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Interventional Effect of Jianpi Bushen Granule Combined with Western Medicine on the Level of Serum Acetylcholine Receptor Antibodies in Myasthenia Gravis Patients

Chao Jiang[a,b], Ping Liu[b]*, Jing-Sheng Zhang[c], Wen-Jing Bao[c], Yan Liang[b], Shao-Bo Qiu[b] and Lin Jiang[d]

[a]Department of Neurology, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, PR China
[b]Traditional Chinese Medicine Department of Longhua Hospital Affiliated to Shanghai University of traditional Chinese Medicine, Shanghai 200030, PR China
[c]Neurological Medical Department of the China Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang 110032, PR China
[d]State Key Laboratory of Bioreactor Engineering & Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, PR China
*Correspondence: Ping Liu, M.D, Ph.D, Professor and Chairman, Department of Traditional Chinese Medicine,Longhua Hospital Affiliated to Shanghai University of traditional Chinese Medicine,650 Wanpingnan Road, Shanghai 200030, People's Republic of China. Tel: 86-13630232169.
Email: liuping23@sina.com

ABSTRACT

Objective: This study is aimed to investigate the clinical significance of regulating effect of Jianpi Bushen Granule (JBG) combined with Western medicine on the level of serum acetylcholine receptor antibodies (AchRAb) in myasthenia gravis (MG) patients, so as to offer a theoretical basis of the targeted therapy with AchRAb.
Methods: We detected the level of anti-AchRAb with enzyme linked immunosorbent assay (ELISA) in the sera from 60 cases of MG patients (randomly divided into the trial group 30 cases, control group 30) for before and after treatment 6 months, and the clinical evaluation carried out by the Quantitative score of myasthenia gravis (QMG) for before and after treatment 3,6 months.
Results: (1) Comparison of the level of serum AchRAb:All included MG patients, the positive rate of serum AchRAb was about 81.67% (49/60), and the trial group was 83.33% (25/30), the control group was 90% (27/30), and the serum AchRAb levels of the trial group (0.994 ± 0.417) was lower than that of the contral group (1.068 ± 0.358), but there was no significant difference between the two groups before treatment ($P > 0.05$). After 6 months of treatment, the serum AchRAb levels of the trial group (0.721 ± 0.280) was also significantly lower than that of the contral group (0.907 ± 0.387), and the serum AchRAb levels was decreased dramatically compared with the same group before treatment, there was significant difference between the two groups ($P < 0.05$). (2)Comparison of QMG scoring:QMG scoring was decreased dramatically in the trial group compared with that of the control group after treatment, there was significant difference ($P < 0.05$), moreover, the total effective rate in the trial group (50%,86.67%)was significantly higher than those in the control groups (23.33%, 40%) after 3,6 months treatment ($P < 0.05$). There was also significant difference in the clinical evaluation by the QMG between the two groups before and after treatment. (3) Adverse reactions: no obvious adverse reactions appeared among the two groups.
Conclusions: JBG combined with Western medicine therapy on MG by regulating the level of patients' serum AchRAb shows definite effects, and it is worthy of further studying.
Key Words: Myasthenia gravis, JBG, AchRAb, ELISIA

Abbreviations: JBG: Jianpi Bushen Granule; AchRAb: acetylcholine receptor antibodiesin; MG: myasthenia gravis; ELISA: enzyme linked immunosorbent assay; QMG: Quantitative score of myasthenia gravis; AchR: acetylcholine receptor; N_2AchRab: nicotinic acetylcholine receptor; TCM: Traditional Chinese Medicine; MuSK: muscle-specific kinase; NMJ: neuromuscular junction

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INTRODUCTION

Myasthenia gravis (MG) is a kind of autoimmune disease, it mainly involves the acetylcholine receptor (AchR) on the postsynaptic membrane of neuromuscular junction, and mediated by the specific nicotinic acetylcholine receptor (N_2AchRab), as well as the complement participates its process. The latest epidemiological data shows that the incidence rate of MG is 8-20/100,000, the number of people contracting this disease in the United States are about 53000–59900, of contracting this disease are about 600,000 in China[1]. According to the theory of immunology, MG patients' serum acetylcholine receptor antibodies (AchRAb) may appear to increase. Moreover, further clinical study also found that the AchRAb were detected in the peripheral blood of MG patients

