Effect of polysaccharides from the roots of *Morinda officinalis* How on physical fatigue

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Abstract

The dried roots of *Morinda officinalis* How is one of the most widely used Chinese traditional tonic herbal medicine. Polysaccharides from the roots of *Morinda officinalis* How (MOP) are its active component and have a lot of pharmaceutical activities. However, anti-fatigue effects of MOP have not yet been tested. This study was carried out to investigate the effects of MOP on physical fatigue in mice. The anti-fatigue effects of MOP were evaluated using a forced swimming test, along with the determination of blood lactic acid (BLA), serum urea nitrogen (SUN), serum creatine kinase (CK), liver glycogen and muscle glycogen contents. The experiment data showed that MOP could increase the swimming time to exhaustion of the mice, as well as increase the liver and muscle glycogen contents, and decrease the BLA, SUN and serum CK contents. These results indicated that MOP had anti-fatigue effects in mice.

Key words: Forced swimming test, blood lactic acid, serum urea nitrogen, serum creatine kinase, liver glycogen, muscle glycogen, mice.

Introduction

Fatigue is a common symptom in sickness and in health, and it is best defined as difficulty in initiating or sustaining voluntary activities. It can be divided into two categories: physical fatigue caused by such things as forced exercise or swimming; mental fatigue caused by sleep deprivation, etc. In busily, strained and economically affluent modern societies, fatigue has become an important and highly prevalent symptom. During the past decades, health scholars and athletic physiologist have been looking for natural active products that not only can improve athletic ability, postpone fatigue and accelerate the elimination of fatigue in human beings, but also have few side effects.

*Morinda officinalis* How (Rubiaceae [madder] family) is a small vine bush plants that grows widely in tropical and subtropical regions, including parts of China, India, Australia and the Pacific Islands. The dried roots of this plant, named “Ba-ji-tian”, are a traditional tonic herbal medicine, and has been used as natural nutrient food and ingredients in Chinese medicine for more than 2000 years. It has been reported to possess the ability to help strengthen the bones and kidneys and enhance the immune system function, strengthen the tendons and bones and relieve rheumatic condition. In the past few years, some biologically active components have already been isolated from this plant, and they include anthraquinones, flavonoids, polysaccharides, glycosides, iridoids, lignans and triterpenoids. Polysaccharides are an important active component, and are confirmed to be responsible for multifont biological function of the roots of *Morinda officinalis* How. It has been shown that polysaccharides from the roots of *Morinda officinalis* How (MOP) have anti-inflammatory, antinociceptive, antimicrobial, antioxidant, antihepatotoxic, hypoglycemic and immunomodulation activities. However, very little is known about anti-fatigue effects of MOP. The present study was undertaken to evaluate the effect of MOP on physical fatigue in mice by forced swimming test.

Materials and Methods

Chemicals and reagent: Commercial diagnostic kit for determination of blood lactic acid (BLA) was purchased from Beijing Leadman Biochemistry Technology Co. Ltd. (Beijing, China). Commercial diagnostic kits for determination of serum urea nitrogen (SUN), liver glycogen and muscle glycogen were purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). Commercial diagnostic kit for serum creatine kinase (CK) was purchased from Biosino Bio-technology and Science Inc. (Beijing, China). All other chemicals used were analytical grade.

Plant material: The dried roots of *Morinda officinalis* How was purchased from a local herb shop (Hangzhou, China). The plant was identified and authenticated by Institute of Botany, Zhejiang Province, China. A voucher specimen has been deposited in the Herbarium of Zhejiang Shuren University (Hangzhou, China). The dried roots were ground to a fine powder with a cyclone mill and stored in dry condition until being used.

Preparation of polysaccharides from the roots of *Morinda officinalis* How: Polysaccharides from the roots of *Morinda officinalis* How (MOP) were extracted as previously described by Zhu et al. with minor modifications. In brief, the ground powder samples were refluxed to remove lipids with chloroform: methanol...
solvent (2:1) (v/v). After filtering, the residues were air-dried, and then refluxed again with 80% ethanol at 80°C to remove some coloured materials, monosaccharides, oligosaccharides, and small molecule materials. The residues were extracted four times in boiled water and filtered. The combined filtrates were concentrated by a rotavapor at 60°C, and then precipitated using 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and lyophilized, resulting in a polysaccharide sample, which was a brown powder, soluble in distilled water and insoluble in organic solvents including ethanol.

