Beneficial Effects of Ginsenosides-Rb_1 on Immune Function of Rats During Strenuous Physical Exercise

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Abstract—Objective: the aim of the present study was to determine the effects of ginsenosides-Rb_1 (GRb_1) on immune function of rats during strenuous physical exercise. Methods: The rats were randomly divided into four groups: one control group and three different doses of GRb1 (25, 50 and 100 mg/kg) treated groups. The animals were intragastrically administered once daily for 28 days. The exhaustive treadmill exercise was conducted on the final day of experimentation, then the spleen and thymus index, and the levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF-α), immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured. Results: GRb_1 increased the spleen and thymus index in exhaustive exercise rats. GRb_1 also increased the levels of serum IgA, IgG and IgM, and decreased the levels of serum IL-1β, IL-6 and TNF-α. Conclusion: GRb_1 have beneficial effects on immune function of rats during strenuous physical exercise.

Keywords- ginsenosides-Rb_1; spleen index; thymus index; interleukin-1β; interleukin-6; tumor necrosis factor; immunoglobulin A; immunoglobulin G; immunoglobulin M; exhaustive treadmill exercise

I. INTRODUCTION

Over the past two decades, the response of the immune system to exercise and sport has evolved into a topic of significant interest to both health and sport professionals. It is widely accepted that regular exercise is known to reduce the risk of chronic diseases, such as diabetes, cardiovascular disease and cancer, and prevent osteoporosis, obesity and aging. However, strenuous physical exercise can induce over-consumption of energy and cause metabolic disorders, resulting in decreased immune functions (1). Several studies have shown that strenuous exercise can cause significant changes in several immunologic parameters. The causes of these changes have usually been attributed to changes in hormone (e.g., cortisol and catecholamines) and cytokine [e.g., interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6)] levels in blood and skeletal muscle (2). Currently, various nutritional strategies have also been used to enhance immune function during exercise. Zinc, glutamine, carbohydrates and antioxidants (primarily obtained as nutrients or nutritional supplements) are among some nutritional strategies that have been examined for their immunopotentiating properties (3).

Panax ginseng is an herbal root that has been used in clinical practice for more than four thousand years in folk medicines of China (4). *Panax ginseng* has several pharmacologic and physiologic effects that are being disclosed gradually. Various clinical and pharmacologic effects associated with its use have been reported, such as anticancer activity, protection against circulatory shock, promotion of hematopoiesis, and modulation of immune functions and cellular metabolic processes involving carbohydrates, fats, and proteins (5). The active constituents of *Panax ginseng* are ginsenosides that include neutral ginsenosides, malonylginsenosides, and oleanolic acid-type ginsenoside (1). At present, more than 30 distinct ginsenosides have now been identified in the ginseng, among these ginsenosides-Rb_1 (GRb_1), -Rb_2, -Ro, -Rg_1, -Rc, -Rd and -Re are highly abundant. In particular, GRb_1, makes up 0.37 - 0.5% of *Panax ginseng* extracts and it has stronger antioxidant potency than the others (7), which suggests that it has beneficial effects on the immune function. Chemical analysis has shown that GRb_1 is found not only in the root but also in the stems and leaves of *Panax ginseng*. This discovery has greatly decreased the cost of GRb_1 production (8). The aim of the present study was to determine the effects of GRb_1 on immune function of rats during strenuous physical exercise.

II. MATERIALS AND METHODS

A. Chemicals and reagents

GRb_1 (it were isolated from *Panax ginseng*, chemical purification > 96.2%) were purchased from the Fanke Pharmaceutical Co. (Shanghai, China). Commercial kits for the detection of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) were purchased from Mr Ng Nanjing Biological (Nanjing, China). Commercial kits for the detection of immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) were purchased from Kemin Biotechnology Co., Ltd. (Shanghai, China).

B. Experimental animals

Male Sprague-Dawley (SD) rats, each weighing 180 - 220 g, were obtained from the Center of Experimental Animal of Hunan Province (Changsha, China). These rats were kept under automatic light and darkness cycle (12 × 12 h), as well as temperature (22 ± 1 °C) and humidity controlled (humidity relative 55 ± 5%). The animals were fed with a standard rat pellet diet and tap water was supplied ad libitium. Experiments were performed according to the guide for the care and use of laboratory animals of Hunan Province, and were approved by the Ethics Review Committee for Animal Experimentation of Institute of Central South University (Changsha, China). The animals were kept under observation for one week prior to the start of treatment.

