

Original Article

Efficacy of tripterygium glycosides on experimental autoimmune myasthenia gravis in rats

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Abstract: This study aimed to explore the therapeutic efficacy of tripterygium glycosides for experimental autoimmune myasthenia gravis (EAMG). EAMG model was established in rats, and the rats were divided into complete Freund's adjuvant group, EAMG model group and tripterygium glycosides treatment group. All rats were evaluated by Lennon scoring and body mass monitoring. We found that the treatment with tripterygium glycosides improved the clinical symptoms of myasthenia gravis, reduced Lennon score but increased body mass. Furthermore, tripterygium glycosides effectively reduced the activities of specificity-activated antigen-reactive T cells and the expression and secretion of pro-inflammatory cytokines such as IL-17 and IFN- γ . In conclusion, tripterygium glycosides are a promising agent for the treatment of autoimmune myasthenia gravis.

Keywords: Tripterygium glycosides, experimental autoimmune myasthenia gravis, T cell receptor, IL-17

Introduction

Myasthenia gravis (MG) is a nervous system autoimmune disease, the main symptom is voluntary exercise muscle weakness and it is aggravated after exercise. Many genetic and environmental factors are involved in the pathogenesis of MG, and it is still difficult to treat MG. Patrick and Lindstrom immunized rabbits with acetylcholine receptor (AChR) as the antigen, and successfully prepared experimental autoimmune myasthenia gravis (EAMG) animal model [1]. In addition, Yoshikawa *et al.* made autoimmune myasthenia gravis rabbit model with human AChR- α 138-167 fragment [2]. Baggi *et al.* successfully established EAMG rat model with rat AChR- α 97-116 [3].

T cell-mediated immune regulation is one of the main mechanisms to maintain immune tolerance, T cell expansion and microenvironment stability. T-cell receptor (TCR) is an important molecule that plays a role in specific immune recognition [4]. When TCR recognizes specific antigen, T cells are activated and secrete a variety of cytokines to regulate immune responses [5]. Therefore, investigating the secretion of

cytokines will help understand the pathogenesis of MG.

Tripterygium glycosides (TG) have anti-inflammatory and immunosuppressive activities for inflammatory and autoimmune disease [6]. However, the efficacy of TG for MG treatment remains unclear. In this study, we aimed to investigate the therapeutic efficacy of TG for EAMG.

Materials and methods

Animals and reagents

Lewis rats (female, 6 to 8 weeks old, body weight 120-140 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and kept in specific pathogen free (SPF) environment, free diet, day and night (12 h) alternating light. Tripterygium wilfordii polycoride (TWP) tablets were purchased from Hunan Xieli Pharmaceutical Co., Ltd. R-AChR- α 97-116 was purchased from Xi'an United Biotechnology Co., Ltd. Complete Freund's adjuvant (CFA) was purchased from Sigma (USA).

Tripterygium on myasthenia gravis

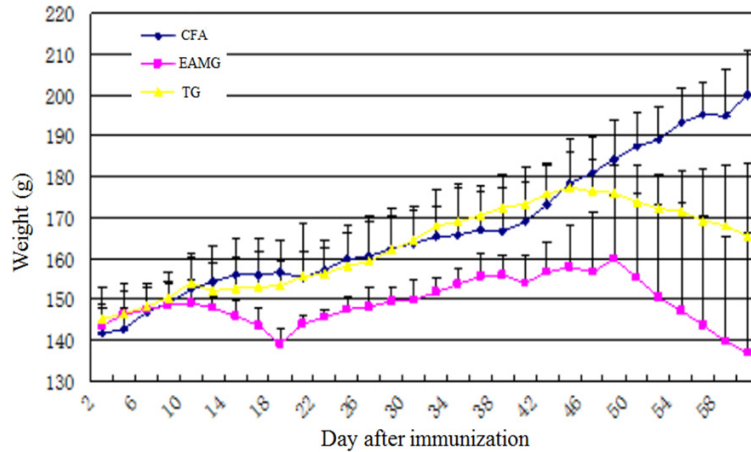


Figure 1. Weight change of rats in each group after immunization (n=10).

Establishment of animal models

Thirty healthy Lewis rats were randomly divided into CFA control group, EAMG model group and TWP treatment group (n=10 for each). All the rats were injected with 200 μ l of immunogen in the tail and the back. The day of immunization was day 1, the second immunization was performed in the same manner 30 days later. TWP was administered at a dose of 8 mg/(kg \cdot d) and administered intragastrically on the 15th day after the first immunization in TWP group, while CFA and sodium chloride was administered in CFA group and EAMG model group, respectively.

After the first immunization, the body mass was measured every other day and double-blind scored according to Lennon's score. After the onset of illness of the rat model, the rats were evaluated according to specific score criteria: 0 point: normal muscle strength in rats, no abnormalities; 1 point: mild activity reduction, biting grasping weakness, fatigue of rats; 2 point: significantly decreased activity, decreased body weight, resting body uplift posture, head and tail drooping, forelimb flexion, unsteady, tremor of rats; 3 point: severe general weakness, no crawling, bite action, body tremor, dying state of rats; 4 point: death of rats.