**Experimental animals:** Male Kunming mice (6-8 weeks old, weighing 18-22 g) were provided by the Experimental Animal Center of Zhejiang Province (SPF grade, Certificate No. 2012487). The mice were kept in stainless steel cages and maintained under standard laboratory conditions of temperature (21±2°C), relative humidity (45±5%), 12 h light: dark cycle (lights on at 07:00), standard rodent diet and water ad libitum. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese National Institutes of Health, and were approved by the Institutional Animal Care and Use Committee (IACUC) at Zhejiang Shuren University.

**Grouping of animals:** A total of 120 mice were divided into four groups equally based on body weight after one week adaptation: normal control group (NC), low dose of MOP-treated group (MOP I, 100 mg/kg), middle dose of MOP-treated group (MOP II, 200 mg/kg) and high dose of MOP-treated group (MOP III, 400 mg/kg). The mice in the NC group orally received 2 ml physiological saline, the mice in the MOP I, II and III groups were given MOP at the dose of 100, 200 and 400 mg/kg body weight dissolved in 2 ml physiological saline for 28 days, respectively. The doses of MOP and 28 days treatment time used in this study were confirmed to be suitable and effective in tested mice, according to preliminary experiments. After 28 days, anti-fatigue activities of MOP were evaluated using a forced swimming test.

**Forced swimming test:** The forced swimming test was performed as described previously by Sun and Wang with some modifications. Briefly, 30 min after the last treatment, the mice were placed in a swimming pool (60 cm in length, 50 cm in width, and 50 cm in high) filled with fresh water. The temperature of the water was maintained at 25 ± 0.5°C, approximately 40 cm deep so that mice could not support themselves by touching the bottom with their tails. A tin wire (5% of body weight) was loaded onto the tail root of the mouse. Exhaustion was determined by observing the loss of coordinated movements and failure to return to the surface within 10 s. After filtering, the residues were air-dried, and then refluxed again with 80% ethanol at 80°C to remove some coloured materials, monosaccharides, oligosaccharides, and small molecule materials. The residues were extracted four times in boiled water and filtered. The combined filtrates were concentrated by a rotavapor at 60°C, and then precipitated using 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and lyophilized, resulting in a polysaccharide sample, which was a brown powder, soluble in distilled water and insoluble in organic solvents including ethanol.

**Sample collection:** After the forced swimming test, the exhausted mice were anesthetized with ether absolute and sacrificed by decapitation. The blood was collected heparinized, chilled tubes and quickly placed under ice cold condition. The whole blood samples were used for the determination of BLA. Serum was prepared by centrifugation (2000×g, 4°C, 10 min) for the determination of SUN and serum CK. Liver and gastrocnemius muscles were quickly dissected and washed in ice-cold saline solution, then the samples were then frozen in liquid nitrogen and kept at -70°C until analysis for determination of liver and muscle glycogen. BLA, SUN, serum CK, liver glycogen and muscle glycogen were determined using commercial diagnostic kits following the manufacturer’s instructions.

**Statistical analysis:** Statistical analysis was carried out using ANOVA followed by post-hoc Turkey’s test (SPSS 15 for Windows). The criterion of significance was set at p < 0.05. All results are given as mean ± SD.

**Results and Discussion**

**The effects of MOP on swimming time to exhaustion of mice:** The forced swimming test was employed in this study to evaluate the anti-fatigue effects of MOP. The lengths of the swimming time to exhaustion indicate the degree of fatigue. It is commonly accepted that swimming is an experimental exercise model. Other methods of forced exercise such as the motor driven treadmill or wheel can cause animal injury and may not be routinely acceptable. To standardize the workload and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal. In this study, the mice had a weight attached 5% body weight in the duration of the swimming to exhaustion. As shown in Fig. 1, swimming time to exhaustion of mice in the MOP I, II and III groups were significantly prolonged compared with that in the NC group (p<0.05), and the increase ratios were 41.01%, 65.32% and 116.92%, respectively. The results indicated that MOP had anti-fatigue effects.

