Effects of ginsenosides-Rb₁ on exercise-induced oxidative stress in forced swimming mice

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Submitted: 13-08-2013 Revised: 24-08-2013 Published: 26-09-2014

ABSTRACT

Background: The fleshy root of Panax ginseng C.A. Meyer (ginseng) is one of the most well-known and valued herbs in traditional Chinese medicine. Ginsenosides are considered mainly responsible for the pharmacological activities of ginseng. The purpose of this study was to investigate the effects of ginsenoside-Rb, (G-Rb,) on swimming exercise-induced oxidative stress in male mice. Materials and Methods: A total of 48 animals were randomly divided into four groups, with twelve mice in each group. The first, second and third groups were designed as G-Rb, treatment groups, got 25, 50 and 100 mg/kg bodyweight of G-Rb,, respectively. The fourth group was designed as the control group, got physiologic saline. The mice were intragastrically administered once daily for 4 weeks. The weight-loaded forced swimming test was conducted on the final day of experimentation. Then the exhaustive swimming time, blood lactate, serum creatine kinase (CK), malondialdehyde (MDA) and antioxidant enzymes in liver of mice were measured. Results: The results showed that G-Rb, could prolong the exhaustive swimming time and improve exercise endurance capacity of mice, as well as accelerate the clearance of blood lactate and decrease serum CK activities. Meanwhile, G-Rb, could decrease MDA contents and increase superoxide dismutase, catalase, glutathione peroxidase activities in liver of mice. Conclusions: The study suggested that G-Rb, possessed protective effects on swimming exercise-induced oxidative stress in mice.

Access this article online
Website:
www.phcog.com
DOI:
10.4103/0973-1296.141818
Quick Response Code:

Key words: Antioxidant enzymes, blood lactate, exhaustive swimming time, malondialdehyde, serum creatine kinase

INTRODUCTION

It is well-established that an aerobic metabolism in biological systems produces pro-oxidant molecules. These pro-oxidant molecules are called free radicals or reactive oxygen species (ROS), including the superoxide radical (O₂), hydroxyl radicals, hydrogen peroxide (H₂O₂) and nitric oxide. Under normal physiological conditions, the cells defend themselves against ROS production has enough endogenous antioxidant reserves. The endogenous antioxidant defense is made up of both non-enzymatic and enzymatic antioxidants. Common non-enzymatic antioxidants include ascorbic acid (vitamin C), tocopherol (vitamin E), reduced glutathione (GSH), melatonin, thioredoxin, uric acid, lipoic acid and bilirubin. Common enzymatic

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antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and GSH reductase. [2,3] Over the past two decades, adequate strong evidence from animal and human studies has shown a strenuous physical activity causes an increase in oxygen consumption and ROS production, then the negative consequence is an imbalance between ROS and antioxidant defense, resulting in oxidative stress, which can lead to damage or destruction of cellular macromolecules such as lipids, proteins and nucleic acids. [4-6] However, under conditions, the excessive formation of free radicals are eliminated by endogenous antioxidants may not be sufficient and should be supplemented with exogenous antioxidant, primarily obtained as nutrients or nutritional supplements. These exogenous antioxidants by scavenging the radicals inhibit the cell and its components damaging and assisting smooth and normal function.[7-9]

Panax ginseng C.A. Meyer (Araliaceae) is a perennial herb of the family Araliaceae. P. ginseng C.A. Meyer is primarily

found in three Northeastern Provinces of China, the Korean Peninsula, Fukushima, Japan and East Siberia of the former Soviet Union. The fleshy root of this plant, commonly known as ginseng, is one of the most well-known and valued herbs in traditional Chinese medicine for over 2000 years. [10] As first recorded in the oldest Chinese herbal compilation (Shen Nong's Materia Medica), ginseng strengthens the vital organs, secures the spirit, anchors the soul, stops fear and fright, eliminates diseases, brightens the eyes, sharpens the senses and benefits intelligence. When taken long-term, it promotes strength, health and longevity.[11] Li Shizhen, a famous Chinese folk doctor in Ming Dynasty, also spoke highly of ginseng as the best tonic in the herbs. Previous studies have shown that ginseng has many pharmacological activities such as anticoagulant, antioxidative, anticancer, anti-fatigue and anti-inflammatory activities, analgesic, central nervous system, neuroprotective and immunomodulation effects. [12-15] Recent investigations have proved that the active constituents of ginseng include ginsenosides, polysaccharides, peptides and polyacetylenic alcohols.^[16] Ginsenosides, a group of saponins with triterpenoid dammarane structure, are considered mainly responsible for the pharmacological activities of ginseng. It has been shown that ginsenosides have antioxidant, anti-inflammatory, anti-apoptotic, anti-fatigue and immunostimulant properties.[17,18] At present, more than 30 distinct ginsenosides have now been identified in the ginseng, among these ginsenosides-Rb₁ (G-Rb₁), -Rb₂, -Ro, -Rg₁, -Rc, -Rd and -Re are highly abundant. In particular, G-Rb, [Figure 1] makes up 0.37-0.5% of ginseng extracts and it has stronger antioxidant potency than the others, [19,20] which suggests that it is beneficial in counteracting exercise-induced oxidative stress. However, the effects of G-Rb, on exercise-induced oxidative stress have not been investigated thus far. Therefore, the research presented

