Anti-fatigue effects of *Panax notoginseng* in simulation plateau-condition mice

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ABSTRACT

Background: *Panax notoginseng* (PN) is one of the most commonly used Chinese herbal drugs. *Panax notoginseng saponins* (PNS) is the main effective components of PN. However, the anti-fatigue effect of PNS in plateau-condition is unknown. **Objective:** Explore the anti-fatigue effects of PNS in mice living under simulation plateau-condition. **Materials and Methods:** Hundred male Kunming mice were randomly divided into five groups (n = 20): one normoxia control group (NCG), one hypoxia control group (HCG), and three PNS groups in low dosage (0.42 g/kg), mid dosage (1.11 g/kg), and high dosage (11.53 g/kg). HCG and PNS groups were fed at a simulated elevation of 5 km. NCG and HCG were intragastric administrated with distilled water. After continuous administration for 10 days, the exhaustive swimming time, glycogen contents in liver, blood lactic acid (BLA), and blood glucose were determined. **Results:** Exposure of the mice to simulation plateau-condition with 5 km altitude for 10 days caused significant decrease of exercise tolerance compared to normoxia environment. The swimming time and glycogen contents in liver were significantly increased at all tested concentration (0.42, 1.11, and 11.53 g/kg). The area under the BLA curve was significantly decreased at the concentration of 0.42 g/ kg. The blood glucose of resting and 0 minutes after swimming were significantly increased by 29.31% and 15.51% ($P < 0.05$) at a concentration of 11.53 g/kg compared to their own control groups, respectively. **Conclusion:** These results indicate that PNS could postpone the appearance of fatigue and accelerate the restoration of fatigue in plateau environment, especially in low dosage (0.42 g/kg) case.

Key words: Anti--fatigue, high altitude, *Panax notoginseng*, plateau

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INTRODUCTION

Panax notoginseng (PN) is one of the most commonly used Chinese herbal drugs. *Panax notoginseng saponins* (PNS) is the main effective components of PN, and *ginsenoside* Rb1 (Rb1) and ginsenoside Rg1 (Rg1) are the two main active *saponins* contained in PNS.[1,2] Rb1, one of the main 20 (S)-*protopanaxadiol* group *saponins*, showed effective anti-inflammatory action, obvious *vasodilating* effect, and tranquilizing function to central nervous system. Rg1 possessed the properties of exciting central nervous system, anti-fatigue, and hemolysis.[3]

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Rapid ascent to a high altitude environment decreases working performance of the sea level residents, which is a general phenomenon of poor acclimatization to hypoxia. The labor efficiency decreased by 12.61% and 18.78% at the altitude of 3500 m and 4500 m than in the plain, respectively.[4] Our results indicated that fatigue was inclined to happen in hypoxia control group (HCG) compared to normoxia control group (NCG). Thus, search for the medicine to strengthen anti-fatigue capability, restore physical strength, and energy fast is crucial. The existing drugs of anti-fatigue in plateau are salidroside, acetazolamide, tyrosine, enalapril, nifedipine, and ginkgo.[5-7]

PNS could alleviate physical fatigue in mice under normoxia environment.[8] PNS increased the activities of superoxide dismutase, decreased the level of lipid peroxides, and promoted metabolism of nucleic acids and protein,[9] which suggested antioxidant and free radical scavenging

activities may play a role in the mechanism of PNS anti-fatigue effect.[10] However, the anti-fatigue effect of PNS in plateau-condition is unknown. The present study was designed to ascertain the anti-fatigue property of PNS in plateau-condition by the determination of exhaustive swimming test, the level of blood lactic acid (BLA) and glucose, and liver glycogen.

MATERIALS AND METHODS

Plant material and preparation of plant extract

The roots of PNS were collected from Wenshan, Yunnan province, China. Voucher specimen (No. 20091201) was deposited at the Key Laboratory of High Altitude Medicine, Ministry of Education, and Third Military Medical University.

The air-dried roots of PNS (100 g) were powdered and extracted twice with aqueous 60% EtOH (1000 ml) under reflux for 1.5 h each time. After filtration and centrifugation (1700×g, 20 min), the combined solution was concentrated by reducing pressure at 50ºC and finally dried in high vacuum to afford about 20.5 g of ethanol extract.[11,12] The ethanol extract was then reconstituted and diluted with distilled water (100 ml).

Materials

The content of liver glycogen was tested according to the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BLA meter (Lactate Pro™ LT-1710) and blood glucose monitoring (ACCU-CHEC Active) were purchased from ARKRAY (Japan) and Roche (Germany), respectively.

