldrich, St. Louis, MO, USA) on day 20, and booster immunized with 300 μg of AChR2α in CFA on day 30 [24]. The control rats were injected with the same volume of PBS and adjuvant without AChR2α. In order to evaluate the therapeutic effect of FTY-720, rats were treated with either vehicle, 0.15 mg/kg, or 1.5 mg/kg of FTY-720 every day. Prednisone dosed at 3.5 mg/kg was treated as the positive control. All rats were weighed twice a week until they were euthanized on day 35.

Repetitive nerve stimulation

Compound muscle action potential (CMAP) decrement was tested using the BL-420F Data Acquisition & Analysis System (Techman, Chengdu, China). Rats were anesthetized, and their temperatures were maintained at 37°C. A stimulating electrode was then inserted near the sciatic nerve for supramaximal stimulation.
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at 5 Hz and 10 Hz. CMAPs were recorded with recording electrodes, which were placed in the gastrocnemius muscle. Decrement values were calculated as the percent of amplitude attenuation between the 1st and 4th CMAPs.

Detection of anti-AChR2α-IgG

Serum samples from Lewis rats were prepared in order to detect anti-AChR2α-IgG using ELISA in accordance with the manufacturer's instructions. In brief, 96-well plates, pre-coated with AChR2α, were incubated with diluted serum (1:5, 50 μL) for 35 min at 37°C, followed by washing three times with wash buffer and incubating with 100 μL enzyme-labeled antibodies for 30 min at 37°C. After additional washing, substrate A and substrate B were added and incubated for 15 min at 37°C. Finally, the reaction was stopped, and the optical density (OD) value at 450 nm was measured.

Immunohistochemical staining

In order to analyze the AChR content of the NMJ, 20-Μm-thick frozen sections of gastrocnemius muscle were prepared and washed with TBST (0.1% Tween 20). The sections were then blocked with 10% goat serum for 2 h at room temperature (RT), and 3 μg/mL α-Bungatotoxin-FITC (Alomone Labs, Jerusalem BioPark, Israel) and anti-neurofilament heavy polypeptide (Abcam, Cambridge, MA, USA) were added to detect AChR and endplate, respectively [25]. After washing, sections were incubated with goat anti-rabbit IgG-Alexa Fluor 594 (Proteintech, Wuhan, China) for 1 h at RT. Finally, sections were observed and photographed under a 200-fold fluorescence microscope, and a quantitative analysis of AChR fluorescence intensity was performed using ImageJ software.

Preparation of lymphocytes

Splenic lymphocytes of Lewis rats from the different treatment groups were prepared by grinding spleens through a cell strainer on day 35, after the first immunization. The freshly-prepared cells were then adjusted to 5×10⁶ cells/mL in RPMI 1640 incomplete medium including 20% FBS at 2×10⁶ cells/mL.

Lymphocyte proliferation assay

A CCK-8 assay (Beyotime, Shanghai, China), in which WST-8 could be reduced to soluble formazan, was used to determine the effect of FTY-720 on lymphocyte proliferation. Briefly, 200 μL of cell suspension from SD rats, pre-treated with FTY-720 (0.5, 1, 5 μM) for 24 h at 37°C, was cultured in 96-well plates. The cells were then stimulated with or without concanavalin A (ConA, 5 μg/mL, Sigma, USA) for 24 and 48 h, respectively, followed by incubation with 10 μL CCK-8 for another 2 h. Cell culture supernatants were then collected for cytokine detection. OD values at 450 nm were measured using a microplate reader. The results were expressed in terms of the stimulation index (SI = ODdrug/ODcontrol).

Furthermore, 5×10⁵ cells from different treated rats were seeded into 96-well plates in the presence or absence of AChR2α (10 μg/mL). After 72 h of incubation, the cells were cultured for another 4 h with MTT. This was followed by the removal of supernatants and the addition of 100 μL DMSO to dissolve formazan, after which the OD values were tested at 490 nm. The results were expressed in terms of the stimulation index.