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up to 85%–90%, the clinical symptoms of MG patients could be improved significantly by taking the plasmapheresis to remove AchRAb; which might be due to the dual role of acetylcholine receptors included autoantigen model and neuromuscular receptors model, and the studies on antigenic structure and channel function aslo attracted the attention of many scientists worldwide[2,3]. However, MG is a cosmopolitan stubborn disease, which may be involved in multiple mechanisms and regulated by multiple genes. Unfortunately, current evidence indicates that the exact pathogenesis of MG was still unclear, and the existing western treatment for MG had different degree of side effects[4]. In addition, alternative treatment with Chinese characteristics has been shown to be effective in MG for many years, and particularly in ocular MG or generalized MG[5–8].

Therefore, to develop a strong effective but less side effects of traditional Chinese medicine compound is the urgent mission, developed by Pro. Jingsheng Zhang under the guidance of TCM PISHEN theory, and had obtained manifest therapeutic effects on whether the clinical or experimental studies till now[5–8]. Then, based on our previous studies, it is necessary to further explore to the internal mechanism of effectively improve the clinical treatment of MG patients by the use of JBG combined with conventional Western medicine, can regulate the patient's immune imbalance through reduced serum AchRAb level in blood, so as to improve the clinical symptoms of patients with MG. The study is reported as follows.

MATERIALS AND METHODS

1. Objects

The 60 patients enrolled were outpatients of special MG clinics of the Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (TCM) and the China Affiliated Hospital of Liaoning University of TCM from November 2010 to April 2012. The age distribution of the objects was defined in 14–75 years old. And the objects were recruited to randomly assigned to 2 groups: the trial group (30 cases) and the control group (30 cases) and the trial group (12 female, 18 male, age 48.70 ± 16.45 y, disease duration 28.03 ± 23.83 mo [mean ± SD]) the controls group (13 female, 17 male, age 49.03 ± 15.26 y, disease duration 30.43 ± 26.69 mo), controls with no history of autoimmune disease were recruited. Statistical analysis showed that the baseline data of the two groups of patients by age, sex, duration, no significant difference, was comparable ($P > 0.05$). All objects were informed, voluntary and signed informed consent.

2. Inclusion and Exclusion Criteria

Inclusion criteria: (1) in line with diagnosis of MG based on the Western medicine diagnostic criteria for autoimmune myasthenia gravis[9], including symptoms of fluctuating muscle weakness supported by an electromyographic pattern of neuromuscular transmission dysfunction by repetitive stimulation, belonging to the modified Osseman A, AA,AB type; (2) patients with Chinese medical diagnosis of flaccidity fitting to the differentiation standard of spleen-kidney deficiency type syndrome[10], Where have the one of the main symptoms and secondary symptoms of more than one, or both, see tongue, pulse can be included; (3) patients were between 14 and 75 years old patients were voluntary; (4) the subjects were informed voluntary and written informed consent to participate the study.

Exclusion criteria: (1) Osseman III, IV, V; (2) Familial MG, congenital myasthenic syndrome and drug (D-penicillamine, interferon induced by MG, etc.); (3) patients who were nursing or pregnant; (4) patients who had comorbid diseases such as cerebrovascular diseases, renal insufficiency, hematopoietic system diseases, and psychiatric diseases; (5) allergies (allergic to more than 2 kinds of food or medicine); (6) The last 1 months participated in other clinical trials of drugs; (7) Once plasma exchange or intravenous gamma globulin in treatment patients in the past three months. In accordance with these exclusion criteria were excluded.

This study was approved by the ethics examination committee of the Longhua hospital and written permission was obtained from all who participated in the study. **(The ethic approval number: 2010LCSY016, and trial registration number: ChiCTR-TRC-13004125)**

3. Drugs Preparation

JBG was provided by Benxi chinese medicine factory Co.Ltd. (Liaoning Province, China). The preparation was a mixture of 11 Chinese herbal medicines shown in Table 1. In brief,

Table 1. The ratio of the components in JBG.