C. Dosing schedule

The rats were subdivided into four experimental groups of eight animals each.

Group I: the rats received 2.0 mL physiological saline by gastric gavage every day.

Group II: the rats received GRb_1 at the dose of 25 mg/kg body weight by gastric gavage every day.

Group III: the rats received GRb_1 at the dose of 50 mg/kg body weight by gastric gavage every day.

Group IV: the rats received GRb_1 at the dose of 100 mg/kg body weight by gastric gavage every day.

GRb_1 were dissolved in 2.0 mL of physiological saline and treatment was continued for 28 days. Following the final administration with GRb_1, the rats were allowed to rest for 30 min, and then removed for the exhaustive treadmill exercise.

D. Exhaustive treadmill exercise

Treadmill exercise was performed as described previously (9) with some modifications. Briefly, the rats were subjected to graded treadmill running starting at 10% grade, 15 m/min for 15 min followed by a gradual increase in the treadmill speed and time to 25 for 15, 30 for 30, 35 for 60, 40 for 30 and 45 m/min for 30 min until exhaustive. Electrical shocks were used sparingly in exhaustive exercise groups to motivate the animals to run. Exhaustion was defined as the inability of the rats to run on the treadmills, despite electrical shock.

E. Biochemical analysis

At the end of exhaustive treadmill exercise, the rats were anaesthetized with ether. Blood was collected in heparinised tubes and serum was then prepared by centrifugation (3000 rpm, 10 min) at 4 °C for the levels of IL-1β, IL-6, TNF-α, IgA, IgG and IgM measurements using commercial ELISA kits according to the protocol recommended by the manufactures. Then the spleens and thymus were rapidly removed and weighed for the spleens and thymus index measurements. The spleen index (SI) and thymus index (TI) of rats were calculated according to the following formula:

$$Spleen \cdot index = \frac{Weight \cdot of \cdot spleen \cdot (mg)}{Body \cdot weight \cdot (g)}$$

$$Thymus \cdot index = \frac{Weight \cdot of \cdot thymus \cdot (mg)}{Body \cdot weight \cdot (g)}$$

F. Statistical analysis

The values are presented as the mean ± standard deviation. The experiments were conducted in at least triplicate and Student's t - test was used for comparing the difference in intergroup measurement data. All analyses were performed using SPSS version 15.0 (Chicago, IL) and the level of significance was noted at $P < 0.05$ level.

III. Results and Discussion

A. Effects of GRb_1 on spleen and thymus index of rats

Spleen and thymus are two important immune organs. Spleen is the largest lymphoid organ in vivo. It is where lymphocytes stay, proliferate and produce immune response. Thymus is an important immune organ in vivo. Degradation and dysfunction of thymus is one of the reasons for decreased immune function (9). Therefore, spleen index and thymus index could be used as the indexes of immunity function.

As shown in Figure 1 and Figure 2, the spleen and thymus index of rats in the Group II, Group III and Group IV were significantly higher compared with the Group I ($P < 0.05$). The results indicated that GRb_1 could effectively upregulate spleen and thymus index in exhaustive exercise rats, which may contribute to its effect in relieving strenuous exercise-induced immunosuppression.

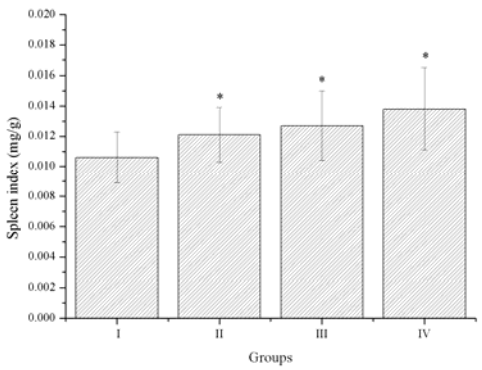

Figure 1. Effects of GRb_1 on spleen index of rats. * $P < 0.05$ when compared to the Group I.