Cell cycle

In order to determine the effect of FTY-720 on the cell cycle, 2 mL of cell suspension from SD rats was cultured in six-well plates with or without FTY-720 (0.5, 1, 5 μM) for 24 h, after which the cells were collected and washed with PBS. After fixation with 1 mL 70% ethyl alcohol for 6 h at 4°C, the cells were stained with 0.5 mL propidium iodide (PI, Beyotime, Shanghai, China) at 37°C in the dark. Finally, the cells were detected using flow cytometry (Beckman Coulter, Brea, CA, USA), and the cell cycle was analyzed with FlowJo software.

Detection of Th cells

In order to detect the distribution of Th1/Th17/Th2 cells, 2 μL of PMA/ionomycin mixture and 2 μL of BFA/monensin mixture (MultiSciences, Hangzhou, China) were added to 500 μL of cell culture media and incubated for 6 h. The cells were then stained extracellularly with anti-CD3-
FITC (eBioscience, San Diego, CA, USA), and anti-CD8α-PE-Cy7 for 30 min at 4°C in the dark. After fixation for 15 min at RT, the cells were stained intracellularly with anti-INF-γ-eFluor 660, anti-IL-17A-PE, and anti-IL-4-eFluor 660 in the permeabilization solution, respectively. In order to detect Treg cells, surface markers on the splenocytes were analyzed by incubation with anti-CD4-FITC and anti-CD25-PE for 30 min at 4°C. After fixation and permeabilization, the cells were stained intracellularly with anti-Foxp3-APC for 30 min at 4°C. Finally, the samples were detected using flow cytometry.

Detection of cytokines

Sera from different treatment groups were prepared to detect TGF-β1 (R&D Systems, Minneapolis, MN, USA), IL-17A, and IL-6 (Bio-Rad, Hercules, CA, USA), and cell culture supernatants were harvested to detect TGF-β1, IL-4, IL-6, INF-γ, and IL-17A via Luminex assay in accordance with the manufacturer’s instructions.

Statistical analysis

Data were expressed as mean ± SEM, and statistical differences among various groups were determined using a one-way ANOVA, with a post hoc test (LSD) performed for multiple comparisons. Statistical differences between two groups were determined using Student’s t-test, with P < 0.05 indicating significant difference.

Results

FTY-720 alleviates AChR2α antigen-induced EAMG in Lewis rats

In order to determine the effect of FTY-720 on EAMG, the body weight, autoantibody titer, RNS, and AChR content of the rats were measured. During the experiment, the rats in different groups exhibited no significant difference in body weight (Figure 1A). The levels of anti-AChR IgG in the EAMG group were significantly increased compared with the levels in the control group (P < 0.001). Prednisone (P < 0.05) and FTY-720 at 1.5 mg/kg (P = 0.052) significantly reduced anti-AChR Abs titer compared with the EAMG group (Figure 1B). To determine the changes in AChR levels in the gastrocnemius muscle, frozen sections were double stained with BTX and anti-neurofilament heavy poly-peptide antibody. The results revealed that AChR fluorescence intensity in the EAMG group was noticeably weaker than that in the control rats (P < 0.05). Treatment with FTY-720 and prednisone significantly strengthened the fluorescence intensity of AChR compared with the EAMG groups (Figure 2A and 2B) (P < 0.05). In addition, gastrocnemius muscle function was tested using CMAP decrement. Consistent with all the above results, there was an obvious CMAP decrement in EAMG rats with supramaximal stimulation at 5 Hz and 10 Hz compared with the control group (P < 0.001 and P < 0.01, respectively). Compared with the EAMG group, both FTY-720 and prednisone noticeably reduced CMAP decrement, although this reduction did not reach statistical significance in the FTY-720 treated groups (Figure 1E and 1F). All of these data suggest that FTY-720 could alleviate the severity of EAMG.

FTY-720 inhibits lymphocyte proliferation

Lymphocyte proliferation was measured to determine whether the therapeutic effect of FTY-720 was related to lymphocyte responses. Splenic lymphocytes of SD rats were pretreated with FTY-720 (0.5, 1, 5 μM) for 24 h, then stimulated with ConA (5 μg/mL) and continuously incubated for 24 h or 48 h. The results revealed that FTY-720 dose-dependently inhibited lymphocyte proliferation (5 μM, P < 0.001; 1 μM, P < 0.05) (Figure 3A and 3B). Furthermore, in order to determine the effect of FTY-720 on the antigen (AChR2α)-specific lymphocyte proliferation reaction, lymphocytes from different treatment groups were cultured with AChR2α (10 μg/mL) for 72 h. As can be seen in Figure 3, splenic lymphocytes from EAMG rats exhibited an obvious proliferation reaction in response to AChR2α compared with lymphocytes from control rats (P < 0.001). Lymphocyte proliferation was markedly decreased, however, in the prednisone group and the 1.5 mg/kg FTY-720 group (P < 0.01 and P < 0.05, respectively) compared with the control group (Figure 3C).