Chinese name	Pharmaceutical name (Latin name)	Amount (g)
Huangqi	Dried root of Astragalus membranaceus (Fisch.) (Radix Astragali)	50
Taizishen	Dried root of Pseudostellaria heterophylla (Miq.) (Radix Pseudostellariae)	25
Baizhu	Dried root of Atractylodes macrocephala Koidz. (Rhizoma Atractylodis)	15
Zhike	Dried immature fruit of Citrus aurantium L. (Fructus Aurantii)	15
Shengma	Dried root of Cimicifuga foetida L. (Rhizoma Cimicifugae)	15
Kuncao	Dried or fresh aerial parts of Leonurus japonicus Houtt. (Herba Leonuri)	30
Fangfeng	Dried root of Saposhnikovia divaricata (Turcz.) (Radix Saposhnikoviae)	10
Danggui	Dried root of Angelica sinensis (Oliv.) Diels (Radix Angelicae Sinensis)	10
Gouqizi	Dried ripe flesh of Lycium barbarum L. (Fructus Lycii)	15
Heshouwu	Dried root of Polygonum multiflorum Thunb. (Radix Polygoni Multiflori)	15
Shanyurou	Dried ripe flesh of Cornus officinalis Sieb (Fructus Corni)	15
Total amounts		215

these were extracted with 1 L of boiled water twice for 1 hr. Poaching liquid was mixed two times. The dregs of the decoction were removed after filtering. The filtered liquid was lyophilized and crushed into a thin powder. The yield of the dried extract was about 38%. JBG was prepared and stored in a granular form before administration to patients. The Drug specifications was 10 g per bag, including 7.4 g of crude drug. Olfactory pyridostigmine tablets (an effective drug generally accepted in MG clinical trials) were provided by Chinese Shanghai Sunway pharmaceutical Co. Ltd., China (No. H31020867). The Drug specifications was 60 mg per tablet.

4. Administration

The trial group was received JBG (take a package each time and was given orally two times per day) combined with olfactory pyridostigmine tablets (60 mg/times, 4 times/day), according to the patient's symptoms was improved, gradually tapering to a relatively low maintenance dose reference to some literature[11]. The control group was only received olfactory pyridostigmine tablets (the same as the trial group). The entire course of treatment was maintained for six months. The dosages of western medicines were gradually reduced to a relatively low maintenance level (olfactory pyridostigmine), but alteration of the study herbal medications was not allowed during the study period. To the end of the trial, we can use the number of JBG recovery to ensure the compliance of the patients taking Chinese herbal and the quality of this study.

5. Sample collection and Determination of Serum AchRAb

Serum samples (5 ml fasting venous blood of each sample) were collected early in the morning after the first (Enrollment) and second (6 mo), rapidly separated serum, were saved at −70°C in order to be measured. The serum samples in our laboratory was coated onto a 96-well microtiter plate (Fudan University neuropathy Institute (20030801) with 0.1 M carbonate bicarbonate buffer (pH9.6) overnight at 4 °C. The plates were blocked with 2% BSA in PBS at room temperature for 30 min. Serum samples diluted 1/800 in PBS/0.05% Tween 20 were added and incubated at 37 °C for 90 min. After four washes, HRP-conjugated goat anti-mouse IgG isotypes (IgG, IgG1, IgG2b (Caltag, San Francisco, CA)) diluted 1/800 in PBS/0.05% Tween were added and incubated at 37 °C for 90 min. Subsequently, ABTS (indicator) solution in 0.1 M citric buffer, pH 4.3, in the presence of H_2O_2 was added, and color was allowed to develop at room temperature in the dark. Absorbance values were measured at a wavelength of 492 nm, usig a Dynatech ELISA reader, and the results were expressed as OD values (P/N > 2.5, positive).

6. The Quantitative score of myasthenia gravis (QMG) Evaluation

Symptoms were observed and recorded by means of the QMG at 3 times during the study: initiation day, end of the 3,6 months (discrepancy within 2 days was allowed), respectively. This scale had 13 items, used to evaluate the fatigue state in patients with MG, (the left, the right score), a total of 6 points, with a four-level scoring system: (1) normal (0points); (2) mild, sometimes (1points); (3) moderate, always (2 points); (4) severe, nearly substantially completely unable to be fit to do some action (3 points)[12]. Every item was scored and the total points were calculated.

