

Original Article

Astragalus on the anti-fatigue effect in hypoxic mice

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Abstract: Objective: Astragalus is a traditional Chinese medicine to improve the function of the body. The purpose of this study is to investigate the effect of astragalus on improvement of anti-fatigue capacity in mice under simulated plateau environment. Methods: Male Kunming mice were randomly divided into the following groups: the control group, astragalus treatment groups in low dosage (LD) (1.0 g/kg-d), mid dosage (MD) (3.0 g/kg-d), and high dosage (HD) (30 g/kg-d). The control group were fed under normoxia environment, and hypoxic mice were fed at a stimulated elevation of 5000 meters. After continuous intragastric administration for 10 days, exhaustive swimming experiment was conducted in the anoxic environment. The swimming time, glucose and lactic acid concentration in blood, glycogen contents in liver, SOD and MDA were determined. Results: Compared with the control group, the swimming time of each astragalus treated group was evidently prolonged ($P < 0.05$), and the area under the blood lactic acid curve was significantly decreased ($P < 0.05$). In the high and middle dose of astragalus group, liver glycogen was obviously increased. After exhausted swimming, glycogen contents in blood and SOD were significantly increased, while MDA was evidently reduced ($P < 0.05$). Conclusion: Astragalus can alleviate physical fatigue in mice under simulated plateau environment. It has an obvious anti-fatigue effect and it's worthy of further study.

Keywords: Astragalus, anti-fatigue, plateau, mice

Introduction

When the plain people quickly access to high altitude hypoxia environment, their work capacity was reduced in general; reaching high altitude of 4500 m, maximum working capacity can be reduced to 50% of that in the plains. The slow recovery of plateau exercise fatigue is an important reason for the reduction in plateau working capacity [1]. Therefore, actively seeking effective drug interventions to promote plateau movement fatigue recovery and improve plateau anti-exercise fatigue ability is significant for the improvement of the plateau work capacity. Traditional efficacies of astragalus were invigorating qi for strengthening superficialities, inducing diuresis to alleviate edema, pus draining and toxin-expelling and promoting granulation, also with the effects of enhancing immune function, protecting liver, diuresis, anti-aging, anti-stress, anti-hypertension and wide antibacterial activity [2-5]. With the increasing research on the application of traditional Chinese medicine in the field of special circumstance medicine, the application of

astragalus in promoting body fatigue recovery has also been concerned [6, 7]. But the anti-movement fatigue effect of Astragalus in high altitude hypoxia environment has not been reported. In this study, the anti-fatigue efficacy evaluation methods of health food [8] was used to observe the anti-fatigue effect of Astragalus on exhaustive swimming mice in simulated high altitude hypoxia, and related biochemical indicators were detected, in order to discuss the application prospects of Astragalus in anti-plateau fatigue.

Materials and methods

Drugs and reagents

Experimental astragalus were from Sichuan. Positive control salidroside extraction was provided by Military Medical Academy II. Liver glycogen detection kit, superoxide dismutase (SOD) kit, malondialdehyde (MDA) kit (Nanjing Jiancheng, China), lactate test strips (ARKRAY Corporation, Japan) and blood glucose test strips (Roche, Germany) were used.

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Instrument

Portable blood lactate rapid detection (ARKRAY Corporation, Japan), blood glucose meter (ACCU-CHEK, Germany) and microplate reader (synergy HT, USA) were used.

Experimental animals

Kunming adult mice (SPF grade), weighing 20 ± 2 g, male, were provided by the Experimental Animal Center, Third Military Medical University. The animal license number was SCXK- (Army) 2007085.

Preparation of astragalus extraction

Astragalus are dried, and grind to powder. Take 100 grams of powder and add 1500 mL 60% ethanol 80°C soaking for 90 min. Ethanol are recovered by 60°C rotary evaporation and get the concentrated solution 2.0 g (crude drug)/ml, and then add water to form different concentrations of the experimental dose. The main active ingredient of astragalus extraction is ASIV, calycosin and thorn formononetin. The results of HPLC quantitative analysis were: astragaloside (0.47 ± 0.01) mg/ml; calycosin (1.45 ± 0.05) mg/ml; thorn formononetin (0.19 ± 0.02) mg/ml.