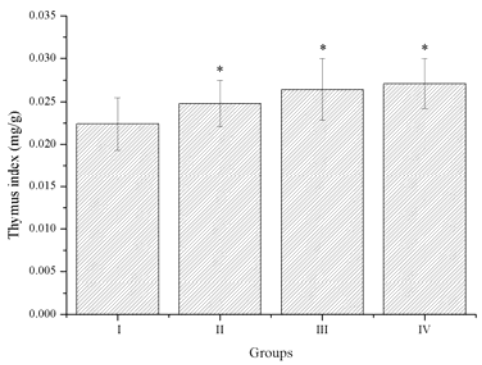

Figure 2. Effects of GRb_1 on thymus index of rats. * $P < 0.05$ when compared to the Group I.

B. *Effects of GRb_1 on serum IL-1β, IL-6 and TNF-α levels of rats*

It is well established that exhausting exercise can result in excessive inflammatory reactions and immune suppression, leading to clinical consequences that slow healing and recovery from injury and/or increase your risk of disease and/or infection (10). Cytokines are termed proteins of low molecular weight that stimulate or inhibit the differentiation, proliferation or function of immune cells. They are divided roughly into two types, ie. immunoregulatory and inflammatory cytokines. Among these cytokines, IL-1β, IL-6, and TNF-α are known to be acute inflammatory cytokines. IL-1β and TNF-α are some examples of pro-inflammatory cytokines. IL-6 can be both pro-inflammatory and anti-inflammatory (11). It is reported that several cytokines can be detected in plasma during and after strenuous exercise, and the increase in IL-1β and TNF-α levels is accompanied by a dramatic increase in IL-6. This release is balanced by the release of cytokine inhibitors (IL-1ra and TNF receptors) and the anti-inflammatory cytokine interleukin-10 (12).

As shown in Figure 3, the serum IL-1β levels of rats in the Group II, Group III and Group IV were significantly lower compared with the Group I ($P < 0.05$). As shown in Figure 4, the serum IL-6 levels of rats in the Group III and Group IV were significantly lower compared with the Group I ($P < 0.05$). Although, the serum IL-6 levels of rats in the Group II were also decreased, no significant difference was observed ($P > 0.05$). As shown in Figure 5, the serum TNF-α levels of rats in the Group II, Group III and Group IV were significantly lower compared with the Group I ($P < 0.05$). The results indicated that GRb_1 could decrease expression of pro-inflammatory cytokines in blood and improve immunity function during strenuous exercise.

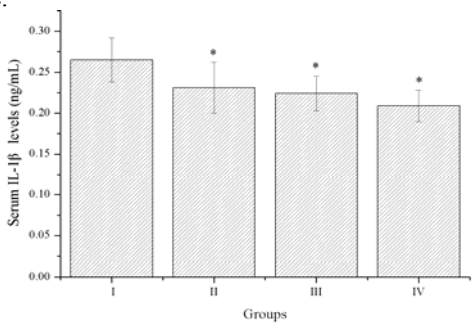

Figure 3. Effects of GRb_1 on serum IL-1β levels of rats. * $P < 0.05$ when compared to the Group I.

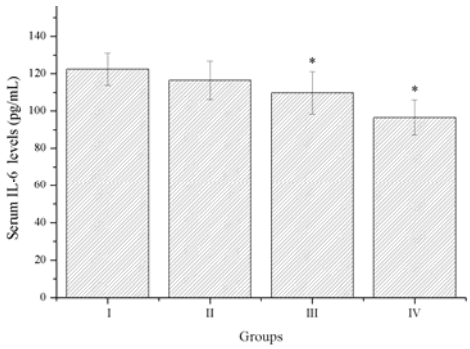

Figure 4. Effects of GRb_1 on serum IL-6 levels of rats. * $P < 0.05$ when compared to the Group I.