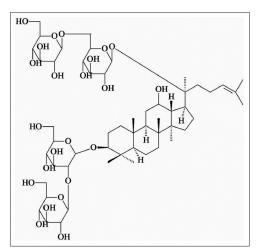


Figure 1: Chemical structure of ginsenoside Rb,

here was designed to evaluate the effects of G-Rb1 on swimming exercise-induced oxidative stress in male mice.

MATERIALS AND METHODS

Main reagents

G-Rb₁ (it was isolated from the root of *P. ginseng* C.A. Meyer, chemical purification >96.2%) was purchased from the Fanke Pharmaceutical Co. (Shanghai, China). The assay kits of blood lactate, SOD, CAT, GPx and malondialdehyde (MDA) were purchased from Jiancheng Institute of Biotechnology (Nanjing, China). The assay kit of creatine kinase (CK) was purchased from Zhong Sheng Biotechnology and Science Inc. (Beijing, China). All other reagents used in this study were of analytical grade and were obtained locally.

Experimental animals

Male Kunming strain mice, weighed 18-22 g, were obtained from the Center of Experimental Animal of Hunan Province (Changsha, China). Animals were housed in plastic cages in a room maintained at 22°C and 55% relative humidity with a 12-h light/dark cycle and allowed free access to laboratory standard diet and water. All the experimental protocols described in this study followed the Institutional Guidelines of Hunan Province and were approved by the Ethics Review Committee for Animal Experimentation of Institute of Central South University (approval number: CNSY 2012-0028).

After 1 week of adaptation period, the animals were randomly divided into four groups, with twelve mice in each group. The first, second and third groups were designed as G-Rb₁ treatment groups, got 25, 50 and 100 mg/kg bodyweight of G-Rb₁, respectively. The fourth group was designed as the control group and got physiologic saline. The mice were intragastrically administered once daily for continuous 4 weeks. The doses of G-Rb₁ and 4 weeks treatment time used in this study were confirmed to be suitable and effective in tested mice, according to preliminary experiments.

Weight-loaded forced swimming test (WFST)

At the final day of experimentation, the mice underwent a weight-loaded WFST as the method described by Zhang et al. [21] with some modifications. Briefly, the animals were placed individually into acrylic plastic pool (50 cm in length, 50 cm in width and 40 cm in high) filled with water to a depth of 30 cm. The temperature of the water was maintained at 25 ± 0.5 °C. The animals were made to swim with a load (tagged to the tail base) of 6% of their body weight. The exhaustive swimming time was used as the index of the exercise endurance. The exhaustion

was defined as animal dropping its head in water within 10 s and being unable to bail out the water surface. [21-23]

Analysis of biochemical parameters

Immediately after WFST, the animals were removed from the acrylic plastic pool. After anesthetization with pentobarbital sodium (0.5 mg/kg bodyweight, i.p.), blood samples were obtained from the orbital sinus for lactate and CK analysis. Then, the livers were removed and then frozen in liquid N₂ for SOD, CAT, GPx and MDA analysis. All the biochemical parameters were determined using respective commercial diagnostic kits according to the manufacturer's recommended instructions.

Serum CK activities were detected a method based on the ability of CK to form ATP, which reacts with glucose to produce glucose-6-phosphate (G-6-P), catalyzed by hexokinase. In the presence of G-6-P dehydrogenase, resultant G-6-P further reacts with NADP + to form NADPH and the wavelength was set at 340 nm. Blood lactate contents were detected using the dehydriding method and the wavelength was set at 530 nm. SOD activities were detected using xanthine oxidase method and the wavelength was set at 550 nm. GPx activities were detected using GSH as substrate by measuring the decrease of enzymatic reaction of GSH (Except the effect of non-enzymatic reaction) and the wavelength was set at 412 nm. CAT activities were detected using the colorimetric method based on the decomposition of H2O2 by CAT and the wavelength was set at 405 nm. MDA contents were detected using the thiobarbituric acid (TBA) method based on its reaction with TBA to form thiobarbituric acid-reactive substances and the wavelength was set at 532 nm.