7. Therapeutic Effect Criteria

The therapeutic effect criteria were formulated according to references[13]. These were as follows: Clinical relative scoring= (absolute score of Pre-treat —Absolute scores of post-treat) /absolute score of Pre-treat ×100%, (1) Cure rate: ≥95%, (2) Basic cure rate:81%~95%, (3) Markedly effective:51%~80%, (4) Improvement rate: 26%~50%, (5) ineffective:≤25%. The total effective rate = (Cure rate + Basic cure rate + markedly effective + Improvement rate. The efficacy of QMG was compared before and after treatment 3, 6 months.

8. Safety Evaluation

We examined the breathing, heart rate and blood pressure once a week, and examined routine blood tests, routine urinalysis, routine stool, hepatorenal function, and electrocardiogram before and after treatment.

9. Statistical Analysis

All the data are analyzed with the SPSS 15.0 software (produced by SPSS Corporation, Chicago, IL, USA). For the measurement data, the paired sample t-test was used for the comparison of the variables in each group before and after treatment; comparison of the variables in groups was done using One-way variance analysis and the q test; and categorical variable analysis was performed with the χ^2 test. Values of $P < 0.05$ were considered significant.

RESULTS

1. Effect of JBG on the level of Serum AchRAb

Twenty-five male and 35 female MG patients were enrolled in this study, 49 patients' serum AchRAb showed positive (81.67%), 25 cases (83.33%) for trial group,27 patients (90%) for the control group, there was no significant difference between the two groups before treatment ($P > 0.05$), was comparable; after 6 months treatment, there were 21 cases's serum AchRAb in the trial group showed positive (70%), 25 cases (83.33%) in the control group, we do statistical analysis using the χ^2 test, $\chi^2 = 0.001$, P=0.970 > 0.05, means that the patients'serum AchRAb positive between the two groups rate was no significant difference before and after treatment, while the patients' serum AchRAb levers in the trial group were significantly lower than that of the control group before and after treatment, and there was significant difference between the two groups, with statistical significance ($P < 0.05$). Moreover, the patients' serum AchRAb levers in the two groups were significantly lower than that of the same group before treatment, there was also significant difference ($P < 0.05$) (Table 2, Fig 1.)

Table 2. Comparison of the patient's serum AChRAb levels among the two groups before and after treatment ($\bar{x} \pm s$).

Item	Trial group (n = 30)		Control group (n = 30)	
	Pre-treat	6 months	Pre-treat	6 months
AChRAb positive	25(83.33%)[a]	21(70%)[bc]	27(90%)	25(83.33%)
AChRAb levels	0.994 ± 0.417[a]	0.721 ± 0.280[bc]	1.068 ± 0.358	0.907 ± 0.387

Notes: [a]$P > 0.05$, compared with the control group (Pre-treat); After 6 months of treatment, [b]$P < 0.05$, compared with the control group; [c]$P < 0.05$, compared with the same group before treatment

2. Comparison of the QMG Scoring and Efficacy between the two groups before and after treatment

After 3 and 6 months treatment, the QMG scores of the trial group and the control group were lower than the same group before treatment, there was difference in the two groups ($P < 0.05$). Moreover, the QMG scores of the trial group were apparently lower than that of the control group ($P < 0.05$), there was also obvious difference ($P < 0.05$, Table 3)

After 3 months treatment, the improvement rate, ineffective rate and the total effective rate of the trial group were 50% (15/30),50%(15/30) and 50% (15/30), respectively; of the control group,23.33% (7/30),76.67%(23/30) and 23.33% (7/30), respectively. The improvement rate and the total effective rate in the trial group were significantly higher than those in the control groups, there was significant differences between the two groups ($P < 0.05$), after 6 months treatment, the cure rate (3.33%), markedly effective rate (30%) and the improvement effective rate (53.33%), as well as the total effective rate (86.67%) in the trial group were significantly higher than those in the control group (0,13.33%, 26.67% and 40%, respectively), there was also significant difference between the two groups ($P < 0.05$). In addition, the improvement rate, ineffective rate and the total effective rate of the two groups were obviously better than the same group before treatment, there was significant difference ($P < 0.05$, Fig 2)

3. Safety

No obvious adverse reactions appeared among the two groups during the treatments.