Experimental methods

Dose and grouping Astragalus are converted into mice dose according to human dose in "Chinese Pharmacopoeia" [9] and then they were divided into high, medium and low dose group (30.0, 3.0, 1.0 g•kg⁻¹•d⁻¹). The high, medium and low dose were 10, 1 and 0.3 times of human dose, respectively. Positive substance salidroside (0.87 g/kg) is consistent with the human dose. 120 mice were randomly divided into six groups (n = 20): normoxia control group (NCG), hypoxia control group (HCG), salidroside positive control group, astragalus treatment groups in low dosage, mid dosage, and high dosage. They were given continuous intragastric administration every day once at 0.25 ml/10 g (body weight) for 10 days. The control group were given an equal volume of saline. In the plains group, the mice were fed in plain environment (300 m altitude). In hypoxia group, they were fed in low-pressure chamber captivity (simulated altitude was 5000 m), the temperature in cabin was $25 \pm 2^\circ\text{C}$ with rela-

tive humidity of $50 \pm 10\%$. Animals were given intragastric administration, cleaning and feeding extravehicular 1 h per day. At 10 days of intragastric administration and 60 min after the last administration in each experimental group, 10 mice were taken to do exhaustive swimming test. Glucose and lactic acid were detected at three time periods: before swimming, swimming exhaustive immediately and 20 minutes after rest; take blood from another 10 mice eyeball to detect serum SOD activity, after taking the blood, the mice were killed by cervical dislocation and dissected quickly to remove the liver. Precold saline was used to wash the blood stains, and then dried the filter paper. The tissue were collected and then detected the liver tissue MDA and liver glycogen content.

In the plain control group, the exhaustive swimming test, blood lactate, blood glucose testing and blood, liver tissue deriving were done in plain condition. The remaining experimental group were completed in a hypobaric chamber with a simulated altitude of 5000 m.

Exhaustive swimming test

60 min after the last administration, 10 mice were taken from each experimental group, and they were placed in a swimming tank with depth of 40 cm and $27 \pm 0.5^\circ\text{C}$ water temperature. Exhausted standard is submerged in the water for 10 s and the head cannot be surfaced. When they were placed on the plane, they cannot be completed the righting reflex. Swimming time was recorded.

Determination of blood lactate and glucose

Blood was collected at the end of the tail and glucose content was detected at three time points: before swimming, exhaustion, 20 min after rest; blood lactate levels was calculated according to the formula: the area under the curve of blood lactate = $5 \times$ (blood lactate value before swim + $3 \times$ blood lactate value immediately after exhaustive swimming + $2 \times$ exhaustive blood lactate 20 min after rest) [8] to calculate the area under the curve of blood lactate.

Detection of SOD activity

SOD activity was detected according to the kit instructions.

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Table 1. Mouse body weight changes during experiments (Mean \pm SD, n = 20, g)

Groups	After 1 day lavage	After 5 days lavage	After 10 days lavage
Normoxia control	19.82 \pm 0.81	22.50 \pm 1.50	23.34 \pm 1.95
Hypoxia control	17.55 \pm 1.23	22.78 \pm 1.43	23.49 \pm 1.85
Salidroside positive control	18.86 \pm 0.97	20.66 \pm 2.50	19.77 \pm 2.89
Astragalus Low-dose	19.98 \pm 2.68	22.02 \pm 2.49	22.68 \pm 2.90
Astragalus Medium dose	19.98 \pm 2.58	21.32 \pm 2.27	22.15 \pm 2.83
Astragalus High dose	19.44 \pm 2.21	21.11 \pm 2.54	21.88 \pm 2.62

Content test of hepatic glycogen and liver tissue MDA

Mice were sacrificed by cervical dislocation. The liver were removed, washed, dried water and homogenized. Content test of hepatic glycogen and liver tissue MDA was done in accordance with the kit instructions.

Statistical data and results

SPSS 11.5 software was utilized to perform the statistical analyses. The data were expressed with Mean \pm SD. ANOVA statistical analysis was completed in total difference and LSD comparison were perform to test the difference between each group. P < 0.05 shows significant difference.