**The effects of MOP on BLA of mice:** Previous studies have indicated that the muscle produces a great quantity of lactic acid when it obtains enough energy from anaerobic glycolysis during high-intense exercise. The increased lactic acid level further reduces pH value, which could induce various biochemical and physiological side effects, including glycolysis and phosphofructokinase and calcium ion release, through muscular contraction. So, BLA is one of the important indicators for judging the degree of fatigue. As shown in Fig. 2, BLA contents of mice in the MOP I, II and III groups were significantly decreased compared with that in the NC group (p<0.05), and the decrease ratios were 20.53%, 40.24% and 49.18%, respectively. The results indicated that MOP effectively delayed the increase of BLA and ameliorates fatigue.
The effects of MOP on SUN of mice: Urea is formed in the liver as the end product of protein metabolism. During digestion, protein is broken down into small peptides and amino acids. The amino acid nitrogen is removed as NH$_4^+$, while the rest of the molecule is used to produce energy or other substances needed by the cell. Circulating ammonia is taken up by the liver and most of it is detoxified in this tissue through the urea cycle. So, SUN is another index of fatigue status. As shown in Fig. 3, SUN contents of mice in the MOP II and III groups were significantly decreased compared with that in the NC group ($p<0.05$), and the decrease ratios were 14.38% and 24.97%, respectively. Although SUN contents of mice in the MOP I group were also decreased, no significant difference was observed ($p>0.05$). The results indicated that MOP might reduce protein catabolism for energy and ameliorates fatigue.

The effects of MOP on serum CK of mice: The normal function of CK in cells is to add a phosphate group to creatine, converting it into the high-energy molecule phosphocreatine. Phosphocreatine may be utilized as a quick source of energy by muscle cells. Most of the CK in the body normally exists in the muscle, an increase in CK in the blood indicates that muscle damage has occurred or is occurring. As shown in Fig. 4, serum CK contents of mice in the MOP I, II and III groups were significantly decreased compared with that in the NC group ($p<0.05$), and the decrease ratios were 20.19%, 25.04% and 57.59%, respectively. The results indicated that MOP could ameliorate skeletal muscle injury damage by exhaustion exercise.

The effects of MOP on liver and muscle glycogen of mice: The determinant role of glycogen stores in the capacity for prolonged exercise has been established for many years. Energy for exercise is derived initially from the breakdown of glycogen in muscle, after strenuous exercise may be depleted and at later stages the energy will be derived from liver glycogen. Liver glycogen depletion might be an important factor in the development of fatigue because as liver glycogen is depleted during exercise there is an inability to maintain blood glucose level, and the ensuing hypoglycemia could result in impaired nervous function. As shown in Fig. 5, liver glycogen contents of mice in the MOP I, II and III groups was significantly increased compared with that in the NC group ($p<0.05$), and the increase ratios were 70.99%, 125.53% and 159.76%, respectively. Muscle glycogen contents of mice in the MOP II and III groups were significantly increased compared with that in the NC group ($p<0.05$), and the increase ratios were 24.34% and 65.13%, respectively. Although muscle glycogen contents of mice in the MOP I group were also increased,
no significant difference was observed (p>0.05). The results indicated that MOP increases liver and muscle glycogen contents by improving glycogen reserve or by reducing the consumption of glycogen during exercise, thereby reducing BLA contents. This may be one of the mechanisms of its anti-fatigue effects.

Conclusions

In conclusion, the data showed that MOP could increase the swimming time to exhaustion of the mice, as well as increase the liver and muscle glycogen contents, and decrease the BLA, SUN and serum CK contents. Taken together, these results indicated that MOP had anti-fatigue effects in mice. However, further study is needed to elucidate the more exact mechanism of anti-fatigue effects of MOP.

References