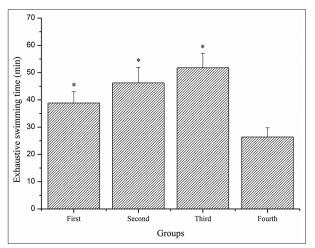


Figure 2: Effects of ginsenoside-Rb₁ (G-Rb₁) on the exhaustive swimming time of mice. Values are means \pm standard deviation, each group contains twelve mice. First group: Low-dose G-Rb₁ treatment group (25 mg/kg bodyweight), second group: Middle-dose G-Rb₁ treatment group (50 mg/kg bodyweight), third group: High-dose Rb₁ treatment group (100 mg/kg bodyweight), fourth group: Control group. *P < 0.05 when compared to the fourth (control) group

Statistical analysis

All the data were expressed as the mean \pm 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. *P* values < 0.05 were regarded as having statistical significance.

RESULTS AND DISCUSSION

Effects of G-Rb₁ on the exhaustive swimming time of mice

As shown in Figure 2, the exhaustive swimming time of mice in the first, second and third groups were significantly prolonged compared with the fourth group (P < 0.05) and the exhaustive swimming time increased by 47.4%, 75.3% and 96.4%, respectively. WFST is commonly used as anti-fatigue and exercise endurance tests. Other methods of forced exercise such as the motor driven treadmill or a wheel can cause animal injury and may not be routinely acceptable. [24] In the current study, the results showed that different doses of G-Rb₁ could significantly prolong the exhaustive swimming time, which demonstrated that G-Rb₁ could improve exercise endurance capacity of mice.

Effects of G-Rb, on the blood lactate contents of mice

As shown in Figure 3, the blood lactate contents of mice in the first, second and third groups were significantly lower compared with the fourth group (P < 0.05) and the blood lactate contents decreased by 21.5%, 45.3% and 65.1%, respectively. Blood lactate is the glycolysis product of carbohydrate under an anarobic condition. Glycolysis is the main energy source for fierce exercise in

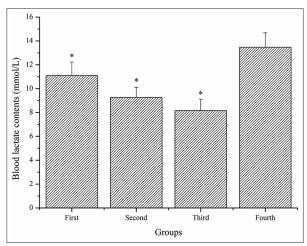


Figure 3: Effects of ginsenoside-Rb₁ (G-Rb₁) on the blood lactate contents of mice. Values are means \pm standard deviation, each group contains 12 mice. First group: Low-dose G-Rb₁ treatment group (25 mg/kg bodyweight), second group: Middle-dose G-Rb₁ treatment group (50 mg/kg bodyweight), third group: High-dose G-Rb₁ treatment group (100 mg/kg bodyweight), fourth group: Control group. *P < 0.05 when compared with the fourth (control) group

a short-time, which can increase lactate production to a point that exceeds the rate of lactate removal. ^[25] Therefore, blood lactate is closely related to workload intensity and is one of the important indicators for judging the exercise endurance. In the current study, the results showed that different doses of G-Rb₁ could significantly accelerate the clearance of blood lactate after swimming exercise, which demonstrated that G-Rb₁ could increase the oxygen uptake and accelerate lactate metabolism, thereby improving exercise endurance capacity.

Effects of G-Rb, on the serum CK activities of mice

As shown in Figure 4, the serum CK activities of mice in the second and third groups were significantly lower compared with the fourth group (P < 0.05) and the serum CK activities decreased by 24.2% and 41.1%, respectively. Although, the serum CK activities of mice in the first were also decreased, no significant difference was observed (P > 0.05). Serum CK is widely accepted as an indicator of muscle damage after exercise. [26] The normal function of CK in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. Phosphocreatine is burned as a quick source of energy by cells. However, the normal function of CK isn't as relevant, in this case, as what happens to CK when muscle is damaged. During the process of muscle degeneration, muscle cells break open and their contents find their way into the bloodstream. Because most of the CK in the body normally exists in muscle, an increase in the amount of CK in the blood indicates that muscle damage has occurred or is occurring.[27] In the current study, the results showed that medium and high doses of

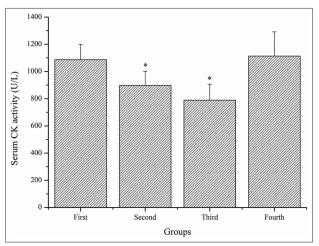


Figure 4: Effects of ginsenoside-Rb₁ (G-Rb₁) on the serum CK activities of mice. Values are means \pm standard deviation, each group contains 12 mice. First group: Low-dose G-Rb₁ treatment group (25 mg/kg bodyweight), second group: Middle-dose G-Rb₁ treatment group (50 mg/kg bodyweight), third group: High-dose G-Rb₁ treatment group (100 mg/kg bodyweight), fourth group: Control group. *P < 0.05 when compared to the fourth (control) group

G-Rb₁ could significantly decrease serum CK activities after swimming exercise. It could be considered that this minimizing muscle damage contributes to improving exercise endurance capacity of mice treated with G-Rb₁.