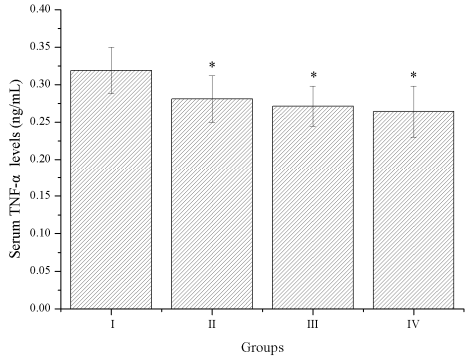

Figure 5. Effects of GRb_1 on serum TNF-α levels of rats. * $P < 0.05$ when compared to the Group I.

C. *Effects of GRb_1 on serum IgA, IgG and IgM levels of rats*

Immunoglobulins (Ig) are antibodies secreted by B lymphocytes and are an important part of the immune system. Ig divided into five isotypes, which can exist in either a membrane bound or a secreted form. These isotypes - IgA, IgD, IgE, IgG and IgM - all have different heavy chains and thus exert different effect or functions (13). Exercise can cause a change in the consistency of serum Ig levels, among these changes are significant variations are in IgA, IgG and IgM (14). Numerous studies have shown that strenuous physical exercise leads to reduced serum IgA, IgG and IgM levels and as a result weakens the immune system. The fall in IgA, IgG and IgM levels may be attributed to the probable inflammation in muscle tissues resulting from intense exercise (15).

As shown in Figure 6 and Figure 7, the serum IgA and IgG levels of rats in the Group III and Group IV were significantly higher compared with the Group I ($P < 0.05$). Although, the serum IgA and IgG levels of rats in the Group II were also increased, no significant difference was observed ($P > 0.05$). As shown in Fig. 8, the serum IgM levels of rats in the Group II, Group III and Group IV were significantly higher compared with the Group I ($P < 0.05$). The results indicated that GRb_1 could reduce the risk of adverse changes in the immune system and improve immunity function during strenuous physical exercise.

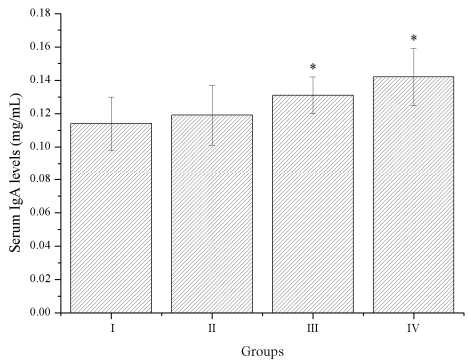

Figure 6. Effects of GRb_1 on serum IgA levels of rats. * $P < 0.05$ when compared to the Group I.

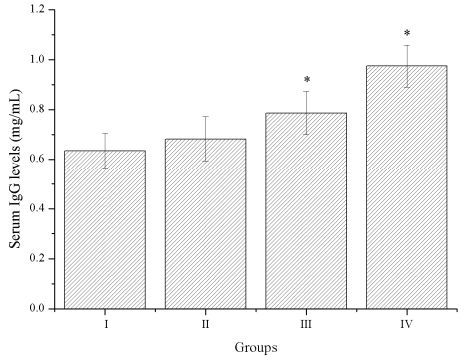

Figure 7. Effects of GRb_1 on serum IgG levels of rats. * $P < 0.05$ when compared to the Group I.

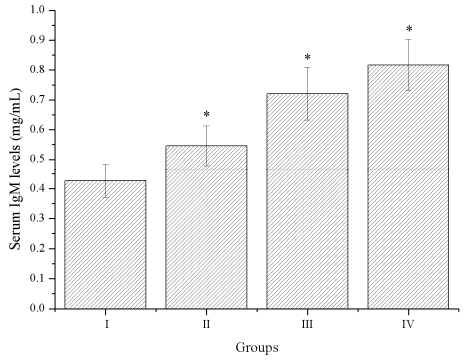

Figure 8. Effects of GRb_1 on serum IgM levels of rats. * $P < 0.05$ when compared to the Group I.

IV. CONCLUSION

In conclusion, GRb_1 increased the spleen and thymus index in exhaustive exercise rats. GRb_1 also increased the levels of serum IgA, IgG and IgM, and decreased the levels of serum IL-1β, IL-6 and TNF-α. The results obtained from this study suggest that GRb_1 have beneficial effects on immune function during strenuous physical exercise. GRb_1 is of potential research and development value in the field of pharmaceutical.

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