Animals, grouping, and treating

Hundred male Kunming mice (weighing 20±2g) purchased from the Center of Laboratory Animal of the Third Military Medical University (Chongqing, China). Mice were divided into five groups (n=20): one NCG, one HCG, and three PNS groups in low dosage (LD), mid dosage (MD), and high dosage (HD). LD (0.42 g/kg), MD (1.11 g/kg), and HD (11.53 g/kg, which are 0.3, 1, and 10 times as much as the recommended dose for people, respectively. HCG and PNS groups were housed in a hypobaric chamber (equivalent to the low pressure chamber of 5 km height), and NCG was fed in normoxia environment. All mice lived at room temperature of 23±1ºC and moderate humidity 50-60% with a 12 h light, 12 h dark cycle (lights on from 6:00 am to 6:00 pm). The experiment protocols were approved by the Animal Care and Use Committee of our institute. LD, MD, and HD were intragastric administered with PNS at the dose of 0.42 g/kg, 1.11 g/kg, and 11.53 g/kg, respectively. NCG and HCG were intragastric administered with distilled water. After continuous administration for 10 days, the exhaustive swimming time, glycogen contents in liver, BLA, and blood glucose were determined. Twenty mice in every group were further divided into two subgroups: group A and B. Every group A (n=10) was conducted for exhaustive swimming test, blood glucose, and BLA. Every group B (n=10) was conducted for liver glycogen test.

Exhaustive swimming test

After the last administration, the mice were allowed to rest for 1 h. Then the exhaustive swimming test was conducted. There is a pump in the tank to make the water circulated. The mice were placed in the swimming tank (90 cm in length, 60 cm in width, and 60 cm in depth) with 50 cm deep water at 25±1ºC water. Mice continued to move their limbs until they succumbed to exhaustion and failed to rise above the surface after 10 s.[13]

Liver glycogen test

Liver glycogen test was conducted 1 h after the last administration in the mice of Group B. The mice were sacrificed and the livers were quickly removed. Their livers were dissected immediately, washed with 0.9% saline, and blotted dry with filter papers. Liver samples (50-100 mg) were weighed accurately. The contents of liver glycogen were tested according to the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute as mentioned above. Glycogen weight (mg) per 100 g of liver was calculated according to the following formula: Glycogen weight (mg/100 g liver) = DU/DS × standard glycogen weight (0.01 mg) × sample dilution (100) × 10 / 1.11, where DU represents the absorbency of the sample and DS represents the absorbency of the standard.

Blood lactic acid test

BLA test was conducted before swimming, 0 and 20 minutes after swimming, the content of BLA was determined with lactic acid meter, and the area under the curve of BLA was calculated. The area under the curve of BLA = 5 × (the level of BLA before swimming + 3 × 0 minutes after swimming + 2 × 20 minutes after swimming).[14]

Blood glucose test

Blood glucose test was conducted before swimming, 0 and 20 minutes after swimming, and the contents of blood glucose was determined by blood glucose monitoring meter.

Statistical analysis

All values were presented as means ± standard deviation (S.D.). The data were statistically analyzed by means of one-way analysis of variance (ANOVA) and Student's *t*-test, using Statistical Package for the Social Sciences (SPSS) v11.5 software. The level of significance was set at $P < 0.05$ and 0.01 for all statistical analysis.

RESULTS

Effect of Panax notoginseng saponins on body weight in mice

There was no significant (P>0.05) difference between PNS groups and HCG on body weight on the first day, the fifth day, and the tenth day (data not shown).

Panax notoginseng saponins prolonged the exhaustive swimming time

Compared with HCG, the swimming times are prolonged by 364%, 254%, and 95% at the concentration of 0.42 g/ kg, 1.11 g/kg, and 11.53 g/kg, respectively [Figure 1]. The swimming time in NCG was significantly longer than that in HCG (4204.90±2393.03 versus 890.64±298.25 s, P>0.01).

Panax notoginseng saponins increased liver glycogen contents

As shown in Figure 2, the liver glycogen contents were greater at all tested concentrations (0.42 g/kg, 1.11 g/kg, and 11.53 g/kg) than that in HCG and the prolonged rates were 102.92%, 96.10%, and 68.91%, respectively(P<0.05). No statistically significant increase of liver glycogen contents was observed in NCG (17.88±7.28 versus 12.32±5.85 mg/g liver, P>0.05).

Effect of PNS on the area under the curve of blood lactic acid

As presented in Figure 3, the area under the curve of BLA was significantly decreased at the concentration of 0.42 g/kg, but increased at the concentration of 11.53 g/kg compared to HCG(P<0.05). The area under the curve of BLA was not significantly decreased in NCG compared to HCG (131.22±43.61 versus 133.05±35.90 %, P > 0.05).

