Int J Clin Exp Med 2015;8(10):19044-19050
www.ijcem.com /ISSN:1940-5901/IJCEM0012201

Original Article

Effects of combined traditional Chinese medicine with immunosuppressive agents for patients with myasthenia gravis

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Received July 1, 2015; Accepted August 27, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Myasthenia gravis (MG) is a kind of autoimmune disease induced by transferring dysfunction of neuromuscular junction. In the present study, we developed an integrated therapy combined with traditional Chinese medicine and immuno suppressive agents to seek for an effective treatment of MG. 220 MG patients were randomly divided into two groups with different therapies. Plasma levels of acetylcholine receptors antibodies (AchRAb) and $CD4^+CD25^+$ regulatory T cells ($CD4^+CD25^+$Treg) were conducted through ELISA and flow cytometry. The amount of AchRAb (8.52±0.96 vs. 5.22±0.46) and $CD4^+CD25^+$Treg (1.94±1.21 vs. 3.21±0.96) in Group A receiving integrated therapy were significantly improved compared with Group B; the clinical performance of group treated with the integrated therapy was also much better. The integrated therapy in the present study could significantly improve the condition of MG with high recovery rate and low recurrence rate, which can be employed in future clinical treatment of MG.

Keywords: Myasthenia gravis, immunosuppressor, traditional Chinese medicine, integrated treatment, clinical performance

Introduction

Myasthenia gravis (MG) is a type of autoimmune disorder due to the antibodies against components of the muscle membrane at the neuromuscular junction [1, 2]. The disease is characterized by fluctuating muscle weakness and abnormal fatigability [3]. MG can occur in patients of all ages and both sexes. The incidence rate of MG varies with the locations, ranging from 1.7 to 21.3 per million [4, 5]. Generally, antibodies directed against the components of neuromuscular junction, including acetylcholine receptor (AchR) and muscle-specific receptor tyrosine kinase are taken as the cause of the autoimmune of MG [6]. Moreover, increasing evidence has revealed the critical contribution of cellular immunity to MG, with peripheral T cells being persistent expanding in MG. Among kinds of subsets of T cells, $CD4^+CD25^+$ regulatory T cell ($CD4^+CD25^+$Treg), which has potent regulatory properties and plays a central role in maintaining immunological tolerance [7, 8], has drawn a lot of attention recent years.

Oral anticholinesterase treatment, such as pyridostigmine, is the first option in patients with mild MG. However, large dose of these drugs may lead to a cholinergic crisis, and the concentration per day should not exceed 450 mg. At present, there are some patients in China received traditional Chinese medicine (TCM) for the treatment of MG [9]. Exclusive application of TCM will lead to less side effect compared with anticholinesterase, however, its efficiency is less than that of immuno suppressive agents to relieve the disease symptom. Moreover, TCM cannot be applied to treat patients with muscle weakness crises and thymus disease.

To develop an effective and safe therapy for treatment of MG, we combined the TCM and western medicine theory. One kind of traditional Chinese (herb heavy agent compatibility bowled yiqi decoction) along with the armour prednisolone and azathioprine were selected in

Table 1. Information of age and gender in the two groups

Group	Gender (Male/Female)	Age (Years)	Course (Months)
A (n=110)	53/57	31.0±1.0	12.1±0.5
B (n=110)	51/59	29.0±0.5	11.8±1.0

Table 2. Concentration of AchRAb (ng/ml), percentage of cells $CD4^+CD25^+$Tregs, and absolutely scoring value between two groups before treatment

Group	$CD4^+CD25^+$Treg	AchRAb	Absolutely scoring value
A	6.52±0.96	3.94±1.59	36.21±0.326
B	6.22±0.46	3.26±1.96	35.87±0.132

the present study for the treatment of MG patients. The levels of acetylcholine receptors antibodies (AchRAb) and $CD4^+CD25^+$Treg, which are representative of the pathogenesis of MG, were investigated through ELISA and flow cytometry. We expected that our work would offer an effective and safe treatment method for MG in clinic.

Materials and methods

Patients

Two-hundred and twenty patients were enrolled in the present study from January 2006 to January 2008 in First Shijiazhuang Hospital. The age of the patients ranged from 16 to 73 years old, including 104 male and 116 females. The course of MG ranged from 2 to 240 months. Patients were classified into Type I, Type II, Type III, and Type IV MG according to the standard criteria [10]. Patients included in the present study should show positive reaction in fatigue test, AchRAb test (higher than 0.5 mg/mL), and Neostigmine test. Moreover, electromyography of the included patients should exhibit attenuation in low-frequency range and no increase in high-frequency range. Patients with thymusectomy, thymoma, other autoimmune disease, and AchRAb amount less than 0.05 mg/mL were excluded. The study was approved by First Shijiazhuang Hospital ethnics committee. The ethics committee approved the relating screening, inspection, and data collection of the patients, and all subjects signed a written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki.