In order to examine how FTY-720 affects lymphocyte proliferation, primary isolated splenic lymphocytes were treated with FTY-720 (0.5, 1, 5 μM) for 24 h followed by PI staining. Flow cytometry was performed to determine the cell cycle phase distribution. The results indicated that FTY-720 significantly increased the per-
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percentage of S phase lymphocytes (5 μM, P < 0.001) (Figure 4D). Meanwhile, the percentage of G1 or G2 phase lymphocytes slightly decreased, and there was no statistical difference between the groups (Figure 4C and 4E). These data suggest that FTY-720 could lead to S phase arrest followed by the inhibition of antigen-activated lymphocyte responses.

FTY-720 regulates the distribution of Th cells

In order to investigate the mechanisms of FTY-720 on EAMG, CD3⁺CD8α⁺INF-γ⁺ Th1 cells, CD3⁺CD8α⁺IL-4⁺ Th2 cells, CD4⁺CD25⁺Foxp3⁺ Treg cells, and CD3⁺CD8α⁺IL-17A⁺ Th17 cells from different groups were detected using flow cytometry. In the EAMG group, Th1 (P < 0.001), Th2 (P < 0.001), Treg (P < 0.05), and Th17 cells (P < 0.001) noticeably increased (Figure 5E, 5F, 5H and 5I, respectively), which might be due to an immune system response to extrinsic antigens. In addition, we further analyzed the ratios of Th1/Th2 and Treg/Th17 in different groups and found a disorder related to Th cell distribution. The Th1/Th2 ratio markedly increased (P < 0.001) while the Treg/Th17 ratio decreased.
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in the EAMG groups compared with the control group (Figure 5G and 5J), indicating that CD4+ T cells differentiated towards Th1 and Th17 cells in EAMG rats. After drug administration, Th1 and Th17 cells markedly decreased in rats treated with FTY-720 in a dose-dependent manner (Figure 5E and 5I) compared with the EAMG group. Similarly, Th2 cells and Treg cells clearly decreased in 1.5 mg/kg of FTY-720 treated rats (P < 0.05 and P < 0.01, respectively), but these cell levels in the 0.15 mg/kg FTY-720 group exhibited no significant difference when compared with levels in the EAMG group (Figure 5F and 5H). Although FTY-720 could downregulate Th2 and Treg cells at high doses, the Th1/Th2 ratios in the 0.15 mg/kg and 1.5 mg/kg FTY-720 groups decreased significantly (P < 0.01 and P < 0.001, respectively), while the Treg/Th17 ratio in the 0.15 mg/kg FTY-720 group increased markedly (P = 0.053) compared with the EAMG groups (Figure 5G and 5J). This indicates that FTY-720 shifted CD4+ T cells from Th1 and Th17 cells to Th2 and Treg cells. These data suggest that FTY-720 pre-treatment may improve EAMG by regulating Th1/Th2 and Treg/Th17 cell balance.