Effects of G-Rb₁ on the antioxidant enzymes activities in liver of mice

As shown in Figure 5, the antioxidant enzymes activities of mice in the first, second and third groups were significantly higher compared with the fourth group ($P \le 0.05$). The SOD activities were increased by 27.4%, 55.1% and 63.3%, respectively. The CAT activities were increased by 35.2%, 77.1% and 126.5%, respectively and the GPx activities increased by 21.1%, 38.0% and 46.1%, respectively. Antioxidant enzymes, which provide the primary defense against ROS generated during exercise, may be activated selectively during an acute bout of strenuous exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. [28,29] The primary ROS produced in the aerobic organisms is O₂ that is a highly reactive cytotoxic agent. O, is converted to H,O, by SOD. H,O, in turn, is converted to molecular oxygen and H2O by either CAT or GPx. In addition, GPx can reduce lipid peroxides and other organic hydroperoxides that are highly cytotoxic products.^[30] In the current study, the results were showed that different doses of G-Rb, could significantly increase antioxidant enzymes (SOD, CAT and GPx) activities in liver after swimming exercise, which demonstrated that G-Rb, had beneficial effects on attenuating the oxidative stress induced by exhaustive exercise.

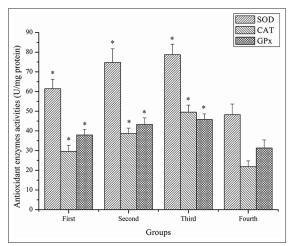


Figure 5: Effects of ginsenoside-Rb₁ (G-Rb₁) on the antioxidant enzymes activities in liver of mice. Values are means \pm standard deviation, each group contains twelve mice. First group: Low-dose G-Rb₁ treatment group (25 mg/kg bodyweight), second group: Middle-dose G-Rb₁ treatment group (50 mg/kg bodyweight), third group: High-dose G-Rb₁ treatment group (100 mg/kg bodyweight), fourth group: Control group. *P < 0.05 when compared with the fourth (control) group

Effects of G-Rb, on the MDA contents in liver of mice

As shown in Figure 6, the MDA contents of mice in the first, second and third groups were significantly lower compared with the fourth group (P < 0.05) and the MDA contents decreased by 29.3%, 38.5% and 52.9%, respectively. The most popular biomarker of oxidative stress is lipid peroxidation. Some confirmatory evidence have indicated that lipid peroxidation could limit different aspects of muscle or cell function by decreasing the fluidity of the membrane, making it more difficult for proteins/nutrients to pass through.^[31] MDA is one of the most readily assayed end products of lipid peroxidation. The analysis of MDA by the TBA assay has been widely employed over many years in biological systems for the assessment of lipid peroxidation.^[32] In response to various forms of exercise many studies have reported significant increases of MDA.[33-35] In the current study, the results showed that different doses of G-Rb, could significantly decrease MDA contents after swimming exercise, which demonstrated that G-Rb, could reduce lipid peroxidation and oxidative damage following exhausting exercise.

CONCLUSION

The present investigation showed that G-Rb₁ could prolong the exhaustive swimming time and improve exercise endurance capacity of mice as well as accelerate the clearance of blood lactate and decrease serum CK activities. Meanwhile, G-Rb₁ could increase antioxidant enzymes activities and decrease MDA contents in liver of mice, which suggested that G-Rb₁ possessed protective effects on exercise-induced oxidative stress in forced swimming

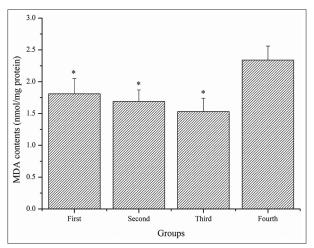


Figure 6: Effects of ginsenosides-Rb₁ (G-Rb₁) on the MDA contents in liver of mice. Values are means \pm standard deviation, each group contains twelve mice. First group: Low-dose G-Rb₁ treatment group (25 mg/kg bodyweight), second group: Middle-dose G-Rb₁ treatment group (50 mg/kg bodyweight), third group: High-dose G-Rb₁ treatment group (100 mg/kg bodyweight), fourth group: Control group. *P < 0.05 when compared with the fourth (control) group

mice. The experimental results provided theoretical support for G-Rb, in the field of sports nutrition.

ACKNOWLEDGMENT

This work was supported by Science and Technology Development Foundation of Zhejiang Province of China (Grant No. 20110406).

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Cite this article as: Qi B, Zhang L, Zhang Z, Ouyang J, Huang H. Effects of ginsenosides-Rb, on exercise-induced oxidative stress in forced swimming mice. Phcog Mag 2014;10:458-63.

Source of Support: This work was supported by Science and Technology Development Foundation of Zhejiang Province of China (Grant No. 20110406), Conflict of Interest: None declared.