ELISA

Blood samples were taken from the rats, and then serum IL-17, IFN- γ and IL-4 and IL-6 levels were detected by using commercial ELISA kits

(R&D Systems Inc, MN, USA) following the manufacturer's instructions.

Statistical analysis

SPSS13.0 statistical software was used to analyze the data. The measurement data were expressed as mean \pm standard deviation ($\bar{x}\pm s$). Single-factor analysis of variance (ANOVA) test was used for the comparison between groups, *t* test was used for paired comparison between groups. $P<0.05$ indicated significant differences.

Results

Comparison of clinical symptoms and body mass changes in three groups

There were no significant changes in the general condition and body weight among the 3 groups of rats after the first immunization. About 15 days after the second immunization, the rats in EAMG model group showed weakness of limbs, decreased activity, body tremor, dull hair loss, reduced appetite, decreased grip strength, and grip strength decreased obviously after repeated gripping. Over time, symptoms of rats gradually aggravated, and reached its peak 10 days after the second immunization. With the gradual aggravation of disease, body mass decrease gradually increased. In contrast, the rats in TG group had relatively slow onset time, mild symptoms, and body mass decline was significantly smaller than in model group (**Figure 1**). 70 days after the injection of immunogen, there were significant differences in clinical scores and body mass between TG group and EAMG group ($P<0.01$), which indicated that TG could relieve the clinical symptoms of EAMG.

Comparison of cytokine secretion in three groups

ELISA method was used to detect the cytokines in the serum of different groups. The levels of IL-17 and IFN- γ in TG group and CFA group were significantly lower than those in EAMG model group ($P<0.01$), while IL-4 and IL-6 were signifi-

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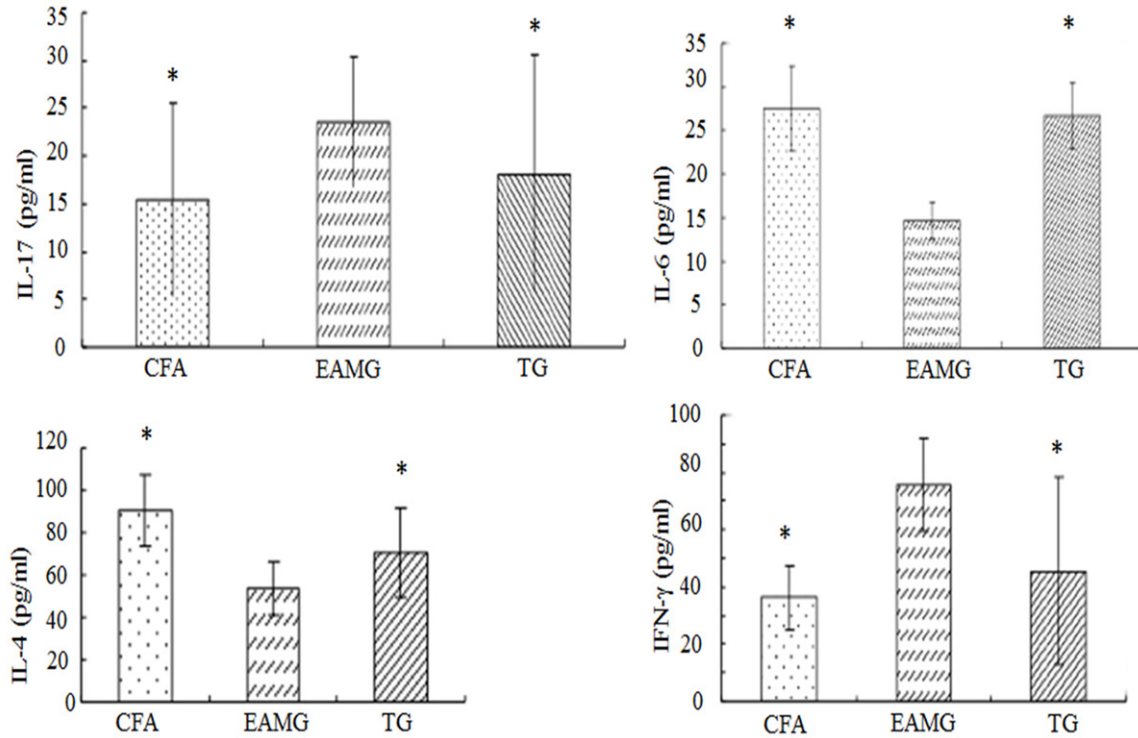


Figure 2. Serum cytokine levels in the rats in each group (n=10). *P<0.05 vs. EAMG group.