Grouping of patients

Patients were randomly divided into two groups equally (Group A and Group B). There was no significant difference in the average age and gender ratio between the two groups (**Table 1**). MG clinical scoring absolute values were tested with all the patients [11]. 2 mL blood sample was extracted from each patient and prepared for AchRAb and $CD4^+CD25^+$Tregs detection.

Detection of AchRAb of all the patients were conducted using ELISA following standard procedure: ELISA plates were coated through overnight incubation with 1 mg/mL of α-coral snake poison (100 μL/well) at 4°C and then washed for 3 times with PBST (PBS containing 0.05% Tween 20). After incubation with 150 uL/well of blocking buffer (PBS containing 2.5% gelatin and 0.05% Tween 20) for 2 h at 37°C, the plate was washed 4 times with PBST, and 100 μL of the samples (AchRAb solutions were diluted 1:100 with ddH_2O) to be tested were added to each well. The plate was incubated at 37°C for 1 h. Goat Anti-human IgG labeled with hot silver peroxide enzyme was dissolved with 0.5 mL ddH_2O and 30 μL solution was mixed with 15 mL PBST. Then 100 μL mixture was added to each well. After washing 3 times with PBST, the wells were added with 4 mg of O-Phenylenediamine in blocking buffer and incubated for 1 h at 37°C. OD values at 490 nm were determined.

Detection of $CD4^+CD25^+$Tregs was conducted using flow cytometry following standard procedure and data was analyzed using System II software.

Clinical treatment

For patients in Group A, treatment was carried out using TCM (heavy agent compatibility bowled yiqi decoction) (one time per day) along with immunosuppressive treatment, including methylprednisolone and azathioprine. The dose of methylprednisolone at the first three days of the treatment was 15-20 mg/kg and then reduced by half per day until 24 mg per day, the treatment would continue for six months. The dose of azathioprine was 2 mg/kg and the treatment continued for seven months. After

Table 3. Treatment effect of the combined therapy on the concentration of AchRAb (ng/ml), percentage of cells $CD4^+CD25^+$Tregs, and absolutely scoring value between the two groups

Group	$CD4^+CD25^+$Treg		AchRAb	
	6 months since the treatment	18 months since the treatment	6 months since the treatment	18 months since the treatment
A	5.50±0.87	8.52±0.96#	2.94±1.21	1.94±1.21#
B	5.26±0.56	5.22±0.46	2.21±0.96	3.21±0.96

#indicates statistically significant difference from Group B.

the cease of the immuno suppressive agents, TCM treatment was still conducted for another one year. Patients in Group B were treated with the same immunosuppressor only, and the administration method was identical with that of Group A.

Six and 18 months since the treatment, MG clinical scoring absolute value, and the amounts of AchRAb and $CD4^+CD25^+$Tregs were recorded as described above. Clinical rating (relative scoring) value of the two groups was calculated as the percentage of difference between the MG absolute clinical scoring before the treatment and those of each sampling time points. And a clinical rating value of 95%-100% indicated healed, 80%-95% indicated basic recovery, 50%-80% indicated significantly effect, 26%-0% indicated improvement, and 0 indicated invalid.

Statistical analysis

All the data were expressed in the form of mean ± SD, and t-test and Kruskal-Wallis test were conducted using SAS version 8.0 with significant level of 0.05.

Results

There was no difference in the values of AchRAb, $CD4^+CD25^+$Tregs, and MG clinical scoring absolute value between Group A and Group B before the treatment (**Table 2**).

Effect of the combined therapy after the six months of the treatment

After the six months since the treatment was started, there were 3 cases and 5 cases being excluded from Group A and Group B, respectively, due to the intolerance of the myelosuppression and side effects of the treatment. The numbers of the patients included in the data analysis were 107 for Group A and 105 for Group A. It was found that there was no difference in the levels of AchRAb and $CD4^+CD25^+$Tregs (**Table 3**). Same result was observed for MG clinical scoring absolute value between Group A and Group B (**Table 4**) (*Uc*=0.845, *P*=0.40).