Results

Body weight of hypoxia mice

During the experimental period, the body weight of mice in each group, compared with hypoxia group, showed no significant difference (P > 0.05), and there was no significant difference between the drug groups as shown in **Table 1**.

Swimming time, liver glycogen and blood lactate after exercise in hypoxic mice

Compared with the hypoxia control group, in low, medium and high dose of Astragalus groups, the swimming time extended 102.65%, 150.79% and 228.24% respectively (P < 0.05); in the middle, high-dose group, liver glycogen increased 71.99% and 132.14% (P < 0.05); in the low, middle and high dose group, the area under the of blood lactate curve decreased 43.42%, 49.71% and 52.62%, respectively (P < 0.05). Although the data showed no significant

difference between groups, there was a dose-dependent increasing trend as shown in **Table 2**.

Blood glucose of hypoxic mice

Compared with the hypoxia control group, in the experimental dose group, the blood glucose showed no significant difference in the resting, exhaustive and 20 min after exhausting three periods (P > 0.05); in hypoxia group, the resting and exhausting blood glucose were significantly lower than the plain control group (P < 0.05). The magnitude of blood glucose before and after each set of motion [(Exhausting blood glucose-resting blood glucose)/resting blood glucose \times 100] suggested that astragalus with middle and high dose can significantly increase blood glucose after exercise (P < 0.05) as shown in **Table 3**.

SOD and MDA in hypoxia mouse

Compared with hypoxia control group, except for the low dose group, SOD activity in middle and high dose of astragalus groups were significantly increased (P < 0.05). MDA content in the liver was significantly decreased (P < 0.05) as shown in **Table 4**.

Discussion

Currently improving the plateau ability to work through drug is a hot area of plateau medical research [10]. At abroad, the use of drugs to improve the efficiency of oxygen utilization and thus to improve plateau work ability was rarely reported. Based on the domestic rich resources of Chinese herbal medicines, plateau medical workers carried out many attempts in this filed. Modern biology mechanism of high altitude affecting the body changes and the views of Chinese medicine practitioners on altitude sickness confirmed that replenishing drugs can improve hypoxia endurance. Rhodiola, Acanthopanax, Dracocephalum Heterophyllum benth, medlar and other traditional Chinese medicines had been confirmed to have anti-anoxia function. Studies have shown that these drugs showed a significantly protective effect on cerebral and myocardial hypoxia, increasing arterial oxygen pressure and oxygen saturation, there-

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Table 2. Effect of Astragalus on mouse exhaust swimming time, liver starch and lactic acid (Mean \pm SD, n = 10)

Groups	Dose (g/kgd)	Swimming time (s)	Liver starch (mg/g hepatic tissue)	Area under curve of Blood lactic acid
Normoxia control	0	1549.64 \pm 590.83	21.45 \pm 7.77	107.0 \pm 25.93
Hypoxia control	0	540.09 \pm 207.11 ^a	12.82 \pm 6.23 ^a	148.05 \pm 19.26 ^a
Salidroside positive control	0.87	1854.0 \pm 420.0 ^b	23.51 \pm 5.47 ^b	106.90 \pm 11.4 ^b
Astragalus Low-dose	1.0	1094.5 \pm 328.82	13.73 \pm 6.48 ^b	83.77 \pm 9.46 ^b
Astragalus Medium dose	3.0	1354.3 \pm 408.55 ^b	22.05 \pm 5.01 ^b	74.45 \pm 13.82 ^b
Astragalus High dose	30.0	1772.8 \pm 250.57 ^b	29.76 \pm 8.27 ^b	70.14 \pm 16.10 ^b

Note: a, P < 0.05, compared to plain control group; b, P < 0.05, compared to anoxia control group.