Effect of PNS on blood glucose

Figure 4 presents the blood glucose before swimming, 0 and 20 minutes after swimming. The blood glucose of resting and 0 minutes after swimming were significantly increased by 29.31% and 15.51%(P<0.05) at the concentration of 11.53 g/kg compared to their own control groups, respectively. But no significant increase was observed in other groups. The

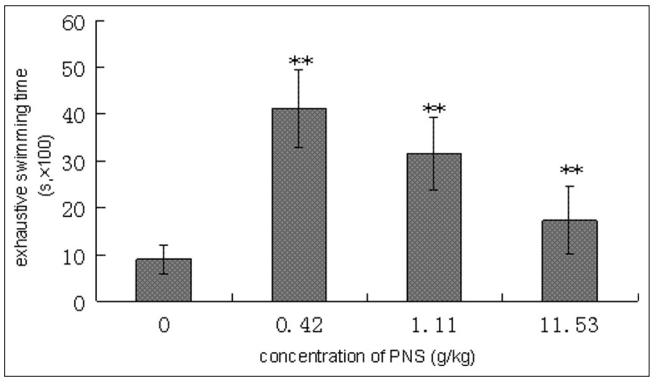

Figure 1: Effect of PNS on exhaustive swimming time in simulation plateau-condition mice. Mice were administered with PNS (0, 0.42, 1.11, and 11.53 g/kg) for 10 days (n=10). Each bar represents the mean±S.D. of swimming time (n=10). **P < 0.01 significantly different from mice in HCG

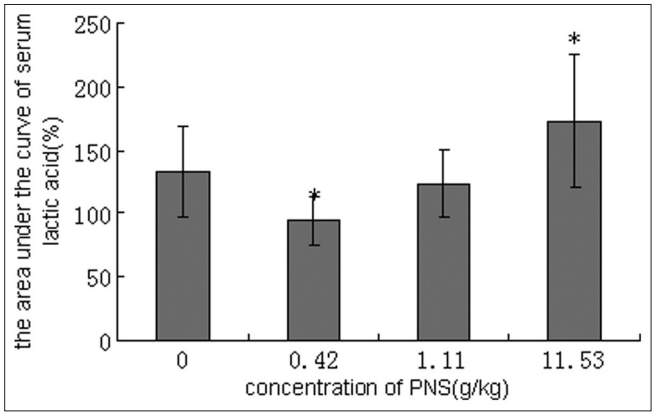

Figure 3: Effect of PNS on the area under the curve of serum lactic acid in simulation plateau-condition mice. Mice were administered with PNS (0, 0.42, 1.11, and 11.53 g/kg) for 10 days. Each bar represents the mean±S.D. of the area under the curve of serum lactic acid (n=10). *P < 0.05 significantly different from mice in HCG

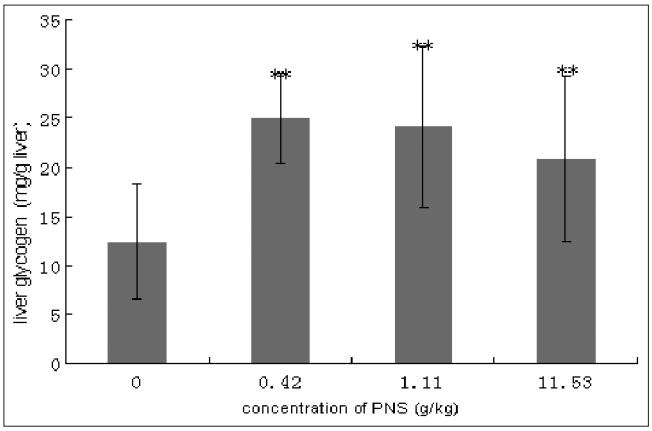

Figure 2: Effect of PNS on liver glycogen contents in simulation plateau-condition mice. Mice were administered with PNS (0, 0.42, 1.11, and 11.53 g/kg) for 10 days. Each bar represents the mean±S.D. of liver glycogen content (n=10). **P < 0.01 significantly different from mice in HCG

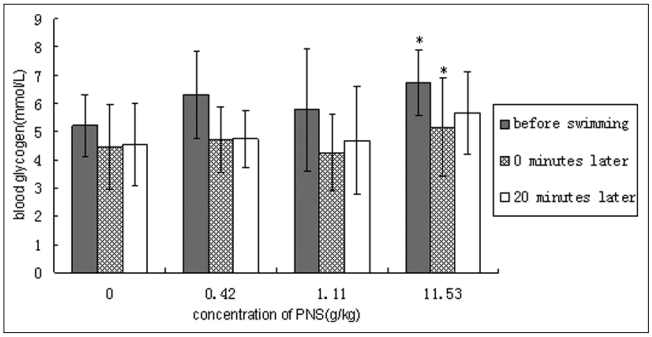

Figure 4: Effect of PNS on blood glucose in simulation plateau-condition mice. Mice were administered with PNS (0, 0.42, 1.11, and 11.53 g/kg) for 10 days. Each bar represents the mean±S.D. of the blood glucose (n=10). *P < 0.05 significantly different compared to own control groups

resting blood glucose was significantly higher in NCG than that in HCG (8.66±1.43 versus 5.22±1.09 mmol/L, $P < 0.01$).