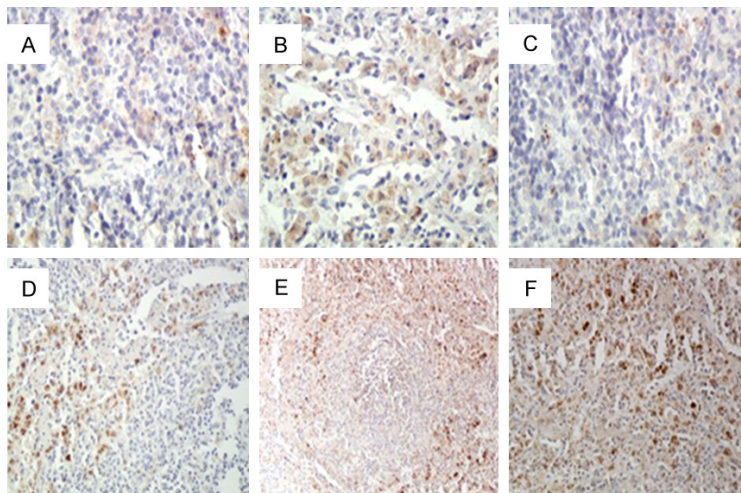
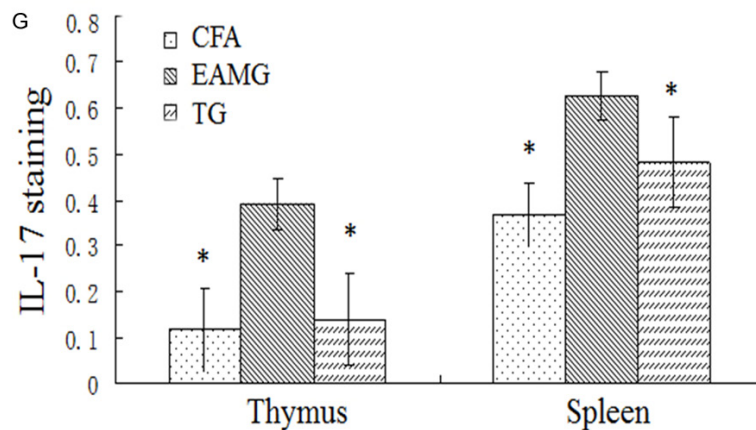


Figure 3. IL-17 expression in the thymus and spleen in the rats in each group (n=10). Immunohistochemical staining of IL-17 in the thymus of CFA group (A), TG group (B) and EAMG group (C). Immunohistochemical staining of IL-17 in the spleen of CFA group (D), TG group (E) and EAMG group (F). (G) Semi-quantitative analysis of IL-17 staining (n=5). *P<0.05 vs. EAMG group.



cantly higher than those in EAMG model group (P<0.01) (Figure 2).

In addition, IL-17 expression in the thymus and spleen were detected by immunohistochemistry. Compared to EAMG model group, IL-17 staining in the thymus and spleen of TG group and CFA group was significantly weaker (Figure 3).

Discussion

In this study, EAMG animal model was established by

immunizing inbred Lewis rats with the combination of R α 97-116 peptide sequence and Freund's adjuvant. EAMG rat model was found to have two phases in the pathogenesis, namely early and late onset phases. The phase of early onset of the disease began on the 10th day of the first immunization and reached a peak on the 14th day, and then symptoms disappeared until the 18th day. The phase of late onset occurred on the 40th day, reaching the peak on the 60th day [3]. In this study, about 1 week after the immunization, EAMG model rats showed symptoms such as weakness of the forelimbs, body tremor, bending, tremor and weight loss. The symptoms of myasthenia gradually aggravated, the extent of body mass decrease was large and rapid. In contrast, the onset of rats in TG treatment group delayed, the symptoms were relatively light, the overall decrease in body mass was significantly smaller than EAMG model group. Taken together, these data indicate that TG could relieve the symptoms of EAMG rats.

T cell-mediated immune regulation is the main mechanism to control the stability of T cell microenvironment and maintain T cell immune tolerance. This mechanism not only can prevent the occurrence of autoimmune diseases through the regulation of autoantigen immune response, but also can regulate the reaction of non-self molecules. For example, there were a large number of activated specific antigen-reactive T cells in the synovium of rheumatoid arthritis (RA) patients [7, 8].

Tripterygium wilfordii belongs to the tripterygium family with anti-inflammatory, antioxidant and immune regulatory activities, and it has been used for the treatment of various autoimmune diseases. Tripterygium wilfordii is recommended as first choice for RA treatment [9, 10]. Active ingredients of Tripterygium wilfordii are complex, containing terpenes, glycosides and alkaloids, alcohol and other compounds including diterpenoids, alkaloids, and triterpenoids. Among them, epoxy diterpene lactone compound triptolide is considered to be one of the most important anti-inflammatory and immunosuppressive components of Tripterygium wilfordii [11, 12]. In recent years, a large number of experimental studies have shown that Tripterygium has immunosuppression and anti-inflammatory effects, and induces apoptosis

and inhibits proliferation of vascular cells. Tripterygium could directly inhibit the proliferation of T cells [13]. Wang et al. showed that Tripterygium could regulate TCR and used for the treatment of RA [14].

In summary, in this study, we established EAMG rat model, and evaluated the efficacy of TG on EAMG rats. Based on the comparisons of the symptoms and cytokine expression in TG treatment group and EAMG group, we concluded that TG is a promising agent for the treatment of MG.

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Disclosure of conflict of interest

None.

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