**FTY-720 alters cytokine secretion in EAMG**

To further investigate whether FTY-720 affects the secretion of cytokines, we first measured TGF-β1, IL-17A, and IL-6 levels in serum samples from different treatment groups. The level of IL-17A, a pro-inflammatory cytokine, increased in EAMG rats compared with the control group, although with no statistical significance, while the levels in the 1.5 mg/kg FTY-720 group markedly decreased compared with the EAMG group (Figure 6B) (P < 0.01). There was no significant difference in immunosuppressive cytokine TGF-β1 among all groups, although there was a slight decrease in the FTY-720 group (Figure 6A). Moreover, the IL-6 level significantly increased in the EAMG group (P < 0.001) and decreased in the 1.5 mg/kg FTY-720 group (P < 0.05) (Figure 6C). Second, we detected TGF-β1, IL-4,
Figure 3. FTY-720 suppresses lymphocyte proliferation. Splenic lymphocytes from SD rats were pretreated with FTY-720 (0.5, 1, 5 μM) for 24 h, and then stimulated with ConA for 24 h or 48 h. Cell viability was determined by CCK-8 assay. In order to perform the antigen-specific lymphocyte proliferation reaction, lymphocytes from Lewis rats of different groups were stimulated with AChR2α for 72 h. Results are expressed as SI, where SI = OD_{drug}/OD_{Control}. The results of stimulation with ConA for 24 h and 48 h are shown in (A and B); the results of stimulation with AChR2α for 72 h are shown in (C). Data are presented as mean ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001 vs. control; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ConA or EAMG).
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Figure 4. Effect of FTY-720 on cell cycle. Splenic lymphocytes were treated with FTY-720 (0.5, 1, 5 μM) for 24 h, stained with propidium iodide, detected using flow cytometry, and analyzed with FlowJo software. The representative fitting graphs of the cell cycle are shown in (A); the cell cycle statistics are shown in (B); and the percent of cells in the G1, S, or G2 phase in the cell cycle are shown in (C–E), respectively. Data are presented as mean ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001 vs. control).

Discussion

MG is an autoimmune disease characterized by skeletal muscle weakness and fatigue. Currently, there are few effective treatments, which encouraged us to explore and discover new therapies. In the present study, we evaluated the efficacy and mechanism of immunosuppressive FTY-720, a novel selective functional antagonist of S1PR1, on EAMG rats. The rats pretreated with FTY-720 had lower anti-AChR Abs titers, higher AChRs, and less CMAP decrement. Since CD4+ helper T-cell subtypes play a crucial role in the pathogenesis of EAMG, we focused our research on the relationship between the therapeutic effects of FTY-720 and the alteration of CD4+ helper T-cell subtypes in rat EAMG. We speculated that FTY-720 might improve the symptoms of EAMG by suppressing lymphocyte proliferation, regulating Treg/Th17 and Th1/Th2 balance, and altering the secretion of cytokines.

As is well known, anti-AChR Abs participate in the pathogenesis of MG. These antibodies induce pathogenicity by activating the classical complement cascade, which leads to membrane lysis and serious damage to the postsynaptic apparatus, including AChR loss, which impedes communication in the neuromuscular junction [2, 10, 26]. Anti-AChR Abs produced by antigen-specific B-cell subsets require activated AChR-specific CD4+ T cells and B cells [27]. In our study, FTY-720 was found to inhibit ConA-induced lymphocyte proliferation in a dose-dependent manner and inhibit AChR2α antigen-specific lymphocyte proliferation. To further investigate the mechanisms of splenic lymphocyte proliferation suppression, we examined the effect of FTY-720 on the cell cycle. Our data suggested that FTY-720 induced the S phase of cell cycle arrest, which might subsequently affect other cellular functions, including proliferation. Specific lymphocytes primarily contain T cells and B cells, and the interaction of activated CD4+ T cells and B cells induce autoantibody production [27]. Taken together, our results indicated that the protective effect of FTY-720 on EAMG may be due to the proliferation inhibition of activated AChR2a-CD4+ T cells and B cells.

Furthermore, naïve CD4+ T cells differentiate into several subtypes when stimulated by dendritic cells or antigens, and both the Th subtypes and cytokines they secrete influence the pathogenesis of the autoimmune response. Th1 cells and their cytokine INF-γ facilitate antigen presentation, activate anti-AChR CD4+ T cells, and induce complement-fixing IgG subclasses [28, 29]. Meanwhile, Th17 cells and IL-17A promote GC formation [30-32] and increase in the IgG1 and IgG3 subclasses [33], whereas Th2 cells and IL-4 suppress the activation of antigen-presenting cells (APCs) and Th1 cells [34]. Treg cells could directly kill antigen-presenting and autoantibody-producing B cells in a cell contact-dependent manner [35], and their cytokine TGF-β could skew the production of antibodies toward non-inflammatory Ig [36].