DISCUSSION

Movement-fatigue may be defined as a situation in which the capacity for work is diminished and efficiency of accomplishment is reduced, resulting from hard physical work. The main mechanism of movement-fatigue is as follows: depletion of energy substances, including liver glycogen consumption and levels of glycemic decrease; accumulation of fatigue material, such as BLA; disorder of internal environment; metabolic control disorder of nervous system, enzyme, and hormone during the process of sport.[15] Body was in oxygen-deficient state after strenuous exercise and the state was deepened under hypoxic environment at high altitude.

The pharmacological effects of PNS included attenuation of cisplatin-induced nephrotoxicity, protection of the injured brain, anti-inflammation due to the reduction of the level of the intracellular free calcium concentration in neutrophils, antioxidant activity,[10] alleviating physical fatigue in normoxia environment.[8] However, the anti-fatigue effect in plateau-condition has not been reported until now.

The present study was designed based on the criterion of testing and evaluating health foods (2003).[16] First, our results indicated that fatigue was inclined to happen in HCG compared to NCG and then the exhaustive swimming time, glycogen contents in liver, BLA, and blood glucose were determined to evaluate the anti-fatigue effects of PNS in simulation plateau-condition mice. The data were statistically analyzed by means of one-way analysis of variance (ANOVA) and Student's *t*-test.

A direct measure of anti-fatigue effect is the increase in exercise tolerance. Swimming to exhaustion is an experimental exercise model to evaluate physical fatigue. It works well for evaluating the endurance capacity of mice.[17] In the present study, PNS could significantly lengthen the exhaustive swimming time in all PNS groups, which indicated that PNS can elevate the exercise tolerance of mice under the simulation plateau-condition. However, the longest exhaustive swimming time appeared in LD of this study, which might be due to the relationship between effects and dose. For example, metronidazole has been used to treat amoeboma of intestine, anaerobic infection, and balantidiasis, whereas therapeutic doses are 1.2 g-1.8 g/day, 0.6 g-1.2 g/day, and 0.4 g/day, respectively.

Carbohydrate is the most important energy sources, which is deposited in the body as blood glucose and glycogen. Glycogen deposited in liver and muscle tissue, which can readily converted to glucose as needed by the body to satisfy its energy needs.[18] Energy for exercise is derived initially from the breakdown of glycogen and circulating glucose released later by the liver.[19] So liver glycogen is a sensitive parameter related to fatigue. It was shown that treatment with PNS increased the level of liver glycogen which provided support for the involvement of liver glycogen as an important parameter in evaluation of PNS anti-fatigue effects in simulation plateau-condition mice. And PNS increased the blood glycogen only in HD, suggesting high concentration of PNS promoted the level of blood glycogen more than liver glycogen.

The accumulation of lactic acid is a reason of fatigue.[20] Lactic acid itself is not a direct reason of the development of fatigue according to some studies, while it is H^+ dissociated from lactic acid that changes internal pH value, which will do harm to certain organs and produce fatigue.[21-23] If any medicine could inhibit the accumulation of lactic acid and accelerate the clearance of lactic acid, that medicine is characteristic with anti-fatigue effects.[24] In the present study, PNS could significantly decrease the area under the curve of BLA which suggested that PNS can accelerate the restoration of energy in simulation plateau environment. However, PNS showed the best effects to undo BLA in LD which is consistent with experiment exhaustive swimming time, suggesting that PNS could postpone the appearance of fatigue and accelerate the restoration of fatigue in simulation plateau environment, especially in LD.

To our knowledge, this is the first study to show anti-fatigue effects induced by PNS in simulation plateau-condition mice. Our results suggested that PNS had significant anti-fatigue effects on hypoxia mice and the strongest effect on most biomarkers was observed in LD (0.42 g/kg). Results of our study indicated that PNS could postpone the appearance of fatigue and accelerate the restoration of fatigue in simulation plateau environment. However, further studies to clarify the detailed mechanisms involved in the anti-fatigue properties of PNS in plateau are necessary.

CONCLUSION

PNS could postpone the appearance of fatigue and accelerate the restoration of fatigue in plateau environment, especially on low dosage (0.42 g/kg).

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