DISCUSSION

Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction usually caused by antibodies to the nicotinic acetylcholine receptor (AChR) and occasionally to muscle-specific kinase (MuSK)[14]. To some extent, acute exacerbation of generalized MG can cause swallowing impairment, respiratory failure, or death and has already deeply caused much hazards to human health, as well as also often lead to high medical costs[1,15]. With the development of research, it has been recognized that AChRAb was mainly circulating antibodies which targeted at the target organ neuromuscular junction (NMJ) postsynaptic membrane AChR, can launch a specific attacks against the postsynaptic membrane unless complements includes, made the postsynaptic membrane simplistic, reduced the NMJ receptor and

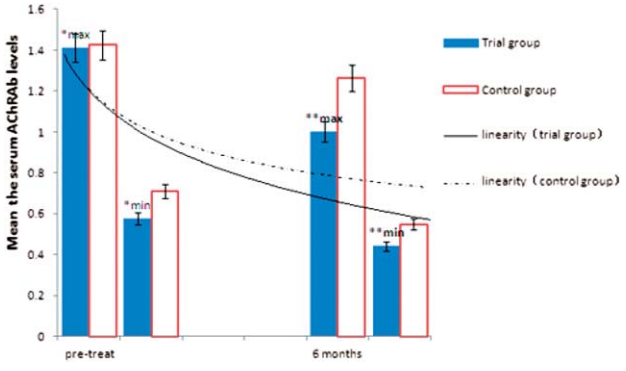

Figure 1. comparison of the level of serum AchRAb among the two groups before and after treatment (*P < 0.05,**P < 0.05)

Table 3. Comparison of Evaluation of QMG Scoring among The two groups ($\bar{x} \pm s$).

Item	Trial group (n = 30)	Control group (n = 30)
Pre-treat	10.80 + 4.92[a]	12.10 + 5.70
3 months	7.90 + 3.82[bc]	10.86 + 4.98
6 months	6.19 + 3.57[cd]	9.41 + 5.18

Notes: [a]$P > 0.05$, compared with the control group (Pre-treat); [b]$P < 0.05$(3 months),[c]$P < 0.01$(6 months), compared with the control group;[d]$P < 0.05$(3 months and 6 months) compared with the same group before treatment

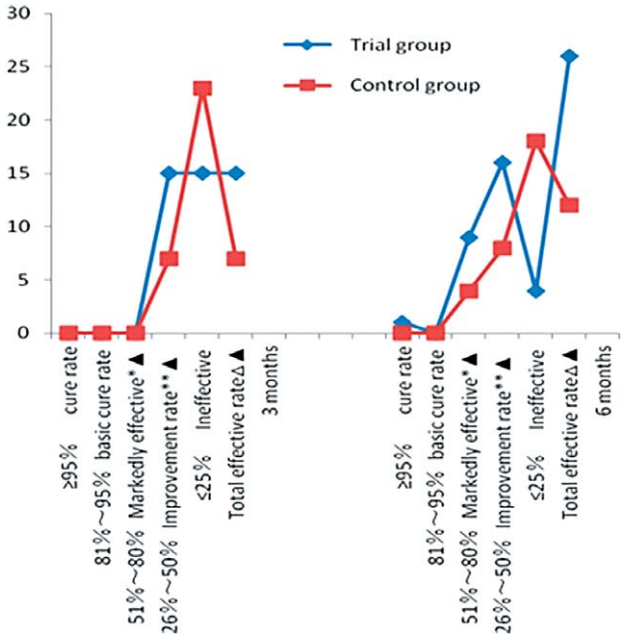

Figure 2. comparison of clinical efficany among the groups before and after treatment [case] After 3 months of treatment, *P > 0.05(Improvement rate), △P < 0.05(Total effective rate), compared with the control group; After 6 months of treatment, **p < 0.05(Improvement rate), ▲P < 0.05, compared with the same group before treatment