Lili Mu et al. found that in EAMG rats, the frequencies of Th1 cells and Th17 cells significantly increased, and the frequencies of Th2 and Treg cells markedly decreased compared with control rats [37]. These findings suggested that naïve CD4+ T cells differentiate toward Th1 and Th17 cells in EAMG rats, and the disequilibrium of Th1/Th2 and Treg/Th17 cells promotes the development of EAMG. Similarly, our data revealed that naïve CD4+ T cells of EAMG rats differentiated towards Th1 and Th17 cells,
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A

Control

EAMG

0.15mg/kg FTY-720

1.5mg/kg FTY-720

3.5mg/kg Prednisone

Th1 cells

1.78

4.15

2.81

2.44

1.74

INF-γ

B

SSC

Th2 cells

2.76%

3.65%

3.81%

3.76%

2.85%

IL-4

C

Foxp3

CD25

5.76%

5.34%

4.85%

5.35%

5.89%

7.89%

5.64%

9.54%

9.51%

8.79%

7.69%

7.39%
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which promoted the pathological development of EAMG. FTY-720 treatment shifted CD4+ T cells from Th1 and Th17 cells to Th2 and Treg cells. The new balance of Th cells indicates, in part, a modified T cell migration, which might be regulated by FTY-720 via the S1P-S1PR1 signaling pathway. However, the effect of FTY-720 on specific Th subsets requires further investigation.

Moreover, IL-6 and TGF-β regulate the differentiation of Treg cells and Th17 cells. TGF-β induces naive T cells into Treg cells, although the synergistic effect of TGF-β and IL-6 induces naive T cells into Th17 cells, which means that IL-6 suppresses the differentiation of naive T cells into Treg cells [38]. Our data revealed that FTY-720 suppressed the secretion of IL-17A, IL-6, and INF-γ, but had no obvious effect on the secretion of TGF-β1 and IL-4. Based on the obtained data and related literature, we speculated that FTY-720 reduced pro-inflammatory or inflammatory cytokines such as IFN-γ, thereby decreasing antigen-presenting activity.
Furthermore, FTY-720 decreased IL-6, thereby facilitating the differentiation of naïve CD4+ T cells into immunosuppressive Treg cells. In addition, FTY-720 downregulated Th1/Th17 cells, thereby reducing the levels of complement-fixing IgG subclasses. Meanwhile, FTY-720 had no marked effect on Th2 and Treg cells, whose abilities could suppress the activation of APC and Th1 cells and kill antigen-presenting and autoantibody-producing B cells, respectively. Finally, by regulating Th cell balance and cytokine secretion, FTY-720 decreased the anti-AChR Abs titer, increased AChR content in the NMJ, and enhanced neuromuscular transmission (Figure S1).

In conclusion, this study demonstrated that FTY-720 exhibits a preventive effect on EAMG by inhibiting lymphocyte proliferation, regulating the balance of Th1/Th2 cells and Treg/Th17 cells, and altering the secretion of cytokines. Although more in-depth research is still needed, the present data may lay the foundation for the development of new drugs to treat MG.

Acknowledgements

This research was supported in part by the National Key Research and Development Program (Grant 2016YFA0502304) and the National Natural Science Foundation of China (Grant 81625020). Researcher Hong-Lin Li was also sponsored by the National Program for Special Support of Eminent Professionals and the National Program for Support of Top-notch Young Professionals. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Disclosure of conflict of interest

None.

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References


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Figure S1. Role of Th cells and cytokines in the pathogenesis of MG [29]. Th1 cells and cytokines induce the production of complement-fixed IgG subclasses. Th1 cytokine IFN-γ facilitates the expression of MHC II molecules, which induce antigen presentation and activate anti-AChR CD4+ T cells. Th17 cells and IL-17A also increase in the IgG1 and IgG3 subclasses, which activates the complement cascade. Th2 cells, however, suppress Th1 cells. In addition, Treg cells could directly kill antigen-presenting and autoantibody-producing B cells in a cell contact-dependent manner by secreting perforin and granzyme. Moreover, Treg cells suppress Th17 cells. In MG, anti-AChR Abs belong to the high-affinity IgG1 and IgG3 subclasses. The Fc region of Abs binds to C1q and triggers the complement cascade, which destroys the muscle membrane and decreases AChR, leading to impeded neuromuscular transmission.