Table 3. Effect of astragalus mouse blood glucose levels

Groups	Dose (g/kgd)	Resting blood sugar (mmol/L)	Exhaustion blood sugar (mmol/L)	20 min resting exhaustion blood sugar (mmol/L)	Range ability (%)
Normoxia control	0	6.42 \pm 2.01	5.72 \pm 2.00	5.98 \pm 2.11	-10.90 \pm 2.36
Hypoxia control	0	4.48 \pm 0.95 ^a	3.77 \pm 1.11 ^a	3.95 \pm 1.51 ^a	-15.85 \pm 1.06 ^a
Salidroside positive control	0.87	3.35 \pm 0.56	3.85 \pm 1.60	3.72 \pm 1.38	14.93 \pm 1.15 ^b
Astragalus Low-dose	1.0	3.86 \pm 0.70	3.19 \pm 0.45	3.05 \pm 0.48	-17.36 \pm 2.15
Astragalus Medium dose	3.0	5.20 \pm 0.96	5.15 \pm 1.58	4.95 \pm 1.70	-0.96 \pm 0.18 ^b
Astragalus High dose	30.0	4.76 \pm 1.25	5.19 \pm 1.07	5.10 \pm 1.47	9.03 \pm 2.78 ^b

Note: a, P < 0.05, compared to plain control group; b, P < 0.05, compared to anoxia control group.

Table 4. Effect of Astragalus on mouse SOD activity and liver MDA content (Mean \pm SD, n = 10)

Groups	Dose (g/kgd)	SOD activity (U/ml)	MDA content (nmol/ml)
Normoxia control	0	197.53 \pm 29.10	6.74 \pm 0.21
Hypoxia control	0	129.10 \pm 26.99 ^a	14.12 \pm 1.08 ^a
Salidroside positive control	0.87	203.94 \pm 24.05 ^b	8.89 \pm 1.21 ^b
Astragalus Low-dose	1.0	153.38 \pm 28.48	12.68 \pm 2.36
Astragalus Medium dose	3.0	180.75 \pm 11.77 ^b	8.61 \pm 0.65 ^b
Astragalus High dose	30.0	192.67 \pm 23.31 ^b	5.69 \pm 0.64 ^b

Note: a, P < 0.05, compared to plain control group; b, P < 0.05, compared to anoxia control group.

by increasing hypoxia tolerance [11, 12]. According to the related anti-fatigue function evaluation methods in "health food function evaluation procedures and test methods" proposed by Ministry of Health, we simulated high altitude environment by low-pressure chamber and established exhaustive swimming mice models to systematically review the anti-fatigue effect of astragalus in a simulated plateau environment; the results showed that in a simulated high altitude environment, low, medium and high doses of Astragalus could significantly prolong the swimming time and promote the elimination of lactic acid after exercise; middle and

high doses can significantly increase hepatic glycogen reserves, maintain blood glucose levels after exercise, increased serum SOD activity and reduce MDA content in liver; between low-dose group and model group, no significant change had been found in glycogen, glucose, lactate, SOD and MDA, which may be related with the too low concentration and poor efficacy. The results show that in high altitude and hypoxia environment, Astragalus

could relieve fatigue and improve labor efficiency. Exercise-induced fatigue refers to the physiological phenomenon of a temporary decline in the maximum contraction or the maximum output power of muscle caused by the movement, which makes the physiological process of the body cannot maintain its performance on a particular level and (or) cannot maintain a predetermined exercise intensity. At present, the mechanism of exercise-induced fatigue is considered to be mainly related with the excessive consumption of materials and energy, accumulation of fatigue substances in the body, internal environment disorders, and the metabolic

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regulation disorders of nervous system, enzymes and hormones during exercise [13, 14].

Astragalus mainly contains saponins, polysaccharides, flavonoids, various amino acids, folic acid and selenium, zinc, copper, and so on. The results of this study showed that Astragalus extract could relieve exercise fatigue and promote the recovery in simulated high altitude environment; mechanism may be related to its roles of enhancing the activity of antioxidant enzymes and reducing the generation of oxygen free radicals. Studies have shown that Astragalus has a strong activity of anti-oxidative stress [15, 16]; flavonoid, a main substance in astragalus, could induce redox reactions by providing a hydrogen atom to scavenge free radical; chemical structure of saponins is similar to that of flavonoids, whose phenolic hydroxyl groups may related with the mechanisms of scavenging free radical.

In summary, this study suggested that Astragalus can significantly alleviate the exercise-induced fatigue of altitude hypoxic mice, with a significant anti-plateau-fatigue effect. This provides an experimental basis for further development and utilization of the ability of astragalus to improve the plateau work capacity.

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Disclosure of conflict of interest

None.

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