increased synaptic space, eventually led to the incidence of MG. It is reported[16–19]that AChRAb can be detected in the serum of more than 85% of generalized myasthenia gravis patients, AChRAb levels and the severity of the clinical manifestations of MG was associated. Although AChRAb were not detected in the serum of about 15% of patients, and these patients were referred to the antibody-negative MG (Seronegative MG, SNMG)[20], the SNMG patients' clinical manifestations in line with MG antibodies patients seemed basically similar, were also sensitive to immunosuppressive therapy, and also copied the MG model after the SNMG patient's serum or IgG to be injected to mice[21]. In addition to AChRab mediated therefore, there was may be other antibodies or molecules involved in the MG pathogenesis. However, the changes of serum AchRAb levels can still be detected in patients with MG in order to guide clinical diagnosis and treatment. At present, nonspecific immunity inhibitor is still mainly adopted in the clinical treatment of MG patients , and wherein the cholinesterase inhibitors therapy on patients is still used as the preferred drug which has affected on the development in the treatment of MG[22,23]. Furthermore, long-term use of steroid drugs caused changes in their image, such as having a moon-like face, being fat and having hair loss, which also affects the patient's selfesteem and psychological aspects, and also often lead to high medical costs[15]. At present there is still a lack of effective prevention and therapeutic measures on MG patients[24,25]. As we all know that traditional herbal medicines have been used for thousands of years and are beneficial in prevention and treatment of many diseases, including MG. Greater attention is being given to such medicines due to their varied biological actions and low toxicity.

According to the theory of TCM, the MG belongs to "TCM Flaccidity"category. The main physiological functions of the TCM kidneys are: storing essence; dominating growth, development and reproduction; regulating water metabolism and receiving Qi. The kidneys also take charge of the bone and manufacturc marrow. Therefore, it is considered as the "foundation of prenatal life". If the kidney essences insufficiency or primary Qi deficiency will affect growth and development as well as muscle function directly, which lead to the disease appearing eventually,"The TCM spleen is the foundation of postnatal life", it is also viewed as the source for the production and transformation of Qi and blood, the TCM spleen dominate muscles, limbs, if this function of the spleen and stomach is deficient, will cannot nourishing muscles and bones, and the limbs will become flaccid and disuse eventually. Our previously related studies[26,27] had also shown that TCM pathogenesis of MG were spleen-kidney deficiency type, which was probably the most common type in clinical research. Under this premise, we developed the basic prescription called "Jianpi Bushen Granules" for the treatment of the MG patients with spleen-kidney deficiency type. Huangqi (Radix Astragali) included in the prescription can regulate immune with two-way function, and to reduce serum AchRAb level by enhancing the inhibitory activity of T lymphocytes, Gouqi (Lycium chinense) are play a regulatory role by increasing the number and activity of T lymphocytes, and Danggui (Radix Angelicae Sinensis) can potent enhance the non-specific immune function but suppress humoral immune[28,29], and so on.

In this study, on the Western conventional treatment's foundation, we plused Chinese herbal compound JBG with invigorating spleen-kidney role to treat MG, in accordance with the principles of evidence-based medicine, using randomized, controlled method to confirmed that the use of Chinese herbal compound combined with Western medicine can significantly improve the total effective rate of the trial group after treatment (50%, 3 months, 86.67%, 6 months), there was significant difference ($P < 0.05$) compared with the control group (23.33%3 months, 40%6 months). We also found that the patients' serum AchRAb levers in the trial group were significantly lower than that of the control group after treatment, there was significant difference between the two groups ($P < 0.05$), with statistical significance. Moreover, the patients' serum AchRAb levers in the two groups were significantly lower than that of the same group before treatment, there was aslo significant difference ($P < 0.05$). Although the positive rate of patients' serum AchRAb was no significant difference between the two groups before and after treatment, of that were more significant reduced compared with the same group before treatment. We speculated that the reason might be because of the relatively small samples, and the relatively short duration of treatment. According to the composition of prescription principles in TCM Formulae, we known that JBG was mainly suitable for the treatment of ocular and systemic myasthenia gravis (equivalent to the TCM of Flaccid patients with spleen-kidney deficiency type), which consisted of Huangqi (Radix Astragali),Gouqi (Lycium chinense), Taizishen (Radix Pseudostellariae), and so on, has invigorating the kidney-spleen as well as elevating Yang Qi functions. Based on the classical theory of TCM, we analysised that Huangqi (Radix Astragali) and Gouqi (Lycium chinense) can reinforcing kidney-spleen and elevating Yang Qi as the principal drugs of this decoction, while compatible effects of the minister drug Heshouwu (Radix Polygoni Multiflori) and Shanzhuyu (Fructus Corni) can nourishing the liver-kidney, Taizishen (Radix Pseudostellariae) can replenishing qi to invigorate the spleen; and compatible effects of the assistant drug Danggui (Radix Angelicae Sinensis) can promoting circulation of Qi and blood. This organic inner joint of the decoction can significantly increase the clinical effectiveness via tonifying the spleen-kidney, to enhancing the nutrition of muscles and bones, and ultimately achieve the purpose for treatment on MG. In addition, our previous studies[5–8,28,30–32] have also shown that JBG can reduce the level of IFN-1 and it's mRNA and then decrease AChRAb level in blood and reduce severity of myasthenia, can promote the growth of new axon in NMJ, increase the number of synaptic vesicles and reduce the synaptic injury in model rat of trial autoimmune MG(EAMG), and can effectively regulate the levels of some EAMG-related differential proteins expressive. In this study, we found that the use of the JBG combined with Western Medicine therapy on Patients with MG can obtain better clinical effects via effectively regulated the serum AchRAb levels of patients,