Effect of the combined therapy after the 18th month of the treatment

After 18 months of the treatment, significant difference was observed for the levels of AchRAb and $CD4^+CD25^+$Tregs between Group A and Group B (**Table 3**). In addition, there were 99 cases with a clinical rating score >50% in Group A, compare with 53 cases in Group B, the difference was statistically significant (**Table 4**) (*Uc*=3.066, *P*=0.00). The image record of 3 cases after 18 month treatments were shown in **Figure 1**.

Discussion

As for most autoimmune disease, the triggering events involved in MG are not clearly understood. Complex disease pathogenesis of MG has obscured the advancement of the understanding of disease, thus delaying the treatment of the individuals. The ultimate goal of the treatment is to achieve complete stable remission, without any myasthenic symptoms or sings without any ongoing treatment for at least 1 year [12, 13]. At present, anticholinesterase drugs, nonspecific immunosuppressants, thymectomy, and plasmapheresis are main treatments for MG. However, the abovementioned treatments are along with serious side effects, such as such as cardiac arrhythmia, osteoporosis, and hypotension [14-16]. Therefore, more rational and effective treatment of MG is badly needed and become a hot spot in the field.

TCM treatment of MG has less side effect but longer effective time compared with immuno suppressive agents. We expected that the combined therapy of TCM and anticholinesterase may have the advantage of both the therapies and become a more effective method in future clinic. In the present study, we found that the combined therapy had a significantly improvement effect on the level of AchRAb, level of

Table 4. Treatment effect of the combined therapy on clinical Rating of two groups after 6 months of treatment (relative scoring system for statistical analysis)

Group	Cases	Healed (95-100)%		Basic recovery (80-95)%		Powerfully (50-80)%		Better (25-50)%		Invalid (<25%)	
		6 months since the treatment	18 months since the treatment	6 months since the treatment	18 months since the treatment	6 months since the treatment	18 months since the treatment	6 months since the treatment	18 months since the treatment	6 months since the treatment	18 months since the treatment
A	107	83	70#	12	14#	9	15#	3	5#	0	3#
B	105	83	20	10	13	9	20	2	8	1	44

#indicates statistically significant difference from Group.

Figure 1. Comparison image results of 3 cases after 18 month treatments.

$CD4^+CD25^+$Tregs, and the clinical rating score (**Tables 3** and **4**). For the data sampling after six-month treatment, no significant difference was detected between the two groups, indicating a curative effect of Group A which was comparable to Group B. However, after the cease of methylprednisolone and azathioprine, patients in Group B showed no further improvement of the symptom while pathogenesis of the patients in Group A kept being relieved (**Table 3**). The same pattern was also detected for clinical rating score (**Table 4**). Moreover, the symptoms of MG were also improved after an 18-month treatment (**Figure 1**) and no obvious recurrence was observed among the patients in Group A. According to previous studies [17], low level of AchRAb and high level of $CD4^+CD25^+$Tregs will lead to the relief of the symptoms of MG, which is identical of the pattern we have observed in the present studies, showing the curative effect of the combined therapy.

Based on the theory of TCM, MG is a type of "Wei Zheng", which can be defined as paralysis [18, 19]. Generally, "Wei Zheng" results from the dysfunction of "Pi" system, which includes organs such as spleen and stomach and plays an important role in the transportation and transformation of resource and energy. The potential function compositions involved in the heavy agent compatibility bowled yiqi decoction adopted in the present study include astragalus, codonopsis pilosula, atractylodes, chinese thorowax root, rhizoma cimicifugae,

angelica sinensis, etc. Most of those compositions can enhance the immunologic functions, which will improve the side effect due to the application of the immunosuppressor in treating MG. Thus, the integrated utilization of TCM along with immunosuppressor had the advantage of both methods while the disadvantages of the both methods were compensated by each other.

In conclusion, the integrated methods of TCM and immunosuppressor could significantly improve the symptoms of MG by regulating the level of AchRAb and $CD4^+CD25^+$Tregs. The effect was comparable to the exclusive use of immunosuppressor with less side effect and functioned much faster than the exclusive use of TCM. Moreover, recurrence was rarely observed in the present study, indicating the stable curative outcome of the therapy. Considering the less economic burden of TCM, the integrated therapy should be taken into account in the future clinical treatment of MG. However, the major functional composition in the heavy agent compatibility bowled yiqi decoction hasn't been identified and further studies need to be conducted to underlie the mechanism of TCM improving MG.

Disclosure of conflict of interest

None.

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