which also verified the existence of a correlation between the level of serum level of AchRAb and clinical symptoms of patients in MG[33], and there was no any adverse reaction appeared in the course of the study. However, this study still can not fully explained that the integrative treatment on MG with a long-term advantage, and also does not provide enough evidence based medicine for the study on MG due to the time and sample size and other factors. Then, exactly which part of the JBG does play a key role in the treatment for MG? Or which channels of JBG does adjust the body's immunological environment and then to display the combined effect of it? Obviously, these should be urgently concerned issue of future studies. Therefore, it is necessary to carry out more in-depth multidisciplinary study on the the fundamental mechanisms of JBG therapy on MG using the more cutting-edged scientific and technical means, such as DNA technology, molecular biology techniques, and so on to furtherly clarify pathogenesis of MG, and provide more effective and well treatment for MG.

Further evaluation on long-term and more in-depth study of JBG will be carried out by continuous follow-up studies.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Phillips LH. The epidemiology of myasthenia gravis. *Ann N Y Acad Sci* 2003,998:407–12.
2. Patkrik J, Lindstrom J, Culp B, et al. Autoimmune response to acetylcholine receptor. *Science* 1973,180: 871–872.
3. Lindstrom JM. Acetylcholine receptors and myasthenia. *Muscle & Nerve* 2000,23:453–477.
4. Tüzün E, Scott BG, Goluszko E, et al. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. *J Immunol* 2003,171(7): 3847–3854.
5. Zhang JS, Sun W, Liu P, et al. Inftuence of Huangqi compound on ultrastructure of neuromuscular iunction in model rat of trial autoimmune myasthenia gravis. *Zhong Guo Lin Chuang Kang Fu Za Zhi* 2006,10(35):20–22.
6. Liu P, Zhang JS.Han B, et al. The Regulation Effects of HuangQi Compound on the level of IFN-Y and it's mRNA in Myasthenia Gravis Patients. *Zhong Yi Yao Xue Kan* 2004, 22(11):2021–2022.
7. Liu P, Ding XF, Zhang YY, et al. Effect of Jianpi Bushen Recipe on the Differential Expressive Proteins in Thymus with Trial Autoimmune Myasthenia Gravis. *Zhong Hua Zhong Yi Yao Xue Kan* 2008, 26(1):66–69.
8. Liu P, Ding XF, Zhang YY, et al. Modulation of Jianjining Recipe on the Differential Proteins expression in rats with trial autoimmune myasthenia gravis. *Zhong Hua Zhong Yi Yao Xue Kan* 2007,5(7): 642–646.
9. Chen HZ. Practical internal medicine. Beijing: People's Medical Publishing Press, 2001: 2380–2501.
10. Zhang BY. Traditional Chinese internal medicine. Shanghai:Shanghai Science and Technology Press, 1985:270–276.
11. Chinese immunological Society of Neuroimmunology branch, Chinese Medical Association Society of Neuropathy branch of Neuroimmunology Unit. Myasthenia gravis diagnosis and treatment of the Chinese expert consensus. *Zhong Guo Shen Jing Mian Yi Xue He Shen Jing Bing Xue* 2012,19(6):401–408.
12. Wang YJ. Neurology Clinical Rating Scale, Beijing: Beijing China Friendship Publishing Company Press, 2005:361–363
13. Xu XH. Neural immunology. Beijing: Peking Union Medical College and Beijing Medical University Press, 1993:124–126
14. Poulas, K, Koutsouraki, E, Kordas, et al. Anti-MuSK- and anti-AChR-positive myastheniagravis induced by d-penicillamine. *J Neuroim* 2012,250 (1-2): 94.
15. Liu FB, Chen XL, Guo L, et al. Evaluation of A Scale of Patient-Reported Outcomes for the Assessment of Myasthenia Gravis Patients in China. *Zhong Guo Zhong Xi Yi Jie He Za Zhi* 2012, 18(10): 737–745.
16. Ohta K, Shigemoto K, Fujinami A, et al. Clinical and experimental features of MuSK antibody positive MG in Japan. *Eur J Neurol* 2007,14(9):1029–1034.
17. Oh S J, Hatanaka Y, Hemmi S, et al. Repetitive nerve stimulation of facial muscles in MuSK antibody-positive myasthenia gravis. *Muscle Nerve* 2006,33(4):500–504.
18. Kuwabara S, Nemoto Y, Misawa S, Takahashi H, Kawaguchi N, Hattori T. Anti-MuSK-positive myasthenia gravis: neuromuscular transmission failure in facial and limb muscles. *Acta Neurol Scand* 2007, 115(2):126–128.
19. Pasnoor M, Wolfe GI, Nations S, et al. Clinical findings in MuSK-antibody positive myasthenia gravis: a U. S. experience. *Muscle Nerve* 2010, 41(3):370–374.
20. Qiu W, James I, Carroll WM, Mastaglia FL, Kermode AG. HLA-DR allele polymorphism and multiple sclerosis in Chinese populations: ameta-analysis. *Mult Scler* 2011,17(4) :382–388.
21. Shigemoto K, Kubo S, Maruyama N, et al. Induction of myasthenia by immunization against muscle-specific kinase. *J Clin Invest* 2006,116 (4):1016–1024.
22. Gajdos P, Chevret S, Toyka KV. Intravenous immunoglobulin for myasthenia gravis. *Cochrane Database Syst Rev* 2012,12(12): CD002277.
23. Li ZY, Zhang W. Review and perspective of pathogenesis and treatment in myasthenia gravis. *Zhong Guo Xian Dai Shen Jing Bing Xue He Shen Jing Wai Ke Xue Za Zhi* 2012,12(2) :113–116.
24. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. *J Clin Invest* 2006,116(11):2843–2854.
25. Richman DP, Agius MA. Treatment of autoimmune myasthenia gravis. *Neurology* 2003, 61(12):1652–1661.
26. Jiang C, LIU P, Liang Y. Discuss on the treatment of myasthenia gravis based on the related theory of spleen-kidney deficiency. *Guoji Zhong Yi Zhong Yao Za Zhi* 2012,34(7):621–623.
27. Li GH. To disscuss the myasthenia gravis with the theory of splenorenal doctrine. *Xin Zhong Yi Za Zhi* 1982,(4):8–12.
28. Liu P, Zhang JS, Zheng F, et al. The Regulating effects of Huangqi compound acetylcholine receptor antibody in patients with myasthenia gravis. *Shen Jing Bing Xue He Shen Jing Mian Yi Xue Za Zhi* 2005,12(2):76–78.
29. Yang TH, Jia M, Mei QB. Immunoloregulation effect of polysaccharide isolated from Angelica Sinensis on mouse. *Zhong Chen Yao* 2005,27(5):563–565.
30. Sun X, Niu GH, Zhang JS. Efect of compound Astragalus membranaceus on Thl, Th2,Tcl and Tc2 subsets in peripheral blood of patients with myasthenia gravis. *Wu Jing Yi Xue* 2010,21(5):420–422.
31. Niu GH, Sun X, Zhang CH, Zhang JS. Effect of Compound AstragalusRecipe on Lymphocyte Subset, Immunoglobulin and Complements in Patients with Myasthenia Gravis. *Zhong Guo Zhong Xi Yi Ji He Za Zhi* 2009,29(4):305–308.
32. Bao WJ, Zhang JS, Qiao WJ. The study of Huangqi compound treatment on 73 cases of myasthenia gravis. *Guang Ming Zhong Yi Za Zhi* 2008,23(2):208–209.
33. Nakajima J, Murakawa T, Fukami T, et al. Postthymectomy myasthenia gravis: relationship with thymoma and antiacetylcholine receptor antibody. *Ann Thorac Surg* 2008,